Supplementary material

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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1S. Quality Assurance and Control

The analytical performance parameters of linearity, detection and quantification limit (LOD and LOQ, respectively), precision, and accuracy were evaluated. With the lack of suitable certified reference material, spiked samples were used to determine the elemental recoveries by used methods (Astolfi et al., 2020a). Method blanks, in-house quality control samples, and spiked and non-spiked real samples (three replicates each) were prepared along with every digested sample batch (see for details Astolfi et al., 2020a). Recoveries for all elements fell within 20% of the expected value, with many of the elements recovering within 10%. The within-run precision for all the elements in honey and most of the elements in other matrices was less than 10%. The intermediate precision was less than 15% for most of the elements in all matrices. After digestion, all the samples were left to cool The volume was completed to 20 mL H_2O for inductively coupled plasma mass spectrometry (ICP-MS) analysis (Astolfi et al., 2020a).

1S.1. Digestion efficiency

A selected tolerance level of the residual carbon content (RCC) in a solution lower than 200 mg L^{-1} was considered appropriate for the subsequent analyses by ICP-MS (Astolfi et al., 2020a).

1S.2. The ICP-MS analysis

The LODs were calculated with three times the relative standard deviation percentage (RSD%) of ten method blanks multiplied by the background equivalent concentration (BEC)/100 (Astolfi et al., 2020b) and the dilution factor used for sample preparation. At regular intervals (every 20 samples) during all analyses, an intermediate calibration standard was analysed as a sample to monitor the instrument drift. A maximum percentage drift of ± 10% was considered acceptable for all the elements. Furthermore, calibration blanks (3% HNO_3) were frequently analysed alongside samples to check for any loss or cross-contamination. An internal standardisation monitored the matrix effects on the sample uptake, and nebulisation with Y, Sc, Rh, In and Th, and measurements were automatically corrected by the respective ICP software. Yttrium was not used as an internal standard for the ICP-MS analyses of the propolis samples because it is contained in the propolis at a concentration of ~0.25 mg kg^{-1} (~0.0025 mg L^{-1} in digests).

2S. Figures and Tables for traffic tracers.

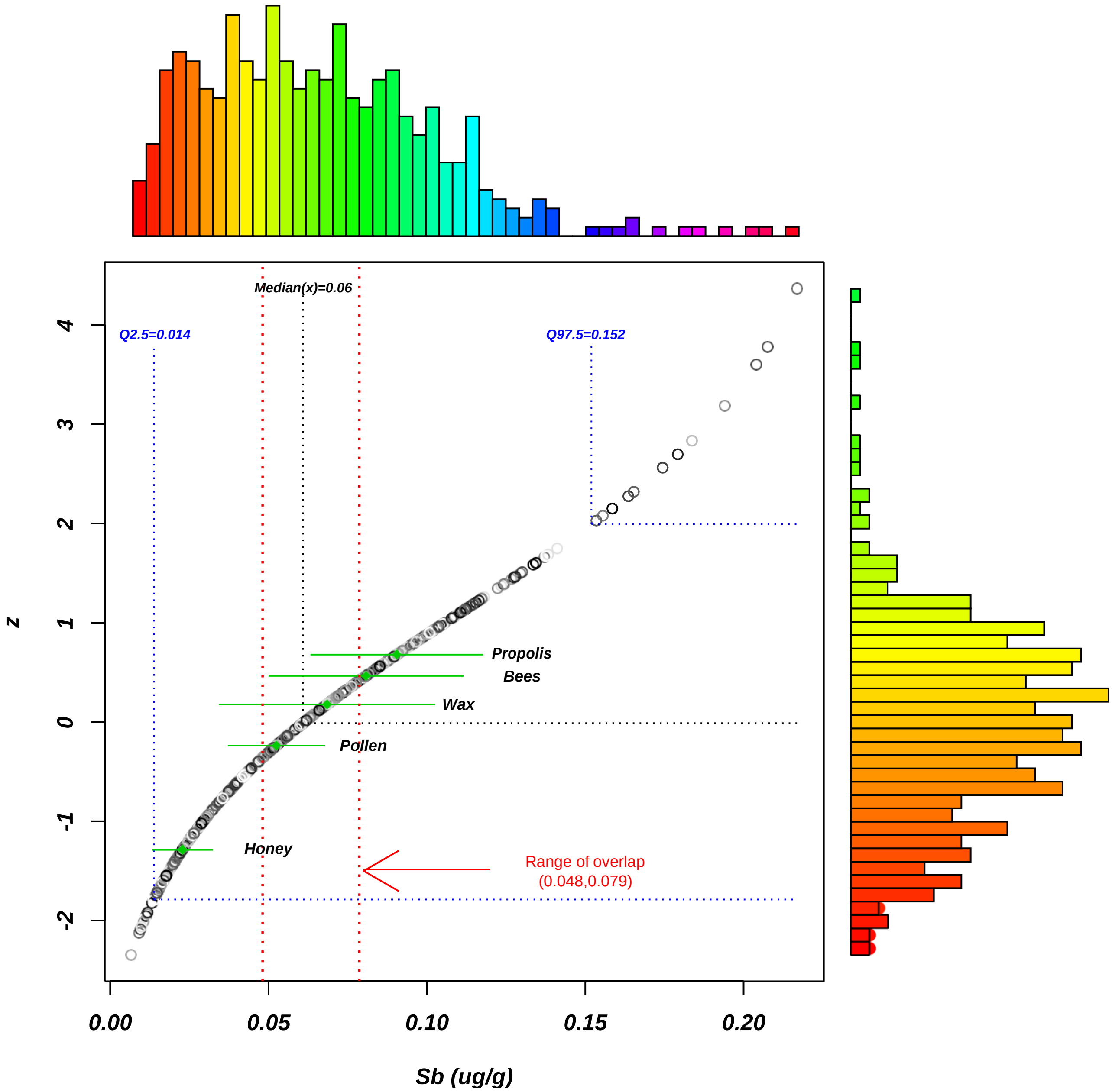

Figure S1. Control chart for Sb built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

