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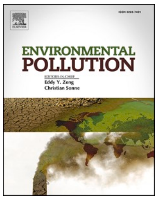

Effects of Covid-19 pandemic lockdown and environmental pollution assessment in Campania region (Italy) through the analysis of heavy metals in honeybees☆

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ABSTRACT

The Covid-19 outbreak had a critical impact on a massive amount of human activities as well as the global health system. On the other hand, the lockdown and related suspension of working activities reduced pollution emissions. The use of biomonitoring is an efficient and quite recent tool to assess environmental pollution through the analysis of a proper bioindicator, such as bees. This study set out to ascertain the impact of the Covid-19 pandemic lockdown on the environmental occurrence of eleven heavy metals in the Campania region (Italy) by analyzing bees and bee products. A further aim of this study was the assessment of the Honeybee Contamination Index (HCI) in three different areas of the Campania region and its comparison with other Italian areas to depict the current environmental pollutants levels of heavy metals. The results showed that the levels of heavy metals bioaccumulated by bees during the pandemic lockdown (T1) were statistically lower than the sampling times after Covid-19 restrictions and the resumption of some or all activities (T2 and T3). A comparable trend was observed in wax and pollen. However, bee, pollen, and wax showed higher levels of Cd and Hg in T1 than T2 and T3. The analysis of the HCI showed a low contamination level of the sampling sites for Cd and Pb, and an intermediate-high level as regards Ni and Cr. The biomonitoring study highlighted a decrease of heavy metals in the environmental compartments due to the intense pandemic restrictions. Therefore, *Apis mellifera* and other bee products remain a reliable and alternative tool for environmental pollution assessment.

1. Introduction

Environmental pollution due to anthropogenic activities has led to direct and indirect contamination of all natural ecosystems. In particular, heavy metal contamination of soil, air, and water is alarming due to its adverse impacts on living organisms (Ćirić et al., 2021). In the latter period, a decrease in concentrations of environmental pollutants was recorded during the Covid-19 pandemic (Singh and Mishra, 2021). Indeed, the Covid-19 pandemic had a critical impact on health, society and economy, but, at the same time, it helped to reduce environmental pollution (Chakraborty and Maity, 2020). This effect could be related to the highly restrictive government measures imposed to contain the spread of the pandemic. The restrictions affected the mobility of people and vehicles and suspended some industrial activities (Zambrano-Monserrate et al., 2020). In Italy, one of the countries significantly hit by this pandemic, the lockdown was set between March 9 and May 3, 2020. The restrictions imposed the closing of factories, schools, shopping malls, blocking public transportation and sporting events, and tourism. Subsequently, with the reduction of the state of emergency, represented by fewer people hospitalized in intensive care, restrictions were gradually reduced until the complete reopening of working activities and mobility. The sudden block of all global anthropogenic activities significantly affected the environmental quality (Karunanidhi et al., 2021). Numerous studies underline the positive effects on water and air quality during the Covid-19 lockdown, comparing the percentage of contaminants found before and during the lockdown (Elsaid et al.,

