12 Years of Honey Surveys in Northern Italy: How Anthropic Activities Can Influence Honey Quality

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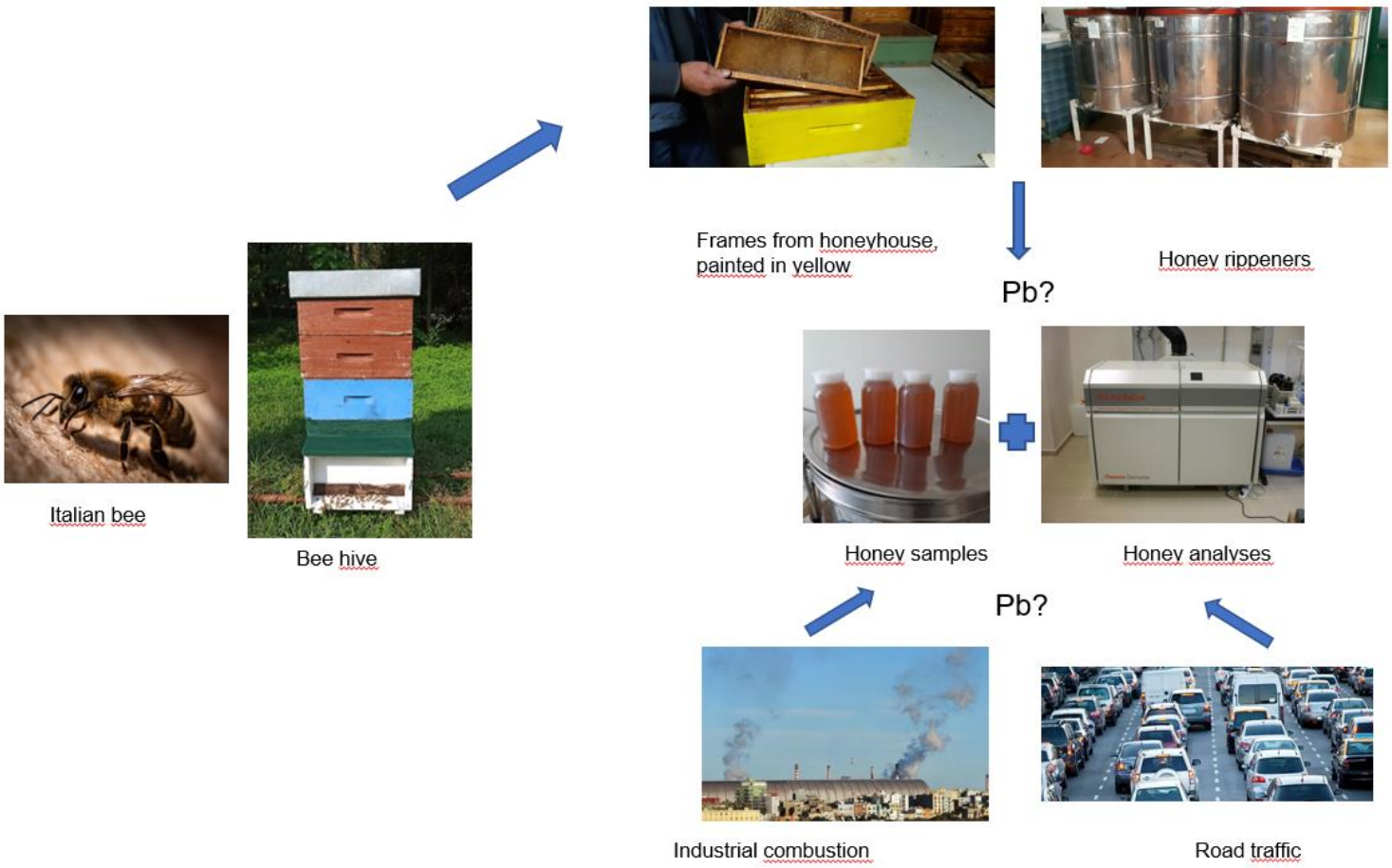
Abstract

Bees and hive products, as honey, can act as indicators of environmental quality. Our research aimed to evaluate historical data of honey quality in Lombardy (Northern Italy) and consider the possible sources of air contamination that can influence it. We collected analytical data from the local Health Protection Agency on residues in 57 honey samples from 2011 to 2022, comparing a rural area and an industrial area. At the same time, we consulted estimated air emissions in the same areas through the INEMAR database used by the local Environmental Protection Agency. Data revealed antibiotic contamination in one case and, regarding heavy metals, lead contamination in several samples in the industrial area. Pb contamination could derive from multiple sources. INEMAR database permitted us to hypothesize that combustion in industry and road transport could have a role in honey contamination, being among the main sources of Pb emission in that area.

Highlights:

- Long term collection of honey chemical data in Lombardy (Northern Italy)
- Comparison between rural and industrial area in Lombardy (Northern Italy)
- In deph-investigation for honey with heavy metal contamination, produced in the industrial area
- Estimated emission loads from anthropic activities in both areas.