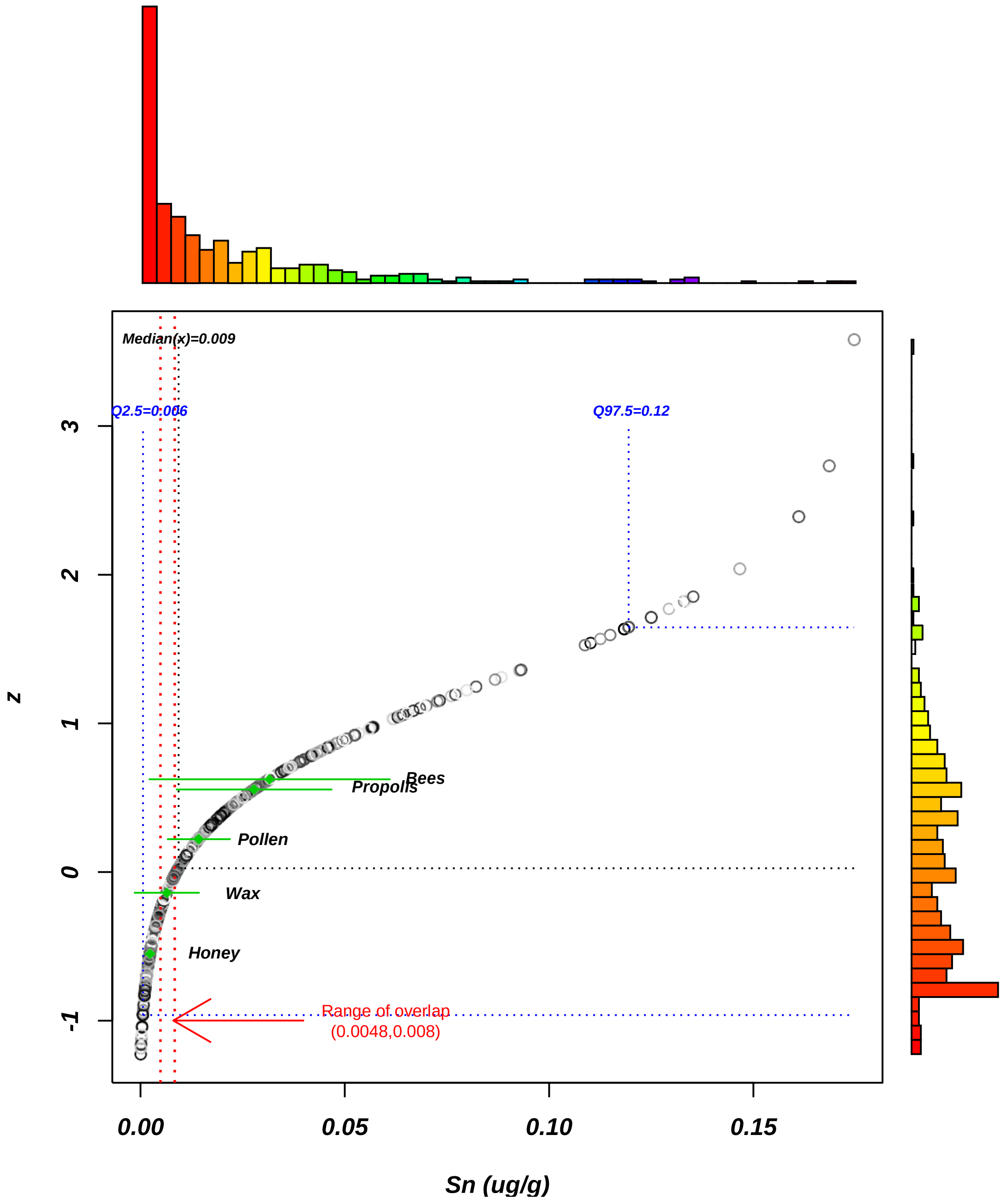

Figure S2. Control chart for Sn built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

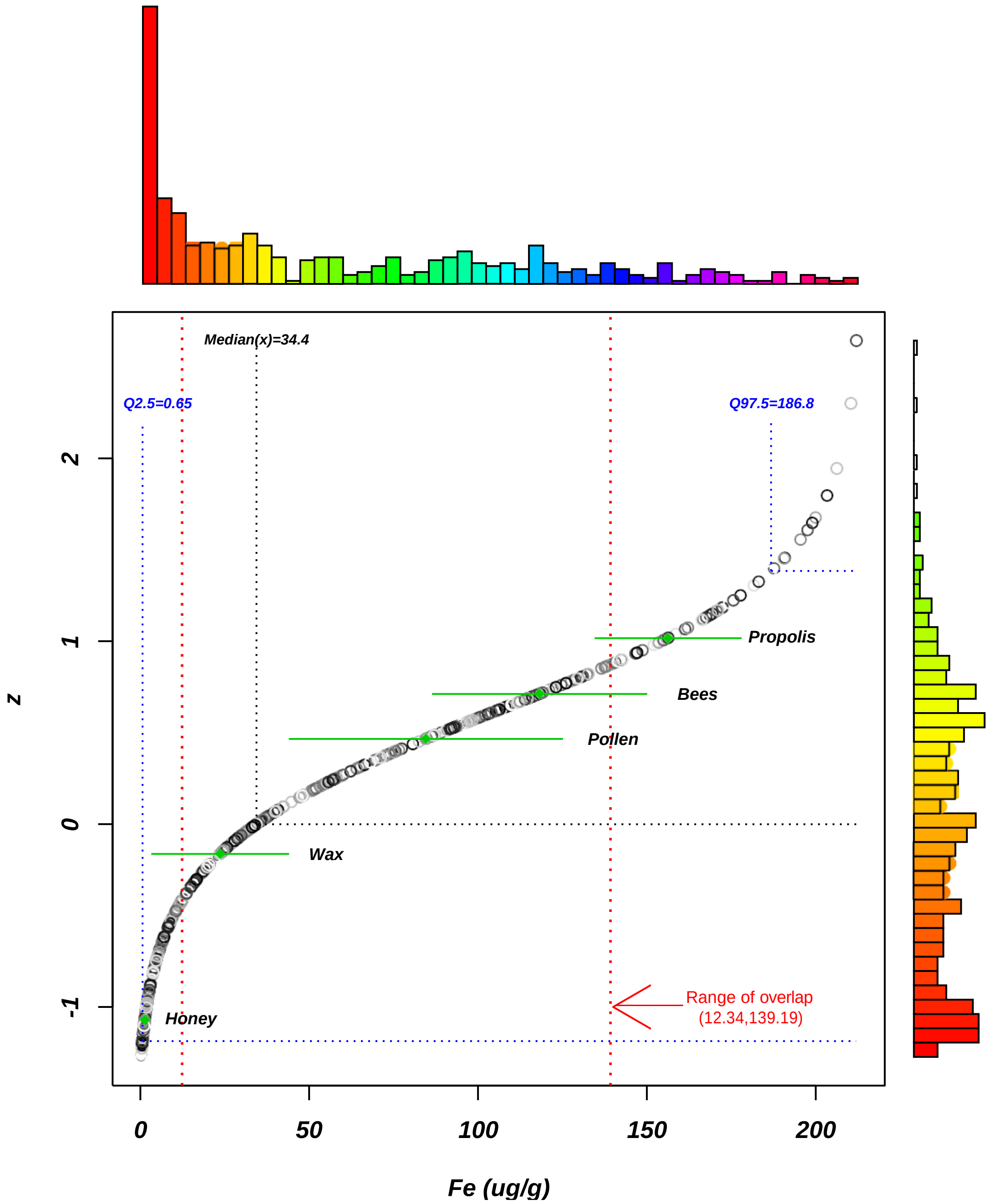

Figure S3. Control chart for Fe built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