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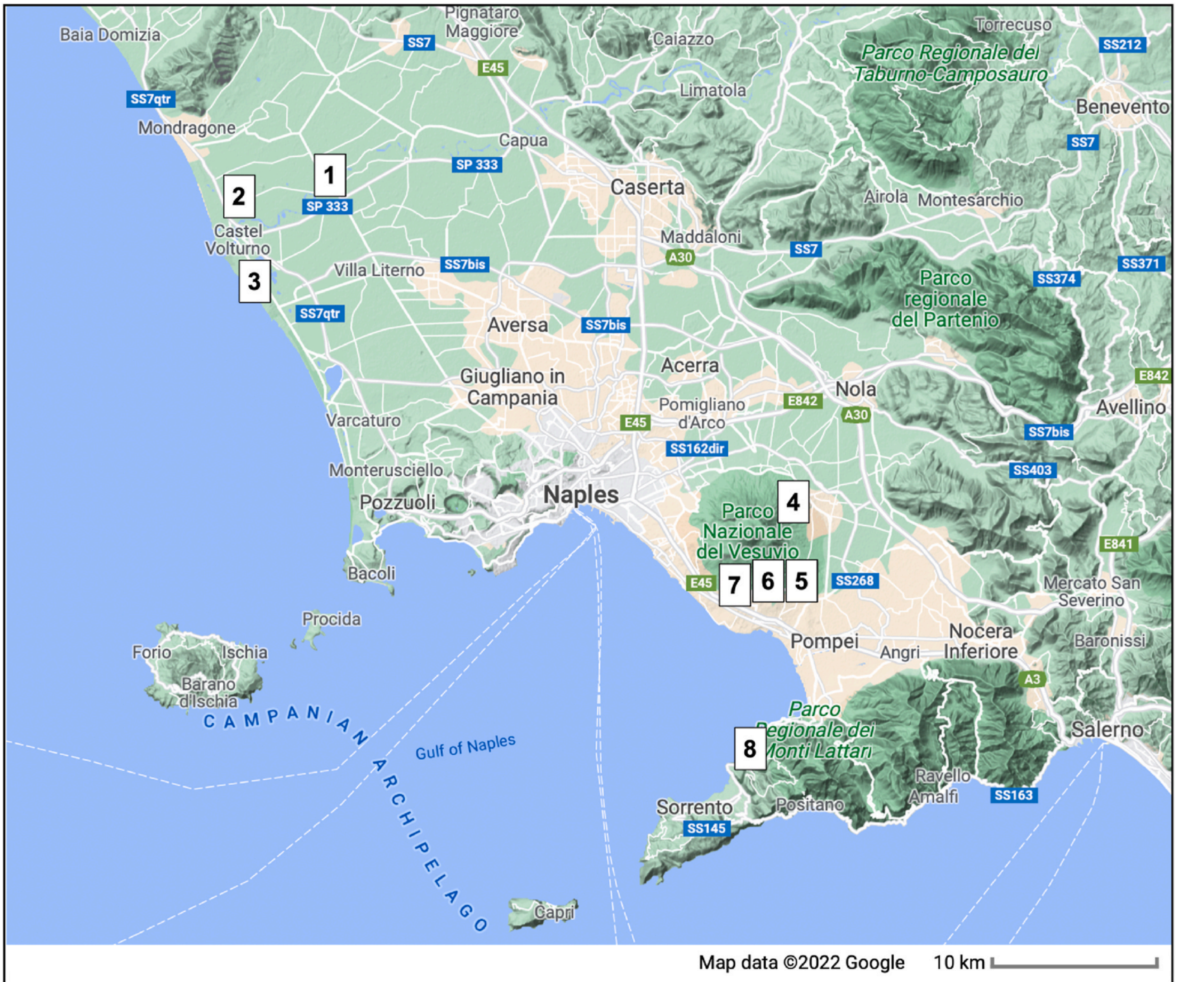
2021; Zambrano-Monserrate et al., 2020). In terms of environmental pollution, greenhouse gas emissions, nitrogen dioxide, black carbon, and water contamination have decreased significantly (Chakraborty and Maity, 2020). In India, several studies conducted on different rivers have found a reduction in industrial waste contamination and an improvement in water quality of about 40–50%, also leading to a reduction in heavy metal concentrations (Chakraborty B. et al., 2021; Chakraborty, S. et al., 2021; Shukla et al., 2021). Therefore, continuous monitoring of ecosystems is necessary and valuable to obtain real-time information on environmental quality. Although different methods are available to assess the environmental quality, an original and efficent approach could be the use of bioindicator species. Among these, bees proved to be a valid bioindicator due to their foraging activities (Giglio et al., 2017); hence, their application in environmental monitoring could be also reliable during Covid-19 emergency. Indeed, bees travel over large areas of approximately 7 km^2 from the colony and encounter various differently contaminated environmental substrates (Goretti et al., 2020; Johnson, 2015; Kastrati et al., 2021). They can be a carrier for heavy metals from the environment to hives in a variety of ways, such as by accumulating airborne particulate matter on their furry bodies during flight, through the water or by picking up heavy metals from pollen and nectar accumulated in the plants due to contaminated soil (Negri et al., 2015; Van Der Steen et al., 2012; Zaric et al., 2018). Studies conducted on the concentration of heavy metals suggest that the accumulation on the body of bees is site-specific, and once transported to the hive, these heavy metals can occur in various bee products such as honey, wax, and propolis (Conti and Botrè, 2001; Omran et al., 2019; Matuszewska et al., 2021), leading to a potential risk to human health as well. The following study is placed in a context of environmental biomonitoring during the Covid-19 pandemic in Italy. The occurrence of heavy metals in bees and bee products in different areas of the Campania region (Italy) was evaluated just after lockdown, after partial restriction, and resumption of activities. Hence, this study set out to ascertain the impact of the Covid-19 pandemic on environmental pollution by heavy metals using *Apis mellifera* and other bee products as potential monitoring tool and the assessment of the contamination level in three different areas of Campania region.

2. Materials and methods

2.1. Sampling

The biomonitoring study was conducted in the Campania region during the 2020 Covid-19 global pandemic. Bees, pollen, and wax were sampled at three different times during the national lockdown: late May (T1), after few months of total shutdown of all anthropogenic activities, late July (T2), partial resumption of activities, and the end of October (T3), total resumption of activities and international mobility. The sampling sites (n = 8) were chosen in three different suburban areas of the region. They were located as follows: one in the municipality of Vico



1. Cancello and Arnone; 2. Mondragone; 3. Castel Volturno; 4. Ottaviano; 5. Terzigno; 6. Trecase; 7. Torre del Greco; 8. Vico Equense

Fig. 1. Area of study and sampling sites (Google Maps, 2022).