Keywords honey, indicator, air pollution, heavy metals, antibiotic



Graphical abstract

Introduction

Bees provide substantial benefits to humans by pollinating plants, producing honey and other products and play multiple roles in nature maintaining biodiversity and as indicators of environmental quality.

The continued expansion of urban areas, deforestation, environmental pollution and the use of chemicals in large quantities have caused a rapid decline not only of the bees but also of other insects such as bumblebees, wasps, dipterans, beetles and ants in several regions.

These declines directly influence ecosystem services and human well-being, especially through food production. In fact, the quantity and/or quality of vegetable yield is directly correlated to pollinators in up to 75% of the major crop species grown (Klein et al., 2007).

Bees are also excellent biotic indicators of environmental quality due to their ethological and morphological characteristics: they require minimal feeding by the breeder and search for food outside the hive; their bodies are covered by hair, making them particularly good at holding materials and substances with which they come into contact. Bees are highly mobile, their wide flight range allows them to monitor a wide area and they are extremely sensitive to most pollutants (Negri et al., 2015, Porrini et al., 2003).

Bees can act as direct and indirect indicators: in the first case, the bees come into contact with compounds that are toxic to such an extent to cause their death, therefore their mortality rate is an index of environmental quality. In the second case, being exposed to contaminants provides information through the residues present on their body or in the hive products, which can be detected by appropriate laboratory analysis. Heavy metals (i.e. Pb, Cr, Cd, Cu, Hg), PAHs

(polycyclic aromatic hydrocarbons), PM (particulate matter), radionuclides, pesticides and antibiotics are examples of the monitored compounds (Al-Waili et al., 2012, Kędzierska-Matysek et al., 2022, Negri et al., 2015, Rondeau and Raine, 2022, Silici et al., 2016). Bees sample various environmental matrices: soil, vegetation, water and air, and it is estimated that each active pollinator completes about fifteen flights in a day and brings back pollen, nectar and propolis to the hive, which they then use and reprocess (Celli, et al., 2003).

Italian and European laws (Law 283/1962, Law 753/1982, Directive EEC 74/409, Commission Regulation 37/2010, Regulation 2003/181/EC, Regulation 396/2005/EC) guarantee the safety of food and, in particular, of honey. In addition, every year the National Residue Research Plan (NRRP) of the Italian Health Ministry plans research activities for residues of banned substances (drugs, environmental contaminants, and prohibited substances) in animal products intended for human consumption, through collaboration with the local Health Protection Agency and its laboratories.

The aims of the present work are i) to collect and evaluate the historical series of analytical data regarding organic and inorganic residues in the honey samples from 2011 to 2022, comparing the Western Milan and Northern Milan-Rho areas in Lombardy, Italy, with agricultural and industrial traditions respectively, from local Health Protection Agency ii) to investigate the possible sources of environmental contamination referable to the contaminants found in honey through the INEMAR database, (INventario EMissioni ARia), the system used by the local Environmental Protection Agency (ARPA Lombardia) to estimate air emissions.

Methods

Area of investigation

It is possible to divide the territory into two macro-areas (Figure 1), identified as:

- Olona Axis (OA) represented by the eastern part of the territory taken as reference, corresponding to the current Northern Milan-Rho district (including the surrounding town of Legnano). This district includes the town of Rho and other municipalities with over 25,000 citizens. These territories are highly industrialized, with numerous plants at high environmental risk with two large oil refineries. Three highways and two important railway lines connecting the province of Varese and Monza - Brianza to the metropolitan area of Milan cross the study area. For this research, this territory is considered an industrial area.

- Ticino Axis (TA) corresponding to the Western Milan district, is characterized by a large number of municipalities with 7,000-8,000 citizens. The area has a prevalent agricultural, cereal (mostly rice) and livestock tradition. The territory is covered by two highways for 10 km and two railway lines for 30 km. The only relevant activities in the area were tanneries (highly polluting through heavy metal residues), which abruptly ceased their activities with the advent of the first legislative provisions regarding the depollution and treatment of industrial waters. On the border of Piedmont region there is a large power plant. Finally, it is worth noting that almost all the municipalities included in this area have been part of the Parco Lombardo of Valle del Ticino, an environmentally protected area, since the ‘80s.

For this study, this territory represents the rural area (Table S-2).

