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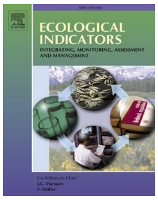

Performance of bees and beehive products as indicators of elemental tracers of atmospheric pollution in sites of the Rome province (Italy)

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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 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). The control charts of the considered elements were built for the first time, 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$_{\text{ower}}$) and the highest (OBI-U$_{\text{pper}}$) 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 environmental contamination cases becomes necessary. To a lesser extent, pollen and propolis showed high levels for several tracers for 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

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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 denote 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 excellent biomonitors/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 et al., 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 for 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 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 a limited sampling period.

The first aim of the work was to determine the levels of fourteen relevant elements that several studies have indicated (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 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 biomass burning emissions 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 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 the probabilistic Johnson's method (Johnson, 1949). By normalisation 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 ($\text{OBI-U}_{\text{pper}}$) and lower ($\text{OBI-L}_{\text{ower}}$) 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).
2. We applied the OBI for ranking the selected indicators according to their median elements' content.
3. The advantage of the OBI is that it considers groups of indicators simultaneously. This is more connected to a holistic approach than classical, deterministic studies.

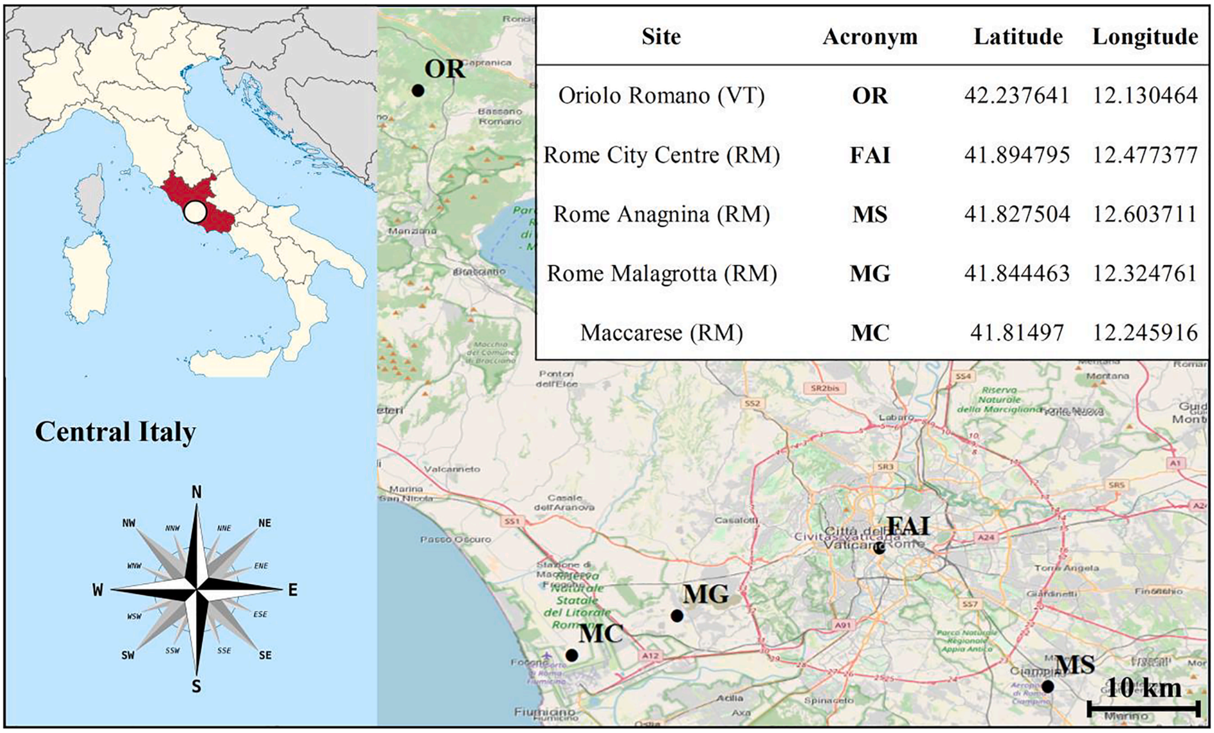
We have already tested the OBI in various studies carried out in the last decade (Conti et al., 2015, 2019a, 2022) concerning marine and atmospheric ecosystems. Recently, Coletti et al. (2022) have also applied the OBI to rank the lithological types according to their average radionuclide content.Therefore, the use of OBI as an integrated tool in environmental management consents to identify the specific biomonitor/indicators needed to study a specific condition of contamination that can arise from natural or anthropogenic activities. It can enhance 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, except the Oriolo Romano site (OR, Viterbo province, Fig. 1), a green area at 60 km from the center of Rome heavily affected by biomass burning contributions. One site was in the centre of Rome, i.e., on the roof of the Apicultural Italian Federation (FAI). For this reason, it was considered a site mainly characterised 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 characterised 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 Fig. 1 for sites' description).