Equense, in the Sorrento peninsula area; four in the Vesuvius area, in the municipalities of Torre del Greco, Terzigno, Trecase, and Ottaviano; three in the Caserta province, in the municipalities of Cancello Arnone, Castel Volturno, and Mondragone (Fig. 1). The sampling was conducted after receiving consent from beekeepers to their anonymous participation in the study. Specimen collection was done respecting the colonies and trying not to interfere with bees' activities. For each apiary, the samples were: about 100 foraging bees; fresh wax (20 g); pollen (20 g) collected from each site using stainless steel pollen-collecting traps positioned at least 24 h before the collection operation.

Some precautions were taken during the sampling phase in order to avoid accidental contamination of the samples: the beekeeping equipment was made of stainless steel and was cleaned from all residues before and after use; the sampling operator made use of new, intact, clean and specific personal protective equipment (PPE); the samples were taken with disposable gloves and scalpels, placed in sterile containers and stored at a temperature of −20 °C.

2.2. Chemical and instrumental analysis

The samples were homogenized using a mixer, and 0.5 ± 0.2 g aliquots of each sample were spiked with 5 mL HNO_3 (65% w/w) and 2 mL of H_2O_2 (30% w/w). Then, the samples underwent wet mineralization via a Milestone microwave for 30 min at 190 °C. At the end of the digestion, the samples were cooled and transferred to flasks. The final volume of 25.0 mL was obtained by adding MilliQ water.

Trace elements detection and quantification were performed using a Thermo Scientific™ ICAP™ RQ inductively coupled plasma mass spectrometer (Q-ICP-MS) with a Burgener Mira-Mist nebulizer, a Quartz cyclonic spray chamber, cooled to 2.7 °C, and skimmer cones. The instrument was operated using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. The operating conditions of the Q-ICP-MS equipment were optimized using a tuning solution (Ba, Bi, Ce, Co, In, Li, U 1.00 μg/L, Thermo Scientific) on masses ^{115}In, ^{7}Li, ^{59}Co, ^{238}U, ^{209}Bi, and ^{140}Ce was used for oxide and doubly charged interference checks. The analysis was performed in KED (Kinetic Energy Discrimination) mode using Helium as collision gas, and the parameters were: plasma gas flow (Ar): 14,8 mL/min; nebulizer gas flow: 0.98 L/min; auxiliary gas flow: 0.85 L/min; ICP RF Power: 1550 W; CeO/Ce = 0.0057. Cell gas flow was 4.8 mL/min for He.

The Q-ICP-MS was used to determine Cd, V, Cr, Mn, Ni, Cu, As, Sb, Ba and Pb in bees, pollen, and wax samples. All samples were analyzed in duplicate, and each sample was measured in triplicate by Q-ICP-MS detection (Kılıç Altun et al., 2017; Aliu et al., 2020; Bereksi-Reguig et al., 2020).

The solutions were prepared using water (18.2 MΩ cm resistivity) purified with Millipore Mill-Q® purification system, concentrated nitric acid (HNO_3 65% m/m, Suprapur®, Merck, Germany) and hydrogen peroxide (H_2O_2 (30% w/w), Suprapur®, Merck). An HNO_3 1% v/v (Suprapur®, Merck, Ultrapure) solution was used to clean the Q-ICP-MS apparatus between quantifications.

A volume of 5 mL of HNO_3 (65% w/w) and 2 mL H_2O_2 (30% w/w) were added to digest both samples and standard solutions. The calibration standards were prepared with multielement standard solution CertiPUR® (Merck, Darmstadt, Germany) 1000 mg L^{-1} at concentrations: 0.5, 1.0, 2.5, 5.0, 10.0 μg L^{-1}. An internal standard mix comprising 50 μg L^{-1} Ge, 5 μg L^{-1} Ir, 10 μg L^{-1} In and 25 μg L^{-1} Y was introduced online with an internal standard mixing kit. The internal standard elements were appropriately matched to analyte elements (de Oliveira et al., 2017; Wetwitayaklung et al., 2018). The Limit of Detection (LOD) was 0.00015 ppm for each metal but Ni, Ba and Cu, where it was 0.00600 ppm and Hg (0.00003 ppm).

2.3. Honeybee Contamination Index

For the assessment of environmental pollution based on heavy metal concentrations in bees was used the Honeybee Contamination Index (HCI) proposed by Goretti et al. (2020):

$$HCI_i = log\frac{C_{bees}}{C_{bees\_i}}$$

where (C_{bees}) is the element concentration in bees and (C_{bees_i}) is the reference threshold limit reported by DiSTAL –UniBo (2010) and Gutierrez et al. (2015). C_{bees_i} varied from high (C_{bees1}) and low (C_{bees2}) reference thresholds of contamination, respectively (Table 1). The lack of data allows assessing the HCI only for Cd, Pb, Cr, and Ni.