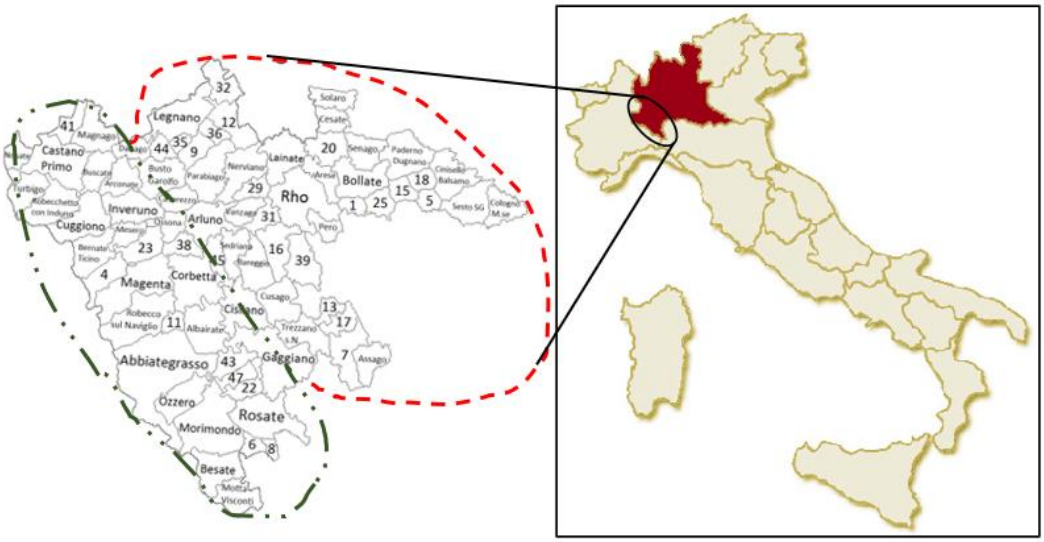


Figure 1 Area of study

Honey Sampling and Analysis

According NRRP, there are two ways of performing honey sampling:

-from the frames of the hive

-from the honey extraction laboratory, where the beekeeper performs the removal of the honey from the frames and transfers it into the so-called “ripeners,” where honey is left to mature for a few days before packaging.

The NRRP provides for the collection of 5 samples of 100 gr of honey, one for official analysis and the others for any subsequent counter-examination in case of an analytical dispute. The number and frequency of sampling in each region are set by the same NRRP (approximately 40 samples/year for the entirety of Lombardy and 2-3 sample for each district).

The macro-categories considered for this study on honey are antibiotics (tetracyclines, macrolides, aminoglycosides, sulfonamides and quinolones), pesticides (including a large number of compounds - see supplemental material) and metals (Lead, Cadmium, Chromium and Mercury) for the period 2011-2022.

The compounds were analyzed by Liquid Chromatography with tandem mass spectrometry (LC-MS/MS), Gas chromatography mass spectrometry (GC-MS/MS), Ion chromatography – high resolution mass spectrometry (IC-HRMS), Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

Analysis data were collected from the New Health Information System and the paper reports available in each district laboratory.

European directives and regulations establish that products of animal origin can contain only residues of antibiotics and other drugs, heavy metals, pesticides and indicate the methods, detection limit and legal limit (Table 1).

There are 377 apiaries and 44 laboratories for honey extraction and hive products in TA while OA has 140 apiaries and 16 laboratories. The presence of about 7,000 hives can be estimated. There is an average of 12 hives per apiary, however this number can vary greatly, even during the same year, depending on the crops and blooms.

Table 1 Investigated compounds, methods and limit of detection, legal limit (more details in Table S1)

Compound	Method	Detection Limit µg/kg	Legal Limit µg/kg
Chloramphenicol	LC-MS/MS	0.3	0,3
Tetracyclines	LC-MS/MS	2.5	5
Sulphonamides	LC-MS/MS	5.0	5
Quinolones	LC-MS/MS	2.5	presence
Aminoglycosides	LC-MS/MS	2.5	1,3-1,6
Macrolides	LC-MS/MS	2.5	5
Amitraz	LC-MS/MS	5	200
Group 1 Pesticides Method 02/292 rev. 3 - 2020	GC-MS/MS	10 *(20 for Iprodione)	10 (100 for coumaphos)
Group 2 Pesticides Method 02/234 rev. 6 - 2021	LC- MS/MS	10	10-50
Group 3 Pesticides Method 02/461 rev. 0 - 2019	IC-HRMS	10	10-50
Lead	ICP/MS	2	100
Cadmium	ICP/MS	2	presence
Chromium	ICP/MS	2	presence
Mercury	ICP/MS	2	presence

INEMAR Database

To investigate the origin of air pollution, the INEMAR (INventario EMissioni ARia) database was used. This system is used by ARPA Lombardia to estimate emission loads and trace the processes that resulted in the presence of a pollutant in the atmosphere (Energy production and fuel processing, Non-industrial combustion, Combustion in industry, Manufacturing processes, Fuel extraction and distribution, Solvent use, Road transport, Other mobile sources and machinery, Waste treatment and disposal, Agriculture, Other sources and removals). As an emission estimate, there are several interacting variables: activity indicators (fuel and paint consumption, amount of incinerated waste and any parameter useful to quantify the emitting activity), emission factors, and data for spatial and temporal identification of emissions.

This database was mainly used for heavy metals to compare the estimated amount in the air and the NRRP outcomes.

Results

For the 2011-2022 period, as part of the NRRP monitoring, a total of 57 reports were obtained, each of them containing hundreds of results, especially for pesticides (range 172-185) (Table 2).