We have placed two (independent) beehives at each site to enhance the requisite variety (n = ten beehives). Six sampling campaigns at the



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. Map of the sampling area. OR—green area; FAI—urbanised (highly populated area); MG—near a landfill; MC—close to Fiumicino airport and MS—on a busy road. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 (Astolfi et al., 2021). After transporting the samples to the laboratory, the wax samples were carefully washed with deionised water to remove any residues. Instead, the bees were not washed to consider the content of the elements both on and within the bees' bodies. 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, making 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 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. Bees and wax samples were lyophilised for 48 h 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. 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). 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 analysed 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., 2020a; Astolfi et al., 2020b; 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 mineralised 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 deionised 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. Through a translation technique, this method allows classifying 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:

$I_{min} = \max(Q_{i,2.5}, Q_{j,2.5})$ with $i = 1, 2,..,k$ and $i \neq j$.

$I_{max} = \min(Q_{i,97.5}, Q_{j,97.5})$ 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:

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

$$OBI - U_i = \frac{Q_{i,97.5}}{I_{max}} \text{with } i = 1, 2, \cdots K$$

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

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

$$OBI - L_i = \frac{I_{min}}{Q_{i,2.5}}$$

The median test and post hoc comparisons have been applied to compare medians.

3. Results and discussion

Both macro-and microelements contents in bees varied in broad ranges. They depended on several factors (Girotti et al., 2020), such as types of soils, 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; Zarić 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 K, Ca, and Si levels in bees and beehive products were the highest of all the elements analysed (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 Zarić 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, leading to some bias. Furthermore, another factor is comparing sites with different characteristics (a column with a short description of the sites is reported in Table SA).

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

On the contrary, the levels of the elements analysed 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 detected elements in wax, pollen, and propolis were generally comparable to or at the 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, the honeybee–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 a 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

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

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). Fig. 3 for K, and S5-S7 for Rb, Cs, and Li, respectively, show the control charts and the overlap metal concentrations for biomass burning markers; then, Fig. 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.

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). At the same time, bees bioaccumulate at significantly higher levels (post hoc of median test $p < 0.05$), i.e. median 23.2 μg/g (Fig. 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