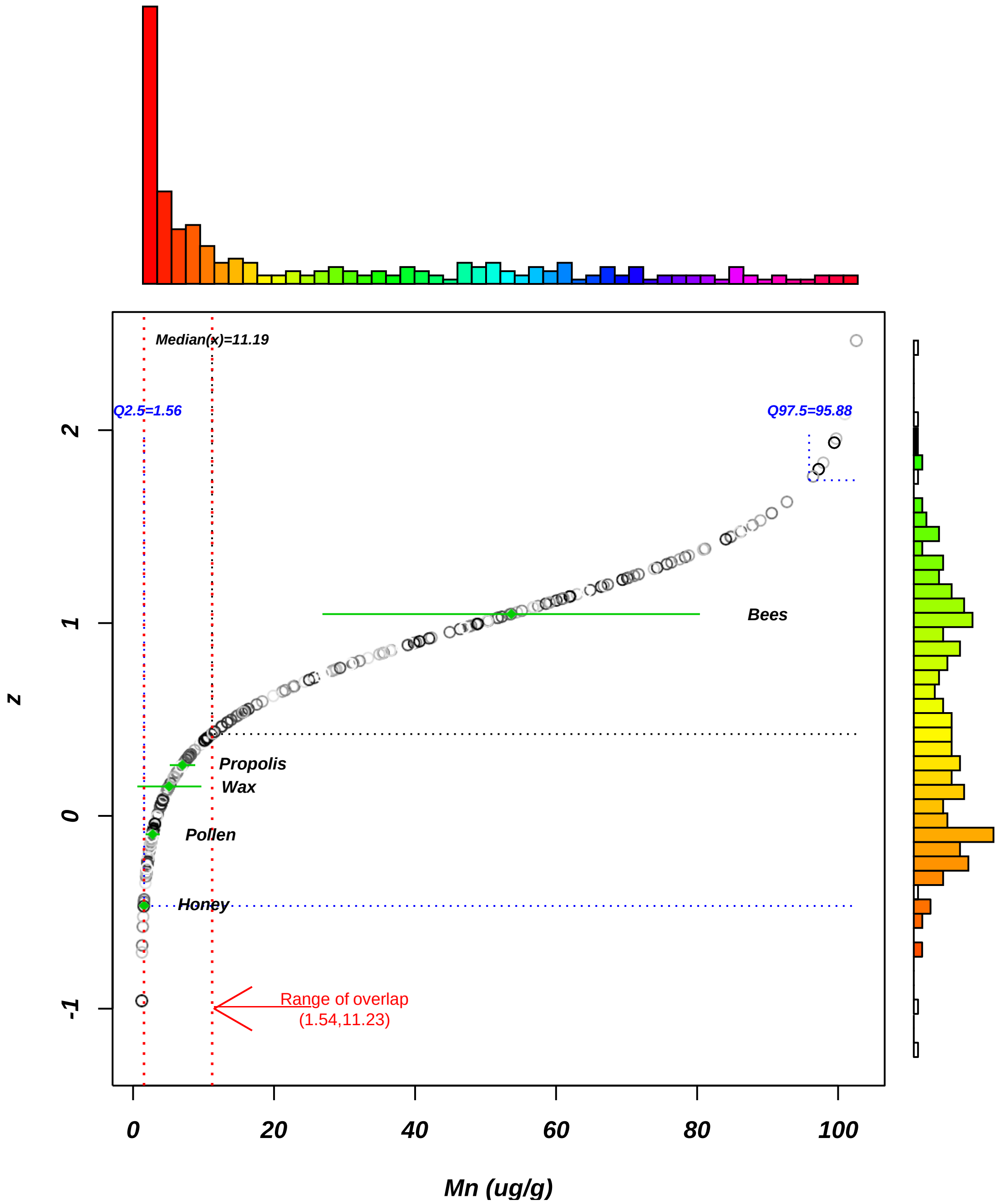

Figure S4. Control chart for Mn built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

Table S1. Q2.5 and Q97.5 percentiles of Sb data distribution (µg/g) and Sb OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	0.04	0.14	2.0	2.9
Wax	0.03	0.29	**2.6**	**5.9**
Honey	<0.03	0.05	**2.6**	1.0
Pollen	0.03	0.12	**2.6**	2.5
Propolis	0.08	0.20	1.0	4.1
Range of overlap	0.05-0.08			

Table S2. Q2.5 and Q97.5 percentiles of Sn data distribution (µg/g) and Sn OBI index.

Matrice	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	0.0049	0.368	1.000	**46.0**
Wax	0.0016	0.062	**3.06**	7.75
Honey	0.0015	0.008	**3.26**	1.000
Pollen	0.0039	0.187	1.26	**23.37**
Propolis	0.0036	0.094	1.37	11.75
Range of overlap	0.008-0.0049			

Table S3. Q2.5 and Q97.5 percentiles of Fe data distribution (µg/g) and Fe OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	69.7	247	2.0	20.0
Wax	3.0	70.3	**46.3**	5.7
Honey	<0.9	12.3	**154.4**	1.0
Pollen	33.1	154	4.2	12.5
Propolis	139	347	1.0	**28.8**
Range of overlap	12.3-139			

Table S4. Q2.5 and Q97.5 percentiles of Mn data distribution (µg/g) and Mn OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	11.2	139	1.0	**92.7**
Wax	0.4	28.8	**28.0**	**19.2**
Honey	0.1	1.5	**112.0**	1.0
Pollen	1.9	5.3	5.8	3.5
Propolis	2.9	13.7	3.8	9.1
Range of overlap	1.5-11.2			