We noticed that, in the 36 reports concerning the rural area, only the pesticide amitraz was detected in one sample under the legal limit. Regarding antibiotics, residues of macrolides, aminoglycosides, sulfonamides, and quinolones were

always absent in all analyses, while tetracyclines were detected in one sample with the maximum concentration of 514.5 µg/L in Cisliano in the 2011.

Pb, Cd, Cr and Hg were detected from 1 (2.7%) to 5 (13.8) cases, always under legal limit.

In the industrial area, antibiotics, pesticides, and Hg were not always detected in all 21 reports, while Pb was present in 7 of them and both Cd and Cr in 3. In these circumstances, Pb exceeded legal limit of 0.1 mg/L reaching maximum of 0.571 mg/L, while for Cr and Cd there is no legal limit and we can only recognize the samples as positive.

In this latter case and considering that there is only a clear legal limit for Pb, an in-depth study was conducted to better understand the origin of the Pb contamination on the mixed-flower and honey sample in Senago.

148 Table 2: Synthesis of the results regarding honey analyses in rural and industrial areas

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		Pb (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Hg (mg/kg)	Tetracyclines (µg/kg)	Macrolides (µg/kg)	Aminoglycosides (µg/kg)	Sulphonamides (µg/kg)	Quinolones (µg/kg)	Amitraz (mg/kg)	Other pesticides (µg/kg)
	Legal limit	0.1	presence	presence	presence	5	5	5	1.6	presence	0.2	10-50
Rural area	Number of positive samples	5	2	4	1	1	0	0	0	0	1	0
	Number of "not detected" samples	31	34	32	35	35	36	36	36	36	35	36
	Total number of samples	36	36	36	36	36	36	36	36	36	36	36
	Number of samples>legal limit	0	0	0	0	**1**	0	0	0	0	0	0
	Maximum	0.088	0.017	0.037	0.006	514.5	0	0	0	0	0.15	0
Industrial area	Number of positive samples	7	3	3	0	0	0	0	0	0	0	0
	Number of "not detected" samples	14	18	18	21	21	21	21	21	21	21	21
	Total number of samples	21	21	21	21	21	21	21	21	21	21	21
	Number of samples>legal limit	**2**	0	0	0	0	0	0	0	0	0	0
	Maximum	0.571	0.04	0.053	0	0	0	0	0	0	0	0

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153 *Case study Senago*

154 The first sample of mixed-flower honey (A) contaminated by Pb came from hives
155 located on meadows in the municipality of Senago in the OA (Figure 2). To further
156 investigate the problem, analyses were also carried out in neighboring apiaries,
157 at 3 km (Bollate) and 13 km (Ceriano Laghetto). The analysis revealed that even
158 in Bollate the 2 mixed-flower honey samples (B and C) were above the legal limit,
159 and the yellow paint, used by the beekeeper for maintenance and waterproofing
160 of the hives themselves, also contained Pb in high concentration (P1).

161 The analyses were expanded to samples of acacia honey contained in ripeners
162 at the extraction laboratory (R1 and R2) and produced before the mixed-flower
163 honey, revealing the presence of Pb in one of the two samples.

164 Two other analyses of mixed-flower honey from ripeners showed the presence of
165 Pb above the authorized limit in both samples (R3 and R4).

166 Additional honey sampling was conducted in the same municipality of Senago,
167 identifying two other apiaries and always exceeding the legal limit (R5 and R6).

168 In this latter case, analyses were extended to hive paints and beeswax used by
169 the insects in constructing the frames, with the result that all were contaminated
170 by Pb, in particular 0.221 mg/kg and 0,895 mg/kg in the beeswax, while in the
171 honey samples Pb was under the legal limit.

172 From a different apiary in Ceriano Laghetto, farther away from the critical area,
173 but still in the industrial area, the honey sample also exceeded the limit for Pb.

174 All honey produced during the investigation period and contaminated by Pb
175 above the legal limit was seized and destroyed.

It is noteworthy that the apiaries in Senago are located close to three high traffic roads, “Autostrada dei Laghi A8,” “Tangenziale Nord di Milano A52” and European Road E64.