2.4. Statistical analysis

Data analysis and graph processing were performed using R Software version 3.6.0 and the following packages: ggplot2, ggsci, FactoMinerR, FactoInvestigate and factoextra (R Core Team, 2019; Nan, 2018; Thuleau and Husson, 2018; Kassambara and Mundt, 2017; Wickham, 2016; Lê et al., 2008).

3. Results and discussion

3.1. Heavy metal concentrations and honeybees contamination index

Heavy metal concentrations (expressed as wet weight) found in honeybees in the three different times are displayed in Table S1. The amount of bee's body water was 68% based on average value reported by Goretti et al. (2020). The samples collected at T3 (the most representative condition of the current environmental situation) showed that Trecase was the city with higher levels of Cd (39.50 μg/kg), V (40.60 μg/kg), Sb (5.25 μg/kg), Ba (1189.10 μg/kg), and Pb (95.55 μg/kg); Torre del Greco showed higher values for Ni (233.00 μg/kg), and As (50.30 μg/kg); Ottaviano reported higher concentration for Hg (42.15 μg/kg), whereas Vico Equense showed higher levels for Cr (147.55 μg/kg), Cu (10,437.95 μg/kg), and Mn (10,531.75 μg/kg). However, statistically significant differences among the sampling areas were observed only for Cu, Cd, and Ba as discussed below. The heavy metals concentrations assessed in bees in Campania were in line with Giglio et al. (2017), which reported similar mean data in the suburban area of Trieste in June of 2013. They reported values (expressed as μg/kg dry weight) of Cd: 52 ± 6, V: 60 ± 16, Cr: 261 ± 16, Ni: 358 ± 37, Cu: 12, 820 ± 920, As: 50 ± 18, and Pb: 127 ± 17. In contrast, a recent study by Goretti et al., (2020) stated higher concentration levels (μg/kg dry weight) for Cd: 300 ± 500, Pb: 340 ± 310, Ni: 1340 ± 1840, Mn: 98, 980 ± 79, 590, whereas similar or lower values were observed for Cr: 270 ± 140 and Cu: 14, 390 ± 2900 from analyses conducted in Umbria region between 2014 and 2015. Instead, Ruschioni et al. (2013) reported mean concentrations (μg/kg) in the range of 20–100 for Cd, 30–150 for Cr, 40–162 for Ni, 50–370 for Pb in ten natural reserves in the Marche Region monitored from 2008 to 2010.

The HCI used to assess the environmental pollution level was calculated following Goretti et al. (2020) methods, based on a reference threshold limit of four heavy metals deriving by DiSTAL-UniBo and Gutierrez et al. (2015). This index was calculated according to the concentrations at T3 for the reason metioned above. The HCI showed a low contamination level for Cd and Pb, whereas, Ni and Cr levels led to an intermediate-high HCI values (Fig. 2). Hence, the detected

Table 1
Maximum and minimum reference threshold limit for Cd, Pb, Cr, and Ni (mg kg^{-1} w.w.) in contaminated honeybees.

Element	C_{bees1} (mg kg^{-1} w.w.)	C_{bees2} (mg kg^{-1} w.w.)
Cd	0.10	0.05
Pb	0.70	0.30
Cr	0.12	0.04
Ni	0.30	0.10