3S. Figures and Tables for biomass burning tracers

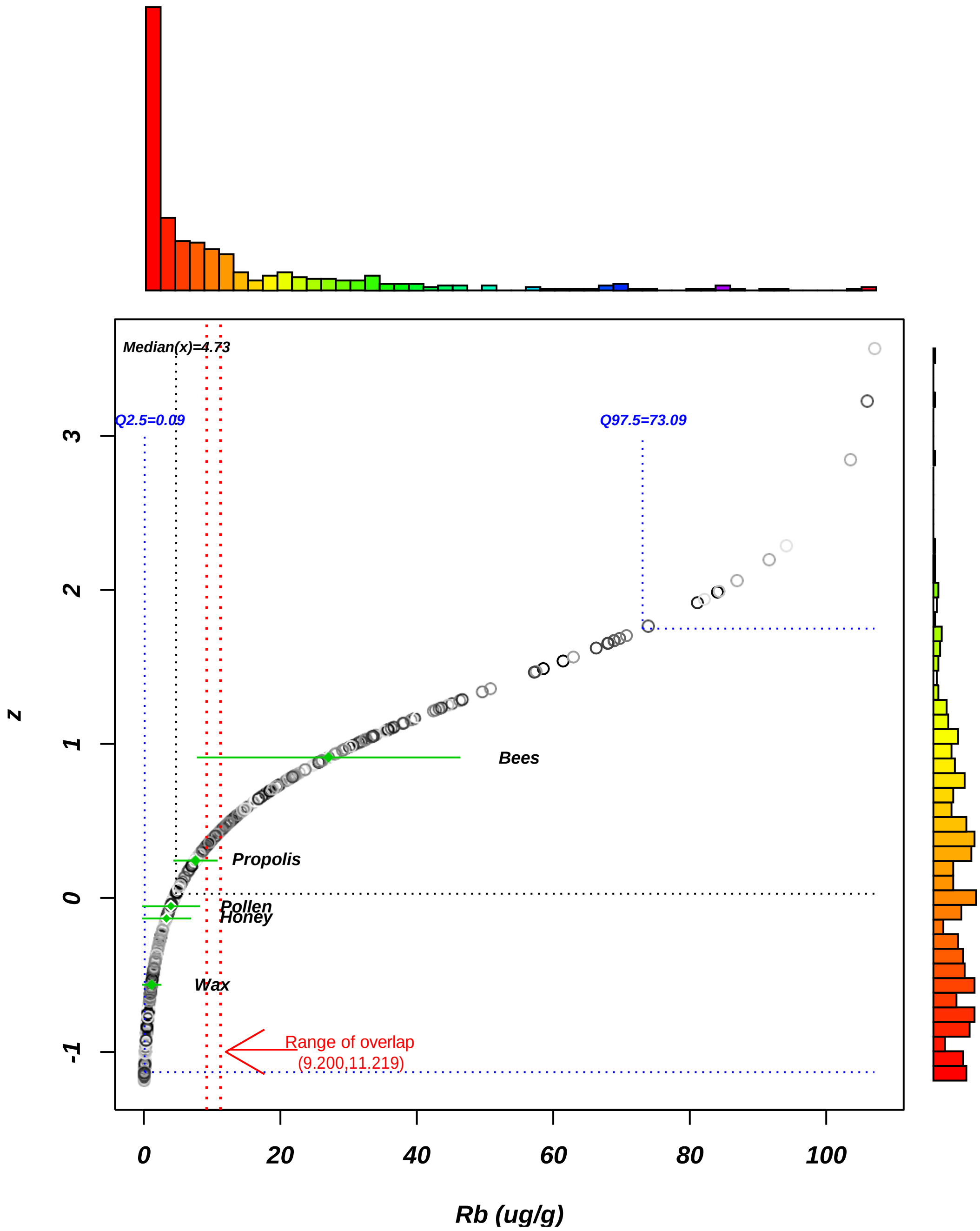

Figure S5. Control chart for Rb built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

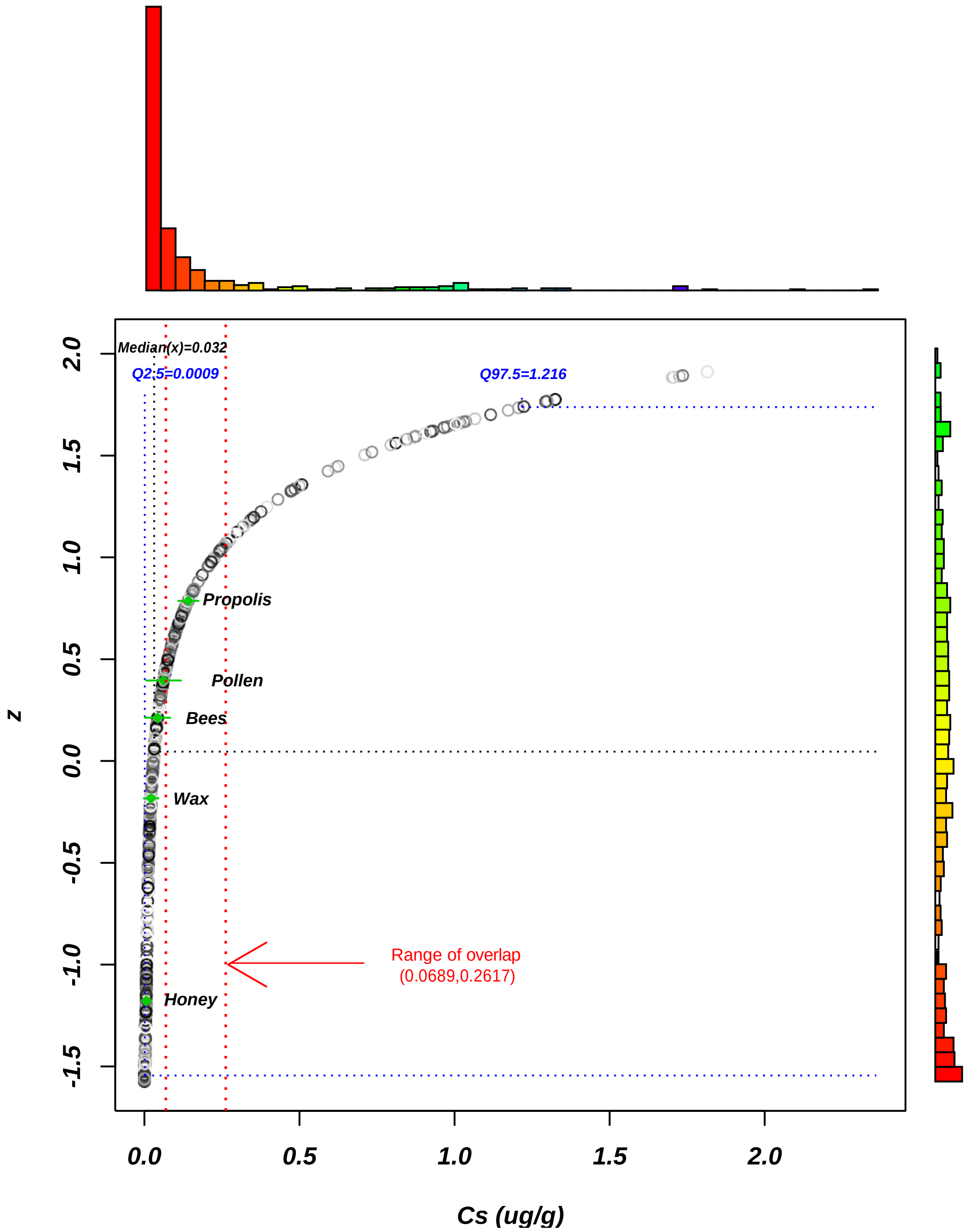

Figure S6. Control chart for Cs built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