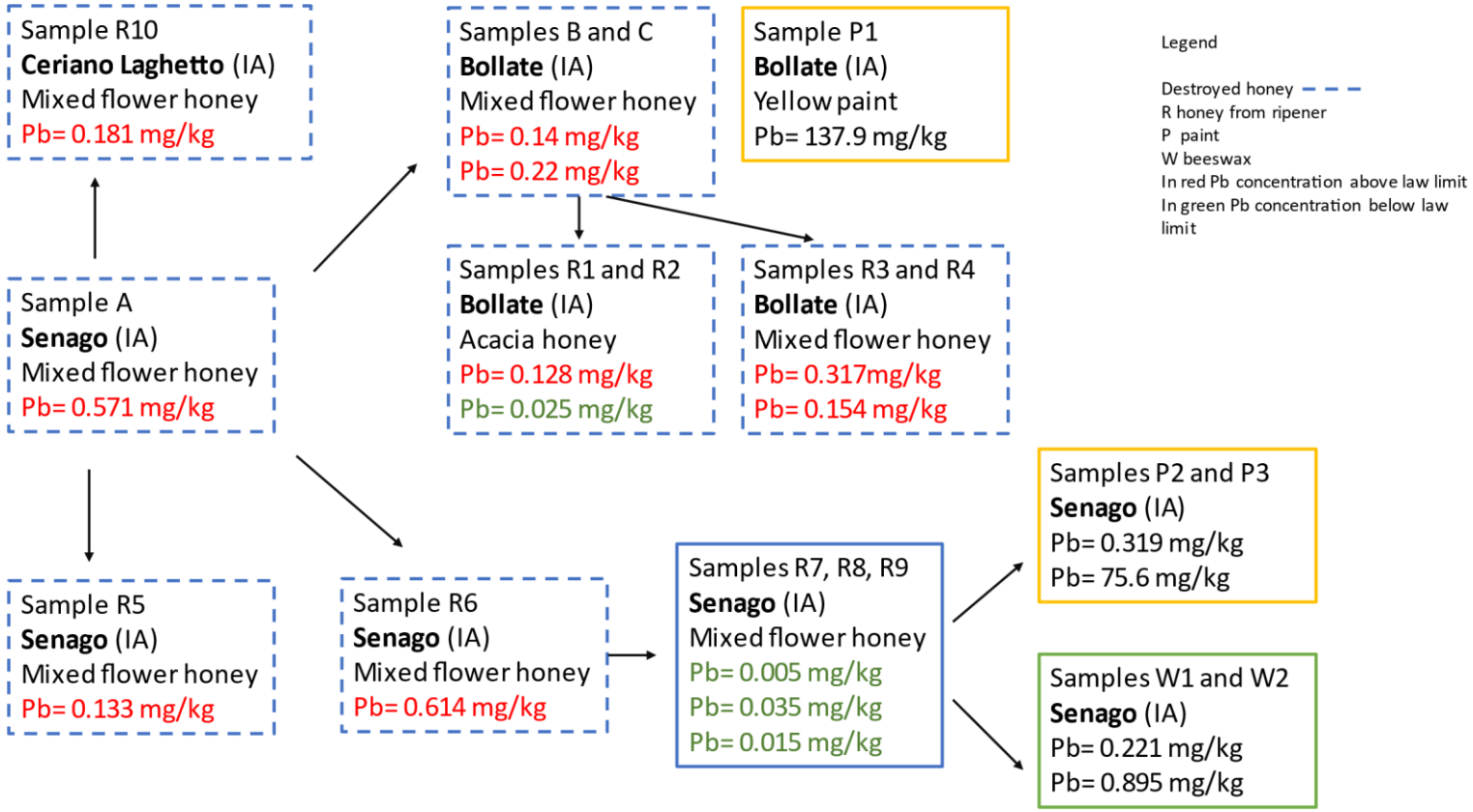


Figure 2 Case study Senago

INEMAR database

Air pollutants can settle on the flowers from which bees obtain nectar and pollen. Based on this observation, the INEMAR database was consulted to estimate how many air emission loads are in the industrial area compared to the agricultural area and what air emission sources are in place, as industrial combustion, manufacturing processes, fuel extraction and distribution, solvent use and road transport.

Considering only heavy metals, the estimated emission loads are of 297.3, 69.7, 29.4 and 467.7 kg/year for Cr, Hg, Cd and Pb respectively in the rural area, 950.1, 63.8, 153.4 and 1949.8 kg/year in the industrial area, 0.2, 0.1, 0.3 and 6.2 kg/year in Senago municipality and 19107.7, 1691.3, 1300.3 and 22476.9 kg/year in the entire Lombardy region (INEMAR, 2019).