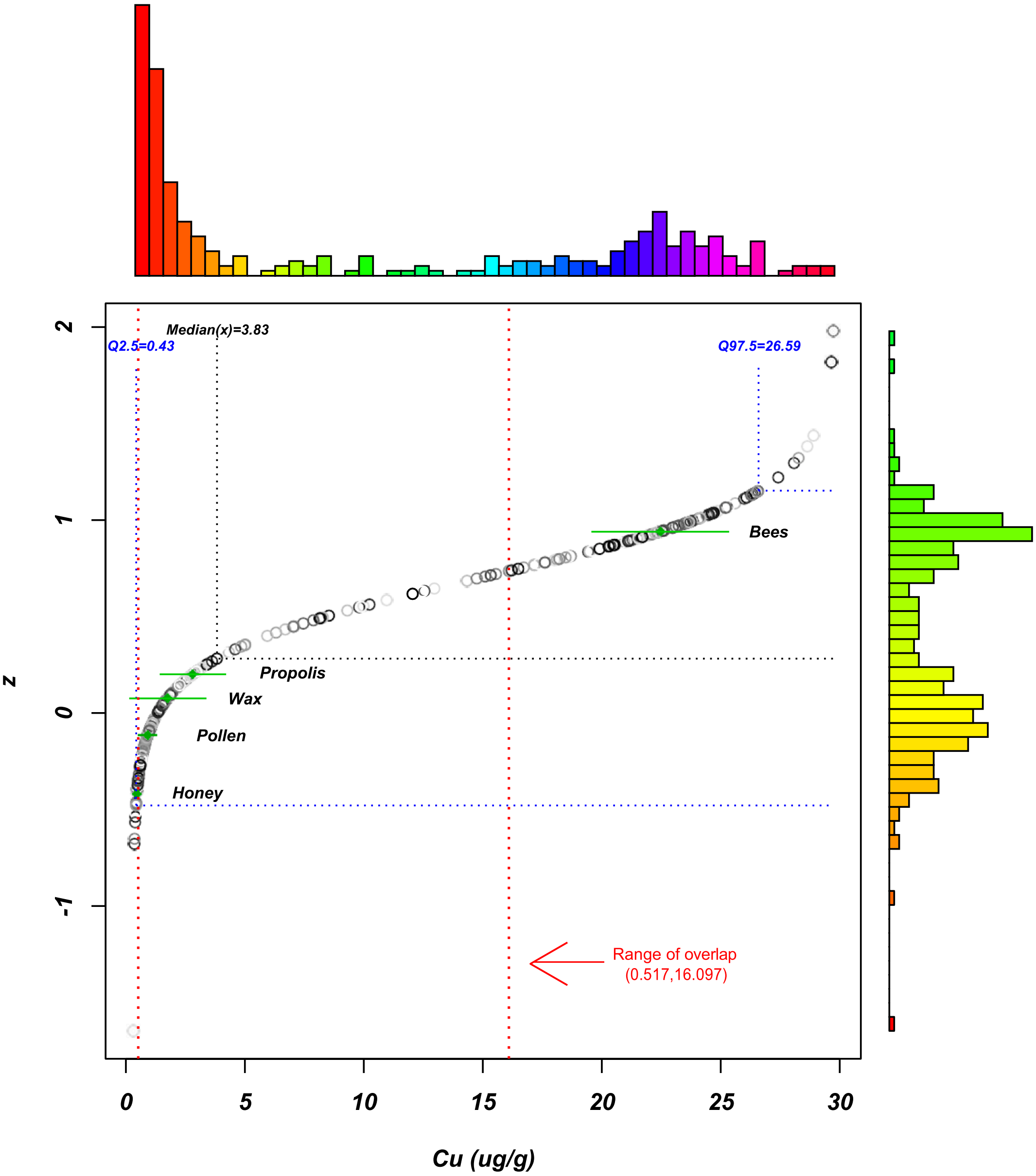


Fig. 2. Control chart for Cu built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (μg/g). Observed values are on x-axes, and values calculated by Johnson’s method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