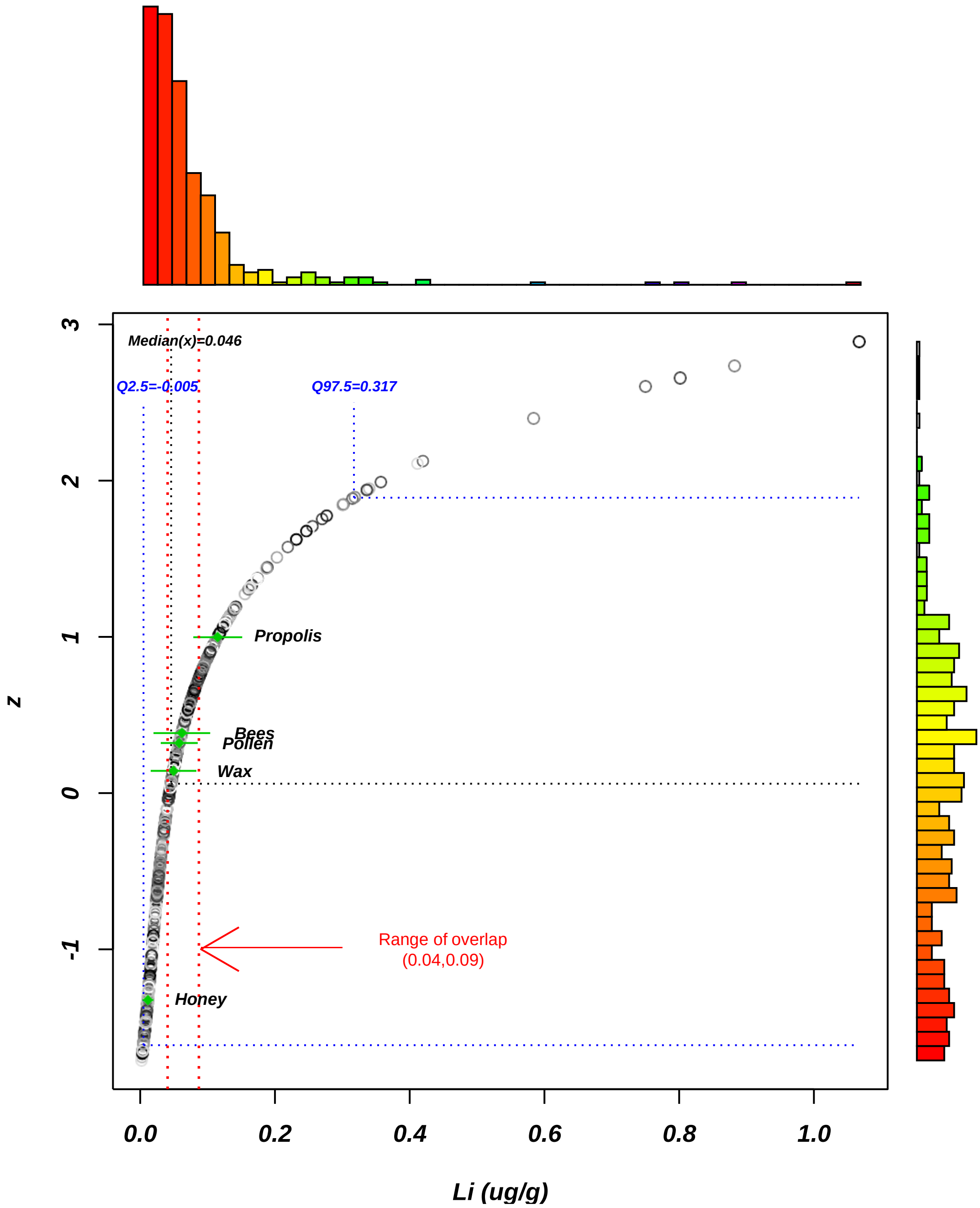

Figure S7.
Control chart for Li built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

Table S5. Q2.5 and Q97.5 percentiles of Rb data distribution (µg/g) and Rb OBI index.

Matrix	Q2.5	Q97.5	OBI-L	OBI-U
Bees	9.20	91.9	1.0	**8.2**
Wax	0.08	43.4	**115.0**	**3.9**
Honey	0.32	14.8	29.0	1.3
Pollen	0.95	18.7	9.7	1.7
Propolis	3.75	11.2	2.5	1.0
Range of overlap	9.20-11.2			

Table S6. Q2.5 and Q97.5 percentiles of Cs data distribution (µg/g) and Cs OBI index.

Matrix	Q2.5	Q97.5	OBI-L	OBI-U
Bees	0.027	1.70	2.6	**6.5**
Wax	0.002	1.01	**34.5**	**3.9**
Honey	0.008	0.262	**8.6**	1.0
Pollen	0.051	1.30	1.4	5.0
Propolis	0.069	0.308	1.0	1.2
Range of overlap	0.069-0.262			

Table S7. Q2.5 and Q97.5 percentiles of Li data distribution (µg/g) and Li OBI index.

Matrix	Q2.5	Q97.5	OBI-L	OBI-U
Bees	0.01	0.34	**9.0**	**8.4**
Wax	<0.01	0.39	**9.0**	**9.6**
Honey	<0.01	0.04	**9.0**	1.0
Pollen	0.02	0.10	4.5	2.5
Propolis	0.09	0.17	1.0	4.2
Range of overlap	0.04-0.09			