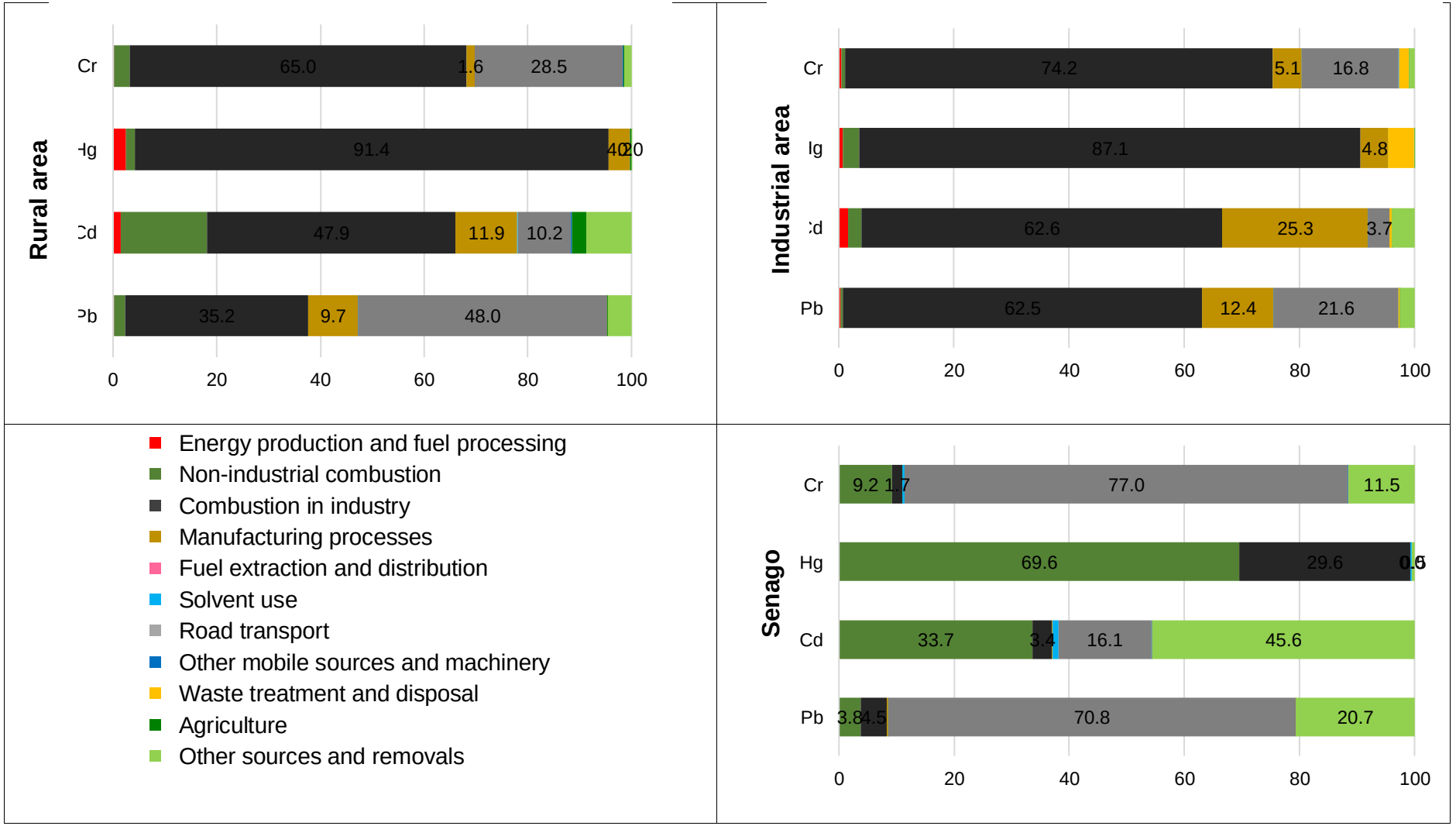
As we can see in Figure 3 A, estimated emissions of Pb (1949.8 kg/year) derive mainly from combustion in industry for 62.5%, then from road transport for 21.6% and manufacturing processes for 12.4% in the industrial area. Otherwise, in the rural area the emission of Pb (467.7 kg/year) derives primarily from road transport (48%) and secondly from combustion in industry (35.2%). If we consider only the Senago municipality, Pb is the most emitted heavy metal and road transport accounts for 70.8%.

These same sources are also encountered for Cd, Hg and Cr, that are emitted mainly by combustion in industry in both rural and industrial areas and secondly by road transport and manufacturing processes.

It's also interesting to notice that comparing emissions per km^2 and emissions per inhabitant in rural area vs Lombardy region, they are more relevant for all heavy metals except Hg in the Lombardy region. Indeed, Hg is widely emitted as

208 combustion in industry for 63.7 kg/year out of a total of 69.7 kg/year, due to the
209 presence of a specific activity in the area that is not found elsewhere in the region.
210 The opposite happens for all heavy metals, if we compare the industrial area with
211 the rest of the region, where both estimated emissions are more abundant in the
212 former. The municipality of Senago represents only a small portion of territory
213 compared to the industrial area, therefore is not representative of the entire
214 industrial context, with fewer missions than the rest of the region (Figure 3 B).

215 A)


Rural area
Cr
65.0
1.6
28.5
Hg
91.4
Cd
47.9
11.9
10.2
Pb
35.2
9.7
48.0
0
20
40
60
80
100
Industrial area
Cr
74.2
5.1
16.8
87.1
4.8
62.6
25.3
3.7
Pb
62.5
12.4
21.6
Energy production and fuel processing
Non-industrial combustion
Combustion in industry
Manufacturing processes
Fuel extraction and distribution
Solvent use
Road transport
Other mobile sources and machinery
Waste treatment and disposal
Agriculture
Other sources and removals
Senago
Cr
9.2
77.0
11.5
Hg
69.6
29.6
Cd
33.7
3.4
16.1
45.6
Pb
3.8
4.5
70.8
20.7