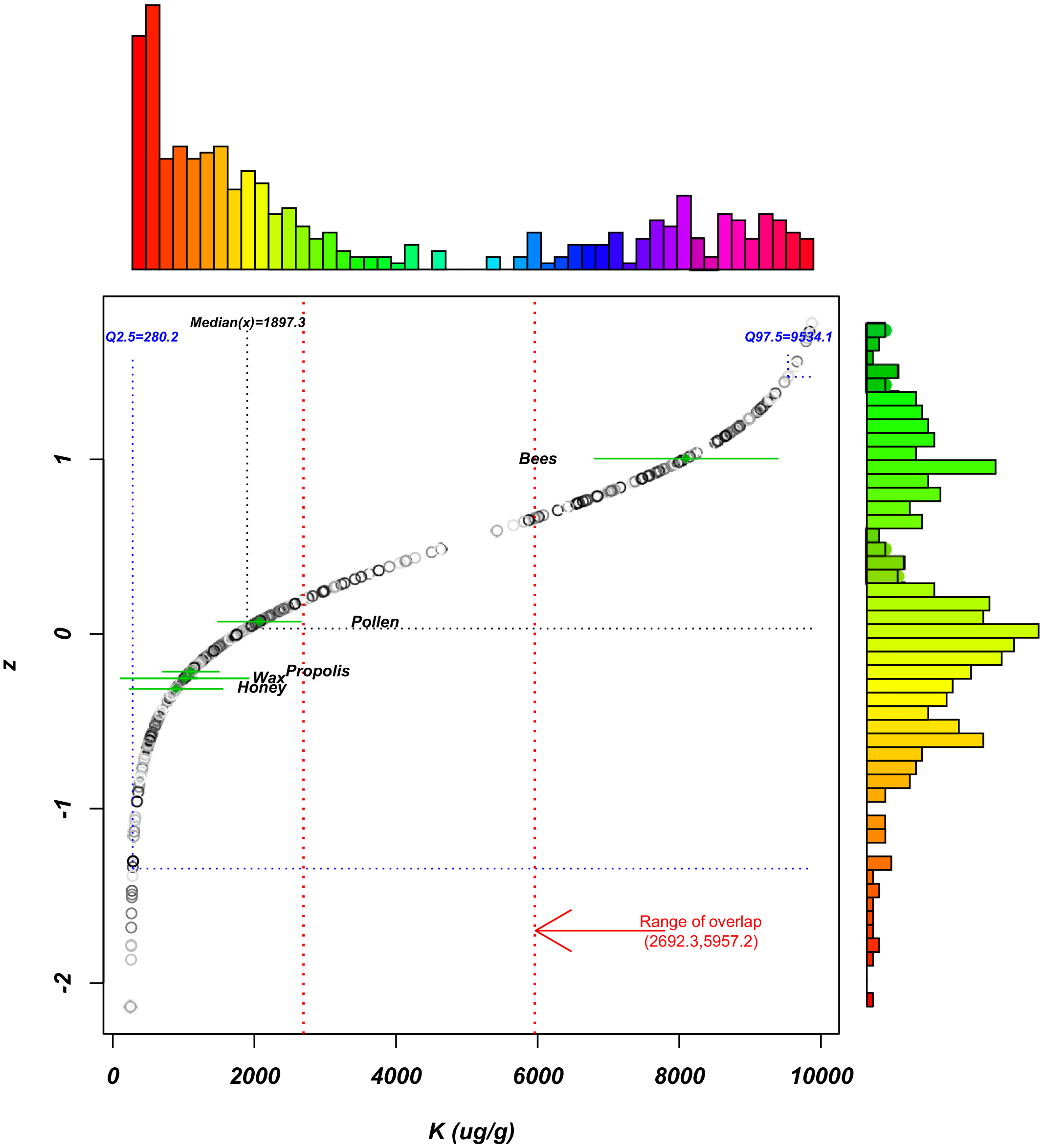


Fig. 3. Control chart for K built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (μg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

obtained after dividing the $\mathrm{Qi}_{,97.5}$ 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) shows that bees have high bioaccumulation Cu surplus (OBI-U = 68.6) and respond better 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.

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 (Table S3)