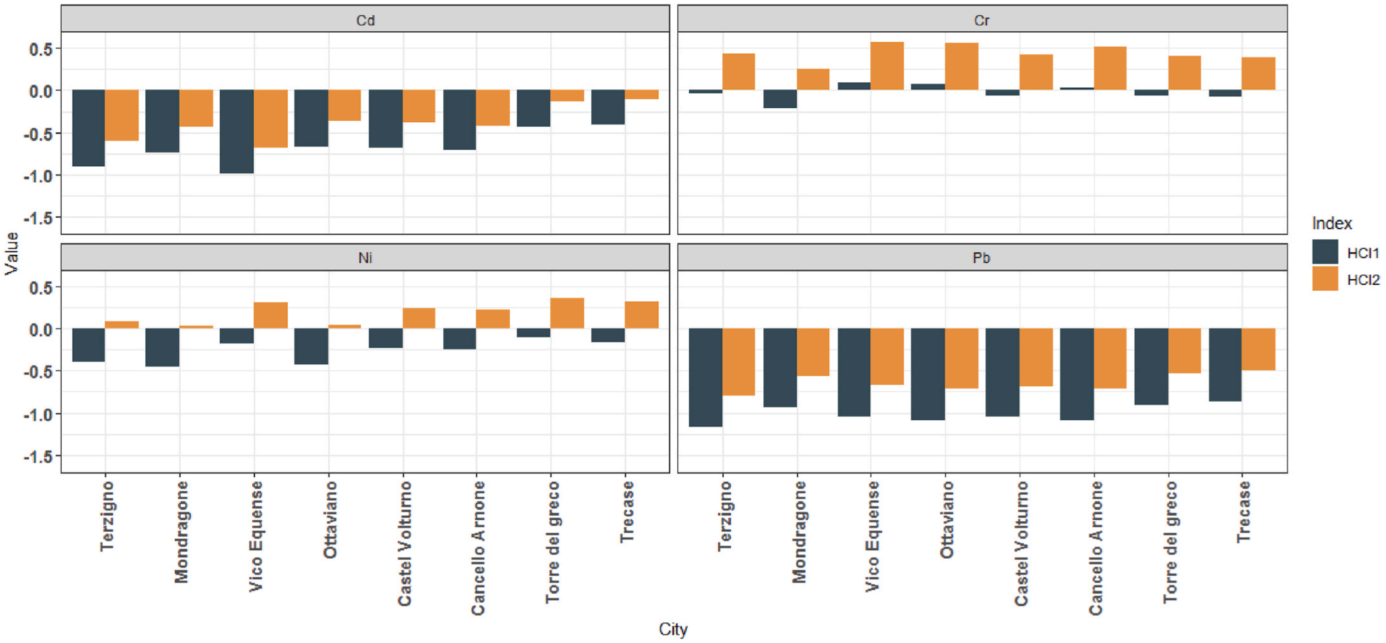


Fig. 2. Level of environmental pollution for Cd, Cr, Ni, and Pb in eight sites of Campania region based on the Honeybee Contamination Index (HCI), calculated through minimum (HCI1) and maximum (HCI2) reference threshold limit.

concentrations of Ni and Cr pointed out a relevant environmental pollution in Campania region.

3.2. Impact of Covid-19 pandemic on environmental pollution

The lockdown due to the Covid-19 pandemic has changed people habits and work activity with effects on air and soil quality. Several measures to contain the Sars-Cov 2 infection were taken such as travel ban (except for health or work reason), school and retail shop closures (except for basic needs shop), suspension of sport, cultural, religious events and sport activities (Italian Presidency of the Council of Ministers, 2020). For this reason, bees contamination during and after different types of social restriction was assessed. The data were arranged according to the three different sampling times (T1, T2, and T3) and were tested for the homogeneity of the variances (Bartlett's test) and normality (Shapiro-Wilk's test). Finally, a one-way analysis of variance with post-hoc Tukey's test was performed to highlight any likely statistically significant difference. Comparing the three sampling times, the levels of the analyzed elements showed significantly different means at the 95% confidence level between the first sampling time (T1) and the other two (T2 and T3) (Fig. 3).

The most striking result to emerge from the data analysis is the lowest environmental concentration for most elements in the samples collected immediately after the pandemic Covid-19 lockdown (T1 time) (Fig. 4). However, it is also worth noting that, unlike the other elements, Cd and Hg showed the highest levels at the T1 period throughout the three sampling times and for most of the sampling sites. This result might be related to the different persistence of these two elements in the environment. Cd concentration was higher in lockdown, as also reported by (Karunanidhi et al., 2021). A similar trend in water samples was also described by Tokatlı and Varol (2021) in three sites in Northwest Turkey. Besides, Hg showed a different trend with the highest levels just after lockdown. Hg concentrations were lower in post-lockdown or partial lockdown. These two metals probably have a more remarkable environmental persistence, and more time could be needed for their decrease to appreciate the effects in the long term (T2 and T3) (Fig. 4). In