216 B)

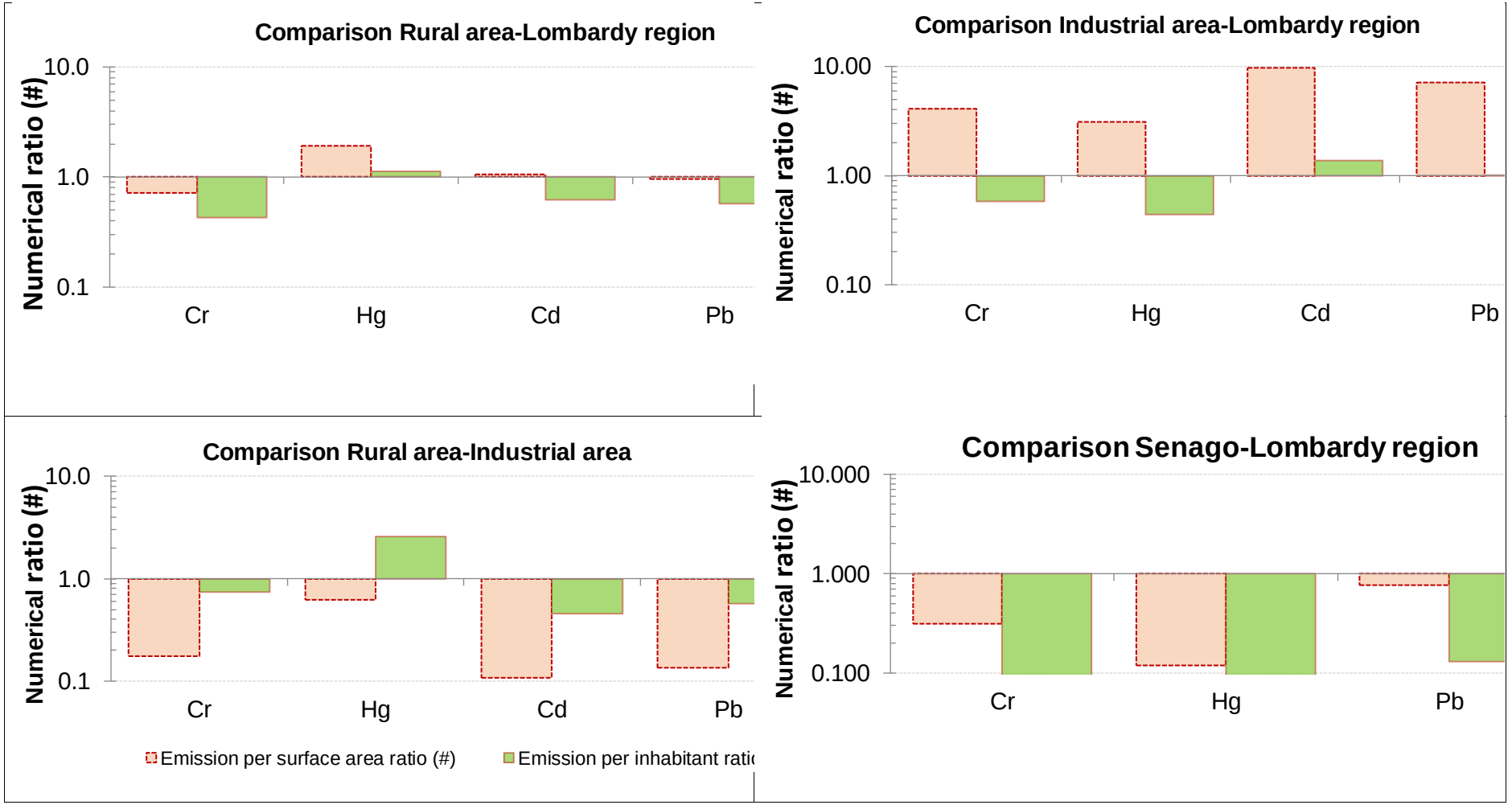


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Figure 3 A) Emission sources and B) comparison of emissions per surface area ratio and emissions per inhabitant ratio between different areas (Inemar database 2019)

Discussion

Bees are very important environmental indicators, revealing pollution conditions through mortality or residues in honey and other hive products. Honey is considered a very important and, at the same time, critical food, and its quality is therefore subjected to continuous monitoring. In this study, attention was focused not on the nutritional properties of the honey, but on its chemical quality, checking the concentration of heavy metals, antibiotics and pesticides according to the annual monitoring provided by the Italian Ministry of Health, in the surroundings of Milan (North Italy), considering two areas, one agricultural and one industrial. The most troubling results concerned the presence of tetracyclines and heavy metals in some honey samples.

In the past years, some beekeepers have fraudulently used antibiotics to eliminate pathogens, such as the bacterium *Paenibacillus larvae*, responsible for American foulbrood, against which the destruction of the hive is mandatory. Several studies report honey contaminated by antibiotics in different countries (Martel et al., 2006, Lima et al., 2020). Antibiotic residues can be tracked for months, so continuous monitoring of these compounds is critical. Adams et al. refer to data on the persistence of lincomycin hydrochloride even 290 days after the hive treatment and Granja et al. found erythromycin residues 3 months after dosing (Al-Whali et al., 2012).