4S. Figures and Tables for soil tracers

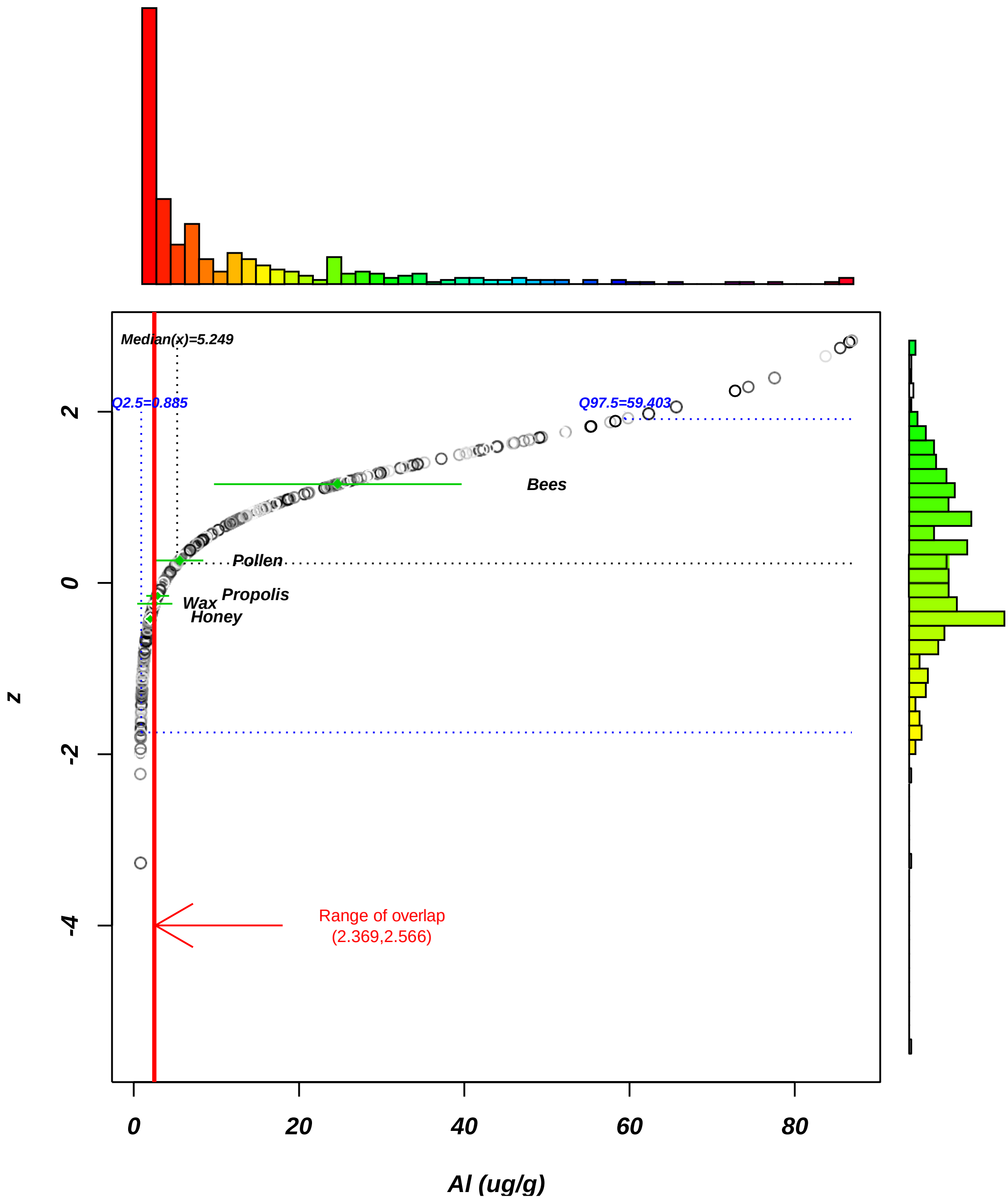

Figure S8. Control chart for Al built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

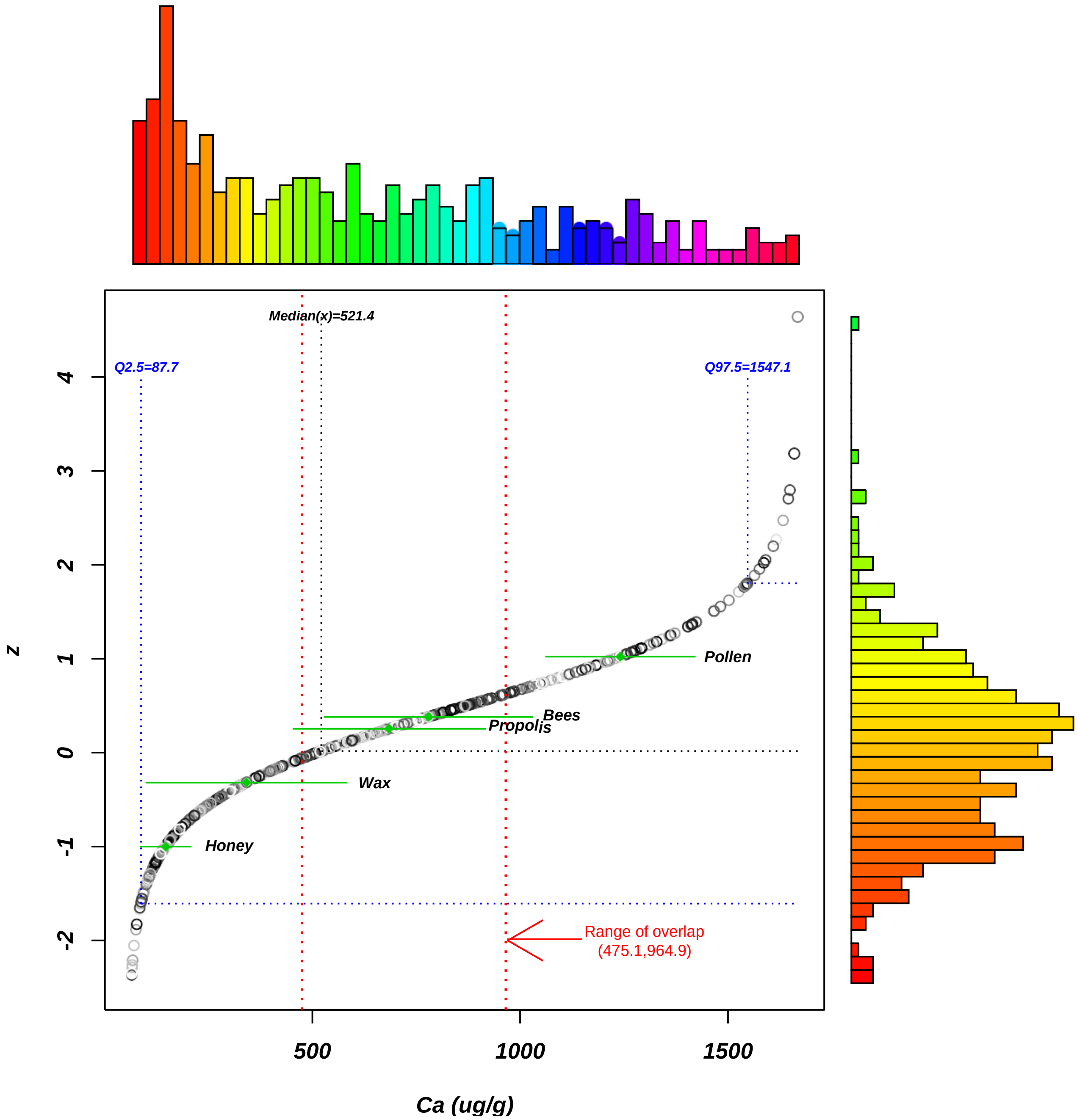

Figure S9. Control chart for Ca built for the five selected biomonitor/indicators with their obtained overlap metal concentrations (µg/g). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation, 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.

Table S8. Q2.5 and Q97.5 percentiles of Al data distribution (µg/g) and Al OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	3	85	1.0	**42.5**
Wax	<1	17	3.0	**8.5**
Honey	<1	2	3.0	1.0
Pollen	<1	8	3.0	3.3
Propolis	2	7	1.5	3.1
Range of overlap	2-3			

Table S9. Q2.5 and Q97.5 percentiles of Ca data distribution (µg/g) and Ca OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	433	1560	2.2	**3.3**
Wax	129	1670	7.5	**3.5**
Honey	52	475	**18.6**	1.0
Pollen	965	1870	1.0	**3.9**
Propolis	410	1459	2.4	**3.1**
Range of overlap	475-965			

Table S10. Q2.5 and Q97.5 percentiles of Ti data distribution (µg/g) and Ti OBI index.