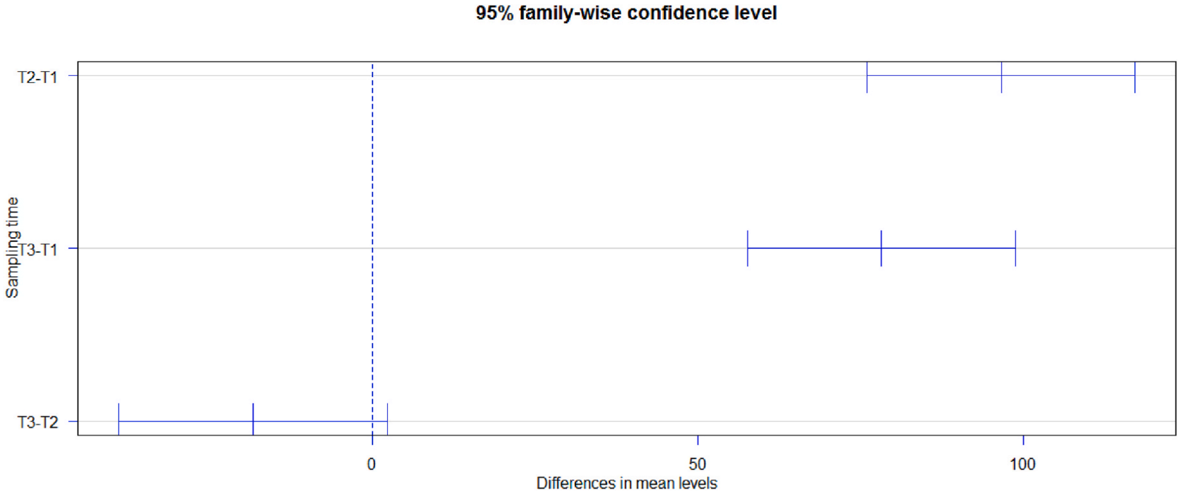


Fig. 3. Differences in mean levels of concentration of heavy metals according to the different sampling times (T1, T2, and T3).

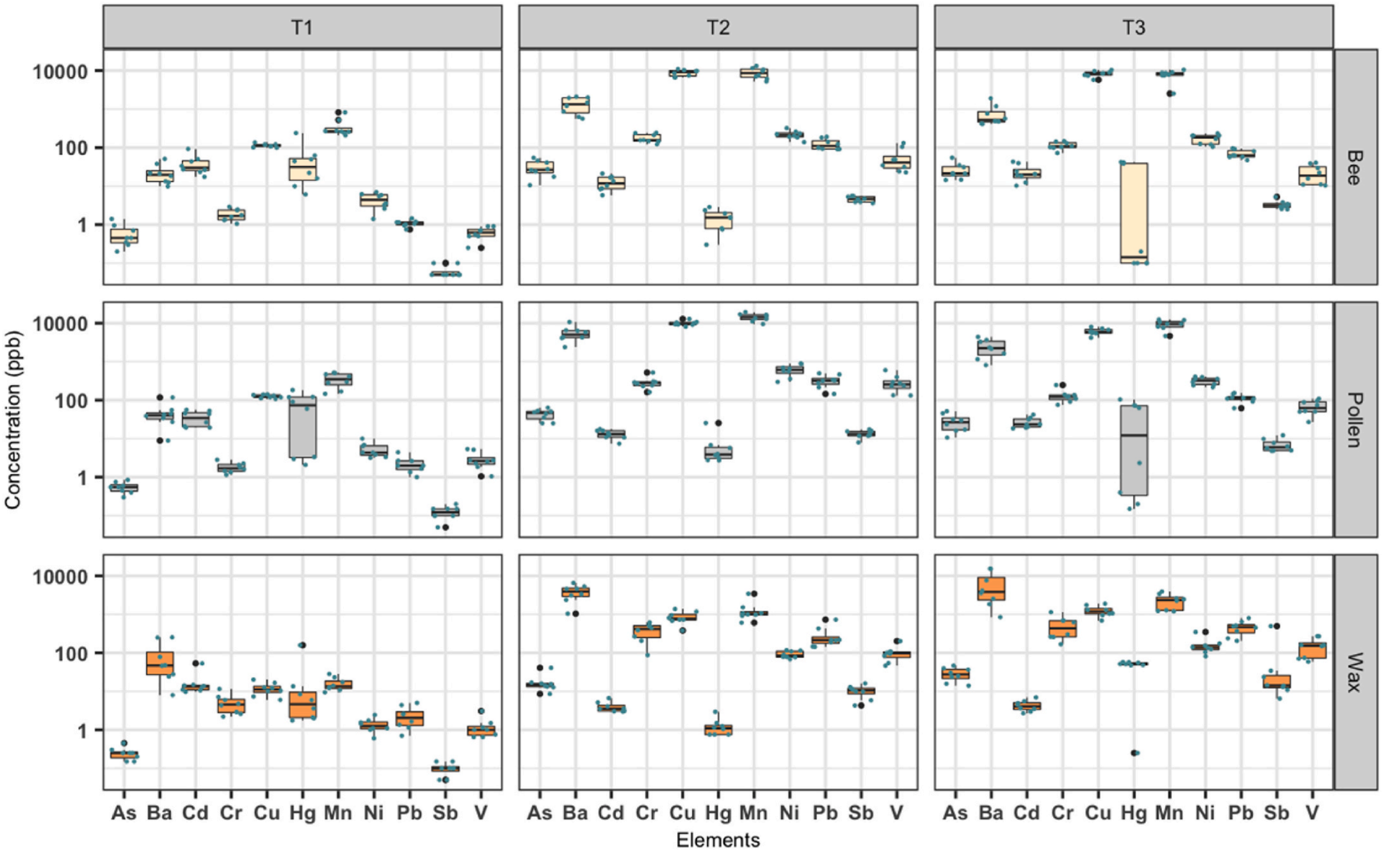


Fig. 4. Heavy metals concentrations (on exponential scale) in three bee product matrices from Campania region according to three different sampling times: just after lockdown (T1), partial restriction (T2), resumption of any activity (T3).