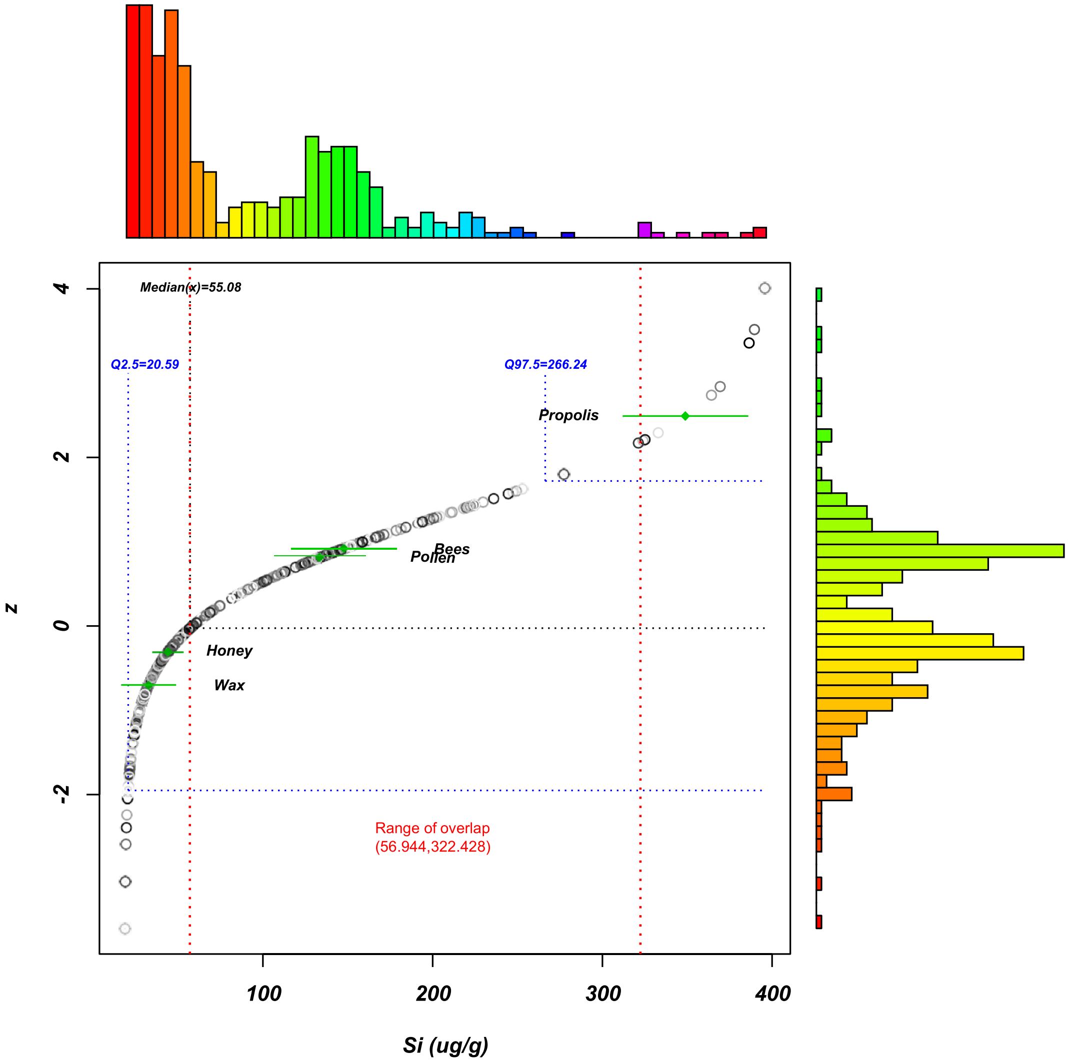


Fig. 4. Control chart for Si built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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			

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			

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			

showed a high Fe bioaccumulation surplus (OBI-U = 28.8), demonstrating its aptitude as a good biomonitor of traffic tracers. On the contrary, honey showed a very high Fe OBI-L value (154.4), confirming its very low 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 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 et al., 2013; Álvarez-Ayuso and Abad-Valle, 2017; Conti et al., 2018).

3.3. Biomass burning tracers and the OBI index in Rome province

Fig. 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, and 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. At the same time, bees could act as good biomonitors of biomass burning tracers in supposedly contaminated sites (see also Figures S5-S7).

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 (Fig. 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). The soil tracers, as mentioned above, make the major contribution to the elemental concentrations of the matrices considered.

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 behaviour 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).

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 good to very high OBI-L values for honey in soil tracers, i.e., Si (10.9, Table 4), Ca (18.6), and 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 does not accumulate the elemental tracers of atmospheric pollution at high levels compared with bees and other beehive products. 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 of 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), and 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. Calcium 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 a high bioaccumulation surplus for Fe (OBI-U = 28.8) and for Ti (OBI-U = 312.9), which 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, botanical sources, and the bee species (Matin et al., 2016). However, due to its chemical composition (mainly amino acids, polyphenols, steroids, and terpenes) and the sticky nature of gum, propolis could be show metal contamination. It might be used as a bioindicator of atmospheric pollution (Finger et al., 2014).

However, it should be noted that multiple additional 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) can link elements. Every element has a threshold 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) suggests that the 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, the resuspension of soils locally contaminated. To cope with 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 biomonitor/indicators is a crucial decisional process that, in turn, encompasses information management (searching, collecting, shaping, interpreting data, etc.) and that enquires for problem-solving that can be 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.

4. 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 emphasised 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 an early warning signal when the contamination process is in its initial stages.

These results underpin our data's hypothesis as baseline data that management decisions about future environmental protection programs can be considered. 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

Marcelo Enrique Conti: Conceptualization, Methodology, Resources, Data curation, Software, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Maria Luisa Astolfi:** Methodology, Resources, Validation, Data curation, Software, Writing – original draft, Writing – review & editing, Supervision. **Giustino Mele:** Resources, Data curation. **Martina Ristorini:** Resources. **Giulia Vitiello:** Resources. **Lorenzo Massimi:** Methodology, Validation, Writing – review & editing. **Silvia Canepari:** Methodology, Resources, Writing – review & editing, Supervision. **Maria Grazia Finoia:** Conceptualization, Software, Writing – original draft.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109061.

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