Matrix	**Q2.5**	**Q97.5**	**OBI-L**	**OBI-U**
Bees	1.196	4.253	4.7	44.6
Wax	0.0852	2.179	66.5	22.8
Honey	0.005	0.095	**1116**	1.0
Pollen	0.8027	6.363	7.1	66.7
Propolis	5.58	29.73	1.0	**312.9**
Range of overlap	0.095-5.58			

Table SA. Elements' concentrations in bees, honey, pollen, wax and propolis of selected papers, expressed as µg/g (mean ± S.D., range)

			Non-exhaust traffic tracers					**Biomass burning tracers**					**Soil tracers**			
	Reference	**Site**	**Cu**	**Sb**	**Sn**	**Fe**	**Mn**	**K**	**Rb**	**Cs**	**Li**	**Tl**	**Si**	**Al**	**Ca**	**Ti**
Bees	*This work**	*Lazio, Italy*	*23.7 ± 4.7 (13.0 – 36.8)*	*0.08 ± 0.03 (<0.03 – 0.21)*	*0.09 ± 0.11 (0.004 – 0.62)*	*135 ± 46 (54 – 258)*	*64 ± 34 (4 – 168)*	*8340 ± 1400 (5360 – 13600)*	*37 ± 29 (6 - 160)*	*0.24 ± 0.43 (0.01 – 1.74)*	*0.08 ± 0.11 (<0.01 – 1.07)*	*0.033 ± 0.11 (<0.001 – 0.87)*	*156 ± 45 (66 – 369)*	*30 ± 20 (<1 – 98)*	*834 ± 298 (418 – 2040)*	*2.4 ± 1.1 (1.2 -6.8)*
	(Grainger et al., 2020)	New Zealand (undisclosed hive)	11 -18			48 – 97	11 – 123	5151 – 10713	8.56 – 16.3	0.030 – 0.121		0.0098 – 0.0637		6.7 – 21.4	537 – 734	
	(Zarić et al., 2022)*	Serbia (protected, city centre and thermal power plant) and Austria (city centre)	15.3 – 31.3	0.003 – 0.3		102 -265	32 -131	7281 – 10397	3.0 -10.3	0.004 – 0.042	0.02 – 0.19	0.0001 - 0.0065		16 – 125	855 -1532	
Honey	*This work***	*Lazio, Italy*	*0.6 ± 0.3 (<0.3 – 1.5)*	*<0.03*	*0.008 ± 0.002 (<0.003 – 0.098)*	*2.2 ± 2.8 (<0.9 – 16.7)*	*0.5 ± 0.4 (<0.1 – 2.5)*	*1030 ± 704 (162 – 3270)*	*4.9 ± 5.3 (0.2 – 26.1)*	*0.06 ± 0.11 (0.001 – 0.53)*	*<0.01*	*0.012 ± 0.020 (<0.001 – 0.111)*	*43 ± 8 (14 – 61)*	*<1*	*171 ± 114 (<50 – 679)*	*0.03 ± 0.02 (<0.01 – 0.15)*
	(Grainger et al., 2020) - multifloral	New Zealand (undisclosed hive)	0.131 - 0.577			0.80 – 4.06	0.18 – 23.5	134 – 3477	0.15 – 9.46	< 0.003 – 0.000851		< 0.018– 0.00282		0.6 – 14.5	366 -377	
	(Conti et al., 2018) – multifloral**	Lazio, Italy (five different provinces, different areas of urbanization)	0.06 – 5.4	<0.0008	0.001 - 0.246	<1 – 4.4	0.09 – 2.8	237 – 6520			0.0011 – 0.024	0.0001 – 0.150		<0.3 – 9.2	<43 – 283	0.001 - 0.230
	(Khuder et al., 2010) – monofloral (2 different ones) and multifloral **	Syria (pollution free places and not)	0.60 – 2.20			1.4 -17.2	0.17 – 1.48	46 - 188	0.186 – 0.932						45 - 125	
	(Astolfi et al., 2020a)** - multifloral	Italy (market samples)	0.06 – 1.01			0.58 – 4.28		89 - 4661		0.0006 – 0.787				0.09 – 6.33	39 -181	
	(Sajtos et al., 2022) – monofloral (3	Hungary (five different sites)	<0.04 – 2.65			0.29 -5.94	0.13 – 0.58	254 – 4455			<0.0138			0.08 – 1.49	15– 141	

	different ones)															
	(Varga et al., 2020)*	Hungary (three different sites)	< 0.0442			<0.37–7.09	0.040 – 0.307	222 – 494			<0.0138			<0.23–1.02	13.6 – 65.6	
	(Sajtos et al., 2019) * – monofloral (10 different ones) and multifloral	Hungary (seven different sites)					0.6 -11.9	322 -2466						0.5 -11.5	24 – 218	
	(Camiña et al., 2008)	La Pampa, Argentina (capital and non-capital)				1.1 – 10.3								n.d. – 5.22	19 – 102	
	(Voica et al., 2020)* – monofloral (4 different ones)	Romania (three different sites)	0.04– 1.19*				0.02 – 6.14*	60 – 859*						1.19 - 3.26*	<1 – 118*	0.07 – 0.36*
	(Hungerford et al., 2020)	Australia (urban, rural, peri-rural)	0.05 – 4.8	0.005 – 0.01	0.025 - 0.48	0.2 – 99.0	0.4 – 38.0	202 - 4600						0.05 – 14.0	21 – 270	
	(Gohar & Shakeel, 2021)	Pakistan (3 different sites) and Turkey (2 different sites)	0 – 3.90	n.d. – 9.56		3.8 – 50.5	0.31 – 3.98	224 – 12197	88 – 16703	1.8 – 84.0	64 – 515				15.6 – 83.6	
	(Stihi et al., 2016) – monofloral (9 different ones)	Romania (different sites)	0.10 – 2.04			1.8 – 10.8		187 - 486							34 - 98	
	(Atanassova et al., 2016) - multifloral	Bulgaria (two different sites)	0.05 – 0.49			0.7 -19.2	0.3 – 4.7	136 - 1900							24 - 94	
	(Squadrone et al., 2020)** – monofloral (4 different ones) and multifloral	Piedmont, Italy	0.30 – 0.76		0.007 – 0.07	1.1 – 2.0	0.61 – 2.2		0.8 – 23					0.5 – 2.6		
	(Grainger et al., 2021)	New Zealand (bush, farm, orchard, rural and urban sites)	0.13 – 1.76			0.33 – 3.05	0.3 – 18.9	230 - 2030	0.64 – 9.78			0.006 – 0.153		0.3 – 19.3	24 - 173	
	(Đogo Mračević et al., 2020) – monofloral (7 different ones)	Serbia (five different sites)	n.d. – 1.6			0.79 - 5.51	0.21 – 7.96	46 – 467						n.d. – 4.56		