Though heavy metals can have natural and mostly anthropic origins, they are considered harmful for human health, being teratogenic, carcinogenic, and neurotoxic. The main sources of lead, cadmium, mercury, and arsenic are motor traffic, exhaust gases and fumes, as well as pesticides and synthetic fertilizers.

Lead is not transported by plants, while Cd originating from the metal industry and incinerators, is transported from the soil to plants and can then contaminate hive products (Kedzierska-Matysek et al., 2022).

Fakhri et al.(2019) reported the results of their meta-analysis based on 45 studies and ranked elements according to their toxicity (Fe > Mn > Pb > Cr > Cu > Ni > Cd > As > Hg) and to hazard quotient (HQ: Pb > Cd > Mn > Fe >Ni > As > Cu > Hg > Cr). Ciobanu and Radulescu (2016) observed specific variations in the heavy metal content of honey, following location of the hives in areas with different impact of pollution. In this way the researchers identified the influence of the pollution sources and the residues of heavy metals.

In our survey, it was intriguing to observe discordant results in the detection of lead in honey samples. This may be due to the fact that lead concentrations were very low and despite very sensitive methods of analysis, non-concordant situations could be observed or that Pb was present in the paint used to waterproof hives from atmospheric precipitation. Another hypothesis could have been that the apiaries were located in areas with significant air pollution. In this case, the INEMAR database was a fundamental support, allowing for the identification of Pb and other compound sources and an estimate their emissive loads. This may also explain why some honey samples from unpainted hives were still contaminated by Pb, and particularly in the Senago municipality, where vehicular traffic is particularly incisive (70.8%).

It is not possible to draw any certain conclusive considerations about the real origin of Pb contamination, but certainly the choice of where to place the hives appears to be of particular importance.

With regard to the presence of lead in the paint analyses, there is no specific legislation regulating the use of paints to waterproof the hives. It is not considered a material capable of coming into contact with food, and therefore not capable of transferring fresh or flaky paint into the hive.

Lead chromate or lead carbonate were the most common lead-based pigments within paints. In Italy, the process of eliminating lead from industrial products dates to 1961, when Law 706 was enacted and banned the use of lead carbonate, lead sulfate and other pigments containing lead, in painting and varnishing work, being compounds toxic to reproduction, harmful and dangerous to the environment. However, the law allowed temporary exceptions for specific activities or if there are some processes in which the use of such products is recognized as irreplaceable. Legislative Decree 81/08 also focused attention to this metal, providing mandatory biological limit values and surveillance procedures for lead and its ionic compounds.

Directive 2011/65/EU banned the use of hazardous substances, including lead, in electrical and electronic equipment for domestic use, thus preventing the use of paints containing these substances in, for example, painting electrical panels and computers. Even the World Health Organization has among its goals the phasing out of lead and its derivatives for all countries. Although these are clear indications, our findings demonstrate that there is no sufficient awareness about this substance and its uses.

As already highlighted, in Italy a legal limit only exists for Pb, but according to Law 753/1982, honey must not contain any organic or inorganic matter foreign to its composition.

In our research, the range concentration for Cd was 6-40 µg/kg and a study conducted in Romania reported overlapping values (0,5-11,60 µg/kg), like those in China (1,34 µg/kg), New Zealand (149,0 µg/kg), Turkey (0,9-17,9 µg/kg) and Poland 5,0 µg/kg. The same is observed for Cr (14-53 µg/kg in our study and 41,595 µg/kg in Romania) and for Hg (6µg/kg in Italy, 1,65 µg/kg in China and 2,72 µg/kg in Croatia) (Oroian et al. 2016).

Finally, it was interesting to use – for the first time – information from different databases and different institutions (Local Health Protection Agency and Environmental Protection Agency). Considering that in the rural area and particularly in the municipalities of San Giorgio/Rescaldina/Magnago/Castano primo, according INEMAR database, Hg emissions are clearly higher than in other territories, it would be interesting to investigate honey quality in those areas, to better understand the influence of air pollution on the concentration of heavy metals in honey.

Conclusion

The 12-year monitoring survey showed that honey marketed in the areas of study is overall of good quality and that any critical situation is immediately followed by interventions aimed at protecting consumer health, destroying batches of honey produced during the same period and investigating the causes of contamination. Health authorities cannot dictate where the hives are placed, but it might be useful to encourage dialogue between authorities and beekeepers, so that the hives could be placed away from high traffic roads and preserve the safety of bees and their products. Another action could be recommending the use of non-toxic

316 paints, which do not contain lead, or paints that have been declared or certified
317 suitable for food use, thus are certainly free of heavy metals.
318 A new insight on monitoring modalities is given, emphasizing the importance of
319 interdisciplinary information and expertise to improve knowledge about honey
320 quality.
321
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