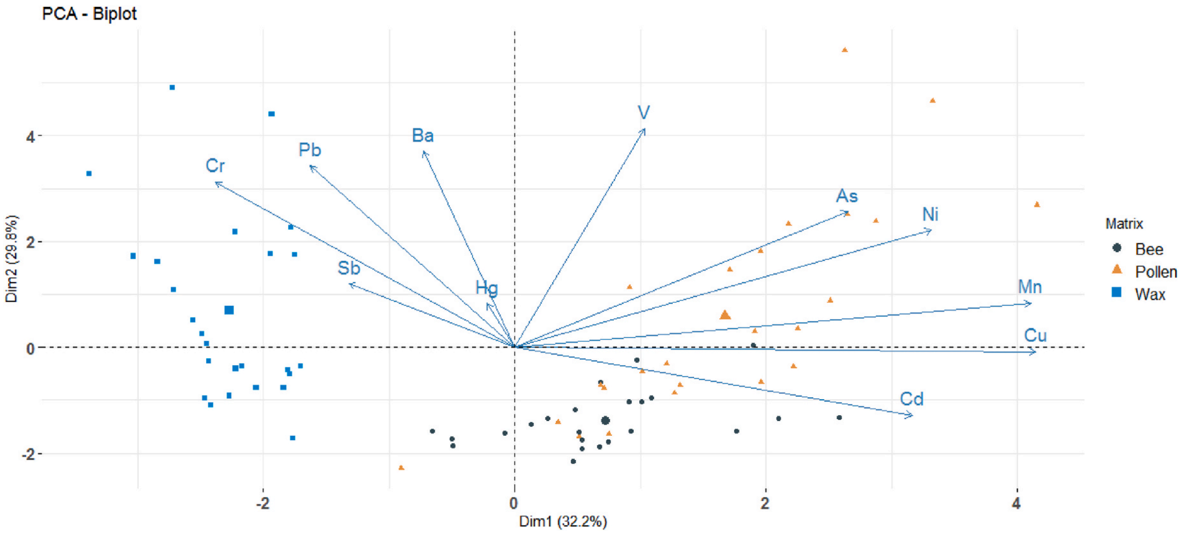


Fig. 5. Principal component analysis (PCA) biplot showing the differentiation of the three bee product matrices by the first two principal axes.

addition, despite the reduction of industrial activities and urban traffic, the increase of atmospheric Hg emissions, as a consequence of intensive use of household appliances (e.g., boilers and heaters) throughout the lockdown, likely led to a different trend in the environmental occurrence of this metal (Huang et al., 2011; Cui et al., 2019 (see Fig. 5).

Regarding Cd, which is naturally present in soil and sediment, almost all of its air emissions come from anthropogenic sources, mainly from non-ferrous metal smelting and refining, fossil fuel combustion and municipal waste incineration (Perugini et al., 2011). Cd air emissions can also come from intensive use of phosphate fertilizers (de Meeûs et al., 2002) that may have been used even during the lockdown period. Another source of Cd contamination of bees is possible through plant uptake and transfer to flowers. The extreme mobility of Cd in the air and in the plant-soil system (Hattab et al., 2014) makes it one of the most toxic metals (Ruschioni et al., 2013). As early as 1991, a study by Yaaqub et al. (1991) stated that much of the Cd occurrence in bees (33%–72%) is due to its widespread occurrence in the air. This theory is consistent with Harrison and Williams (1982), who state that highly mobile Cd is transferred primarily by large-scale atmospheric transport. No statistically significant difference emerged among the sampling sites, except for Cu and Cd ($p < 0.001$) and Ba ($p < 0.05$) that showed higher levels in the Vesuvius area as regards Cd and in the Sorrento peninsula, for Cu and Ba: these differences could depend upon either anthropogenic activities (agricultural runoff) or the site-specific characteristics of the volcanic area (Memoli et al., 2018).