	(Tahboub et al., 2022)	Jordan (market samples)	0.14 – 2.34		0.034-0.186	7.0 – 49.3	0.13 – 13.4	27 - 2404		0.0002 – 0.0379	0.0003 – 0.0729	0.0002 - 0.0134		2.7 – 21.9	27 - 205	
	(Bayram et al., 2020) - monofloral (13 different ones)	Turkey (seven different sites)	0.08 – 2.42	n.d.	n.d. – 0.3		0.1 – 27.8	442 – 5400	0.15 – 4.33	n.d. – 0.08	n.d. – 0.60		3 – 172		5 -115	
	(Meister et al., 2021) – unifloral (3 different ones)	Wairarapa, New Zealand (five different sites)	0.13 – 0.30			0.7 – 1.2	1.1 – 4.2	463 - 1108						5 - 11	60 – 68	
	(Hategan et al., 2021)	French and Romania – monofloral (11 different ones)	n.d. – 8.67	0.0005 – 0.040	0.01 - 242.05	0.3 – 148	0.2 – 36.5	64 – 3238	0.1 – 14.5		n.d. – 0.68			0.02 – 90.19		
	(Kędzierska-Matysek et al., 2021) – monofloral (4 different ones) and multifloral	Lublin, Poland	0.47 – 0.95			0.00139 – 0.00185	0.72 – 5.58	0.7 – 1.2								
	*This work***	*Lazio, Italy*	*1.0 ± 0.4 (0.5 – 2.3)*	*0.06 ± 0.02 (<0.03 – 0.13)*	*0.042 ± 0.032 (0.006 – 0.159)*	*86 ± 40 (20 -197)*	*3.2 ± 1.5 (1.6 – 8.1)*	*2030 ± 484 (1200-31400)*	*6.1 ± 5.3 (0.6 – 19.5)*	*0.28 ± 0.40 (0.01 – 1.32)*	*0.06 ± 0.03 (<0.01 - 0.10)*	*0.035 ± 0.090 (<0.001– 0.429)*	*129 ± 32 (48 – 221)*	*4 ± 2 (<1 – 9)*	*1310 ± 218 (664 – 2000)*	*2.7 ± 1.6 (0.64 – 8.92)*
	(Grainger et al., 2020)	New Zealand (undisclosed hive)	9.0-25.0			65-385	32.4-61.5	5275 – 7077	6.0-18.0	<0.003				26.5-68.4	1730 - 4059	
	(Matuszewska et al., 2021)	Poland (inner village)	4.33-5.17	0.008-0.009		47.0-51.3	16.7-33.3	4166-4300					33.8–46.7	22.6 – 29.7	1226 – 1250	
Pollen	(Astolfi et al., 2020a)**	Italy (market samples)	5.82			24.0		3703		0.0142				3.68	547	
	(Lilek et al., 2021)*	Slovenia (four macro-regions)				81 – 145	14.2 – 93.1	2710-11400	12.4-43.1						1080 – 2360	
	(Pavlova et al., 2022)	Albania and Bulgaria (serpentine sites)	9.45 – 9.59			103 -246	29.5 - 29.6	5234-5260							1506 – 1811	
	(Swiatly-Blaszkiewicz et al., 2021)	Poland (west-central)	4.4 - 5.7			49.0 -68.0	16.0-62.0	4400 - 4600							680 - 1500	
	(Mayda et al.,	Turkey (five	9.3 –	0.01 –	16 – 105	84 – 258	17 – 110	5429 – 8994	6.1 – 30.1	0.03 -0.11	0.01 –	n.d. –	47 -	3 -341	236 – 447	

	2020)	different sites)	20.0	0.08							2.38	0.75	416			
	(Pohl et al., 2020)	Various countries	0.1 – 27.7	0.01 – 0.50	1.72 – 6.46	2.6 -1180	0.1 – 367	0.4-38.0	29.4 -89.7		1.91 – 2.35	n.d. – 0.02	0.1- 10.5	0.1 - 836	0.13-5.19	
	(Atanassova et al., 2016)	Bulgaria (two different sites)	3 - 17			53 - 79	15 -17	3533 -3746							1500 - 2211	
Wax	*This work**	*Lazio, Italy*	*2.2 ± 4.6 (<0.3 - 37.0)*	*0.08 ± 0.03 (<0.03 – 0.37)*	*0.343 ± 0.023 (0.016 – 6.92)*	*29 ± 23 (2 -129)*	*3.8 ± 10.7 (<0.1 – 61.0)*	*1400 ± 1960 (19 -9480)*	*6.6 ± 12.8 (0.05 – 179)*	*0.14 ± 0.34 (0.001 – 2.36)*	*0.08 ± 0.13 (<0.01 – 1.07)*	*0.028 ± 0.069 (<0.001 – 0.474)*	*37 ± 21 (4 – 128)*	*5 ± 9 (<1 – 58)*	*559 ± 414 (88 – 1930)*	*0.69 ± 0.59 (0.03 – 2.47)*
	(Astolfi et al., 2020a)*	Italy (market samples)				0.15				0.000091						
	(Gajger et al., 2019)	Croatia (rural, city and industrial sites)	12.8 – 40.9			56 -286	16.6 – 32.9		11.1 – 15.0							
Propolis	*This work***	*Lazio, Italy*	*3.1 ± 1.7 (0.9 – 7.9)*	*0.12 ± 0.04 (0.04 – 0.25)*	*0.970 ± 0.026 (0.10 – 3.69)*	*243 ± 25 (137 – 528)*	*7.3 ± 3.0 (2.7 – 15.7)*	*1250 ± 822 (539 – 3910)*	*7.7 ± 3.5 (2.6 – 14.9)*	*0.156 ± 0.067 (0.063 – 0.321)*	*0.12 ± 0.04 (0.07 – 0.22)*	*0.009 ± 0.004 (0.004 – 0.019)*	*354 ± 31 (321 – 1080)*	*4 ± 3 (2 – 12)*	*785 ± 327 (398 – 1630)*	*11.6 ± 7.7 (5.1 – 35.4)*
	(Matuszewska et al., 2021)	Poland (inner village)	1.60 – 1.69	0.031 – 0.042		86 – 143	6.87 – 7.53	647 – 767					101 – 157	86-127	293 – 453	
	(Arslan et al., 2021)	Turkey (two different sites)	2.01 – 2.45			428 – 508	5.30 – 7.47	1156 – 2607			0.20 – 0.22			407 – 408	269 – 429	
	(Hodel et al., 2020)*	Brazil (nine different sites)	0.6 -11.6					0.23 – 7.94								
	(Mutlu et al., 2022)	Turkey (seven different regions)			n.d. - 3.69	117 - 627	3.4 -14.1	952 - 1636					268 - 785	122 -475	665 - 2974	n.d. – 62.6

* dry weight, ** wet weight

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