3.3. Effects of Covid-19 restrictions on elements concentration in bee matrices

The effect of pandemic restrictions was also evaluated in bee product matrices such as pollen and wax. Hence, the concentrations of elements were measured at three times (Tables S2, S3). Levels in pollen were the highest at T2 and the lowest at T1 for most of the elements, whereas Cd and Hg still reported a different behavior (T1>T3>T2) (Fig. 4). The concentration detected in pollen fully confirms the same trend observed in bees for all elements. Likewise, Cd in wax was higher in the samples collected just after the Covid-19 pandemic lockdown and, along with Hg (T3>T1>T2) reported the lowest levels during partial-lockdown. The rest of the elements reported the highest concentrations in samples collected after the resumption of activities (Fig. 4). The levels of most contaminants in pollen and wax confirm a decrease in pollution after substantial pandemic restrictions, as emerged from the analysis of bees. In pollen and partially in wax, Cd and Hg levels increased just after the lockdown, confirming the trend highlighted in bees. In addition, the lowest levels of Cd and Hg occurred in partial lockdown both in bees and bee product matrices, suggest that the decrease of these two metals could be appreciated in the long term. Bees and bee product matrices contamination may be affected by seasonality due to weather conditions: the rainfall that usually occurs in winter-autumn lead to higher elements levels of contaminants than in spring-summer (Roman, 2010; Lambert et al., 2012). As a matter of fact, in Campania region the rainfall are more abundant and frequent in September–October rather than July and May (CAR, 2022). However, the results of this study showed an opposite trend highlighting that the reduction in environmental pollution might have been influenced by pandemic restrictions rather than seasonality.

3.4. Principal component analysis (PCA)

In order to highlight any matrix contribution in the accumulation of heavy metals, a Principal Component Analysis (PCA) was performed. The plane described by the first two components (Dim 1 and Dim 2) accounts for more than 60% of the total variance, whereas the individuals are clearly separated according to the qualitative variable “Matrix”, which better illustrates their distance on the plane. On this basis, a hierarchical clusterization of the individuals revealed two clusters: the first is characterized by high values of Cr, Pb, Ba, Sb and low values of Cu, Mn, Cd, Ni, and As and was identified by the wax. The second cluster is characterized by high values of Cu, Mn, Cd, Ni, and As and low values of Cr, Pb, Ba, and Sb: both bees and pollen belong to this cluster, revealing a causal relationship between contamination of pollen and bioaccumulation in the tissues of bees. A possible explanation might be that the bees accumulate environmental pollutants during their foraging activity, mainly through pollen collection. These results can be partly motivated by the study of Zhelyazkova et al. (2004), which evaluated how the concentration of some contaminants changes in the hemolymph of bees. Among these, Mn and Cd were predominant, respectively 21.7 and 17.7 times higher than in bees not subjected to contaminated feeding. The co-occurrence and the proximity of Cu and Cd in the PCA plane may suggest their agricultural origin, whereas Ni and As are likely to have a volcanic origin.

4. Conclusions

The study mainly focused on assessing the contaminant levels just after strict lockdown, partial-lockdown, and post-lockdown. The analysis of bees, pollen, and wax suggests that levels of most contaminants decreased during the intense restriction, likely due to the reduction of industrial and urban activity. However, Cd and Hg showed an opposite trend. Overall, the limitation set during the Covid-19 pandemic lockdown had a counter-effect that reduced anthropogenic activities, resulting in a lower occurrence of heavy metals in the environment. In contrast, an immediate increase in the levels of these chemicals emerged along with the resumption of these activities.

As regards biomonitoring, *Apis mellifera* resulted in an efficient bioindicator for monitoring heavy metals and toxic elements; however, the analysis of bee products may also support this assessment. The HCI allowed estimating the environmental pollution in the examined areas for some elements by comparing with reference threshold limits (Cd, Cr, Ni, and Pb). All sites showed low contamination levels for Cd and Pb and an intermediate-high level of Ni and Cr in one city in each area. However, the concentrations of Cd related to the Vesuvius area were statistically higher than in the other two areas, whereas higher levels of Cu and Ba occurred in the Sorrento peninsula.

In conclusion, whilst the restrictions due to the Covid-19 pandemic impacted the socio-economic sphere, an improvement of environmental quality was confirmed, likely through the reduction of some sources of anthropogenic pollution, generally related to work activities and vehicle emissions. Overall, although the primary purpose of this study was to gain an insight into the impact of the Covid-19 pandemic on environmental pollution, these findings also strengthen the idea that the use of bees as a biomonitoring tool may well have a bearing in urban-scale environmental investigations, with more manageable and less costly analyses.

Credit author statement

Marcello Scivicco: Methodology, Investigation, Writing – original draft. **Agata Nolasco**: Writing – original draft preparation, Writing – review & editing, Data curation; **Luigi Esposito**: Writing - Review & Editing; **Andrea Ariano**: Writing- Original draft preparation, Writing – review & editing, Data curation, Investigation; **Jonathan Squillante**: Writing – original draft, Writing – review & editing, Data curation; **Francesco Esposito**: Writing- Original draft preparation, Validation, Data curation, Writing – review & editing, Formal analysis, Supervision; **Teresa Cirillo**: Writing – review & editing, Supervision, Resources. **Lorella Severino**: Conceptualization, Writing – review & editing, Resources, Supervision, Project administration

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119504.

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