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Honey bee (*Apis mellifera* L.) colonies as bioindicators of environmental SARS-CoV-2 occurrence

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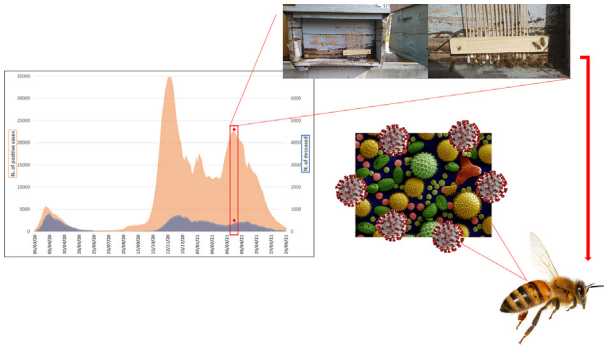
HIGHLIGHTS

- Atmospheric particulate matter (PM) plays a role in SARS-CoV-2 transmission.
- *Apis mellifera* colonies are used as bioindicators for environmental sampling.
- SARS-CoV-2 was detected in the PM carried by honey bee foragers.
- *A. mellifera* colonies can be used in the environmental detection of airborne pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

SARS-CoV-2 is responsible for the COVID-19 pandemic. Airflows sustain the infection spread, and in densely urbanized areas airborne particulate matters (PMs) are deemed to aggravate the viral transmission. *Apis mellifera* colonies are used as bioindicators as they allow environmental sampling of different nature, PMs included. This experiment demonstrates for the first time the possible use of honey bee colonies in the SARS-CoV-2 monitoring. The trial was conducted in Bologna on 18 March 2021, when the third wave of the Italian pandemic was at its peak and environmental conditions allowed high PM concentrations in the air. Sterile swabs were lined up at the hive entrance to sample the dusty material on the body of returning foragers. All of them resulted positive for the target genes of viral SARS-CoV-2 RNA. Likewise, internal samples were taken, but they resulted in no amplification of the target sequences.
This experiment does not support speculations about the role of honey bees or their products in SARS-CoV-2 transmission. However, it indicates a novel use of *A. mellifera* colonies in the environmental detection of airborne human pathogens, at least in a densely urbanized area, deserving better understanding and possible integration with data from automatic air samplers.

1. Introduction

The COVID-19 pandemic originated from the SARS-CoV-2 outbreak reported in China in December 2019 (Lai et al., 2020). The infection reached Italy in February 2020, when the first case was officialised (Grasselli et al., 2020), and quickly spread nationwide. Initially, it affected mainly the North of the country (Distante et al., 2020). Emilia-Romagna lies in that area and eventually turned into the third most

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severely hit Italian administrative region. On a regional population of approximately 4.4 million inhabitants (http://dati.istat.it/Index.aspx), by 31/04/2021 Emilia-Romagna witnessed 346,820 positive cases and 12,380 fatalities (Gamberini et al., 2021; Vignatelli et al., 2021). Official records report 27,790 and 86,856 new cumulative infections, respectively in the course of the first (March 2020) and third (March–April 2021) pandemic wave (Italian Ministry of Health) (Ministero della Salute and Istituto Superiore di Sanità, 2021).

Like other coronaviruses, SARS-CoV-2 is an ssRNA viral strain with a crown-like external layer of spike proteins (Wang et al., 2020a). As the virus misses a lipidic membrane, its stability substantially depends on high relative humidity (Wu et al., 2020a; Wu et al., 2020b). Aerosols generally promote transmission, which may occur through droplets (<5 μm) and droplet nuclei (5-10 μm) (Leung et al., 2020). Before falling, droplets may float in the air for long distances, depending on airflow, temperature, and humidity (Seto, 2015), but droplet nuclei persist airborne only a few hours and tend to disperse within a narrow range (<1 m) (Leung et al., 2020; Li et al., 2020). Thus, aerosols and their size may influence the dispersion of airborne pathogens like SARS-CoV-2 (Zhao et al., 2019).

Atmospheric particulate matter (PM) plays a role in SARS-CoV-2 transmission, as it may bear the virus and foster its spread (Comunian et al., 2020). Conventionally, PM is defined by the particle size. PM_{10} and $PM_{2.5}$ are respectively particles and fine particles with a 50% cut-off aerodynamic diameter of 10 and 2.5 μm. Studies indicate a high incidence of new SARS-CoV-2 infections in conditions of remarkable air pollution, especially in dense metropolitan areas connotated by high levels of smog (Liu et al., 2021; Travaglio et al., 2021; Wang et al., 2020b; Wu et al., 2020c; Yao et al., 2021; Zhu et al., 2020). In northern Italy, the SARS-CoV-2 RNA was found in filters used in monitoring the air quality (Belosi et al., 2021; Borro et al., 2020; Chirizzi et al., 2021; Fattorini and Regoli, 2020; Setti et al., 2020). This leads to the hypothesis of PM-mediated vehiculation of the virus.

Emilia-Romagna is one of the four Italian regions encompassing the Po Valley, where the intensity of industrial and urban settlements, road traffic, and agricultural activities act as almost incessant fume generators. On the other hand, the whole area distinguishes for limited air circulation and a relatively stable climate. These conditions hinder the dispersion of air pollutants and promote smog accumulation, especially in the winter (Chirizzi et al., 2021; Contini et al., 2015; Ferrero et al., 2010).

Honey bee (*Apis mellifera*, L. 1758) colonies are frequently used as bioindicators in environmental monitoring plans (Bargańska et al., 2016; Rissato et al., 2007; van der Steen et al., 2012; van der Steen, 2016). Individual morphology and behaviour make honey bees suitable for this purpose for their: i) high mobility within the explored area; ii) large populations of flying colony members; iii) high sensitivity to chemical pollution; iv) frequent flights of forager bees; v) ubiquity; vi) flight radius approximately 1.5 km wide, corresponding to 7 km^2; vii) body covered by hairs and bristles, capturing pollen and other particles during the flight (Celli and Maccagnani, 2003; Herrero-Latorre et al., 2017; Porrini et al., 2002), PMs included (Capitani et al., 2021; Negri et al., 2015; Pellecchia and Negri, 2018). That makes possible the detection of environmental contaminants: radioactive fallouts (Barišić et al., 1994, 1992), heavy metals (Conti and Botrè, 2001; García et al., 2006; Perugini et al., 2011; Ruschioni et al., 2013; Skorbiłowicz et al., 2018; van der Steen et al., 2016, 2015), pesticides (Balayiannis and Balayiannis, 2008; Ghini et al., 2004; Porrini et al., 2014, 2003), and plant pathogens (Ghini et al., 2002; Girotti et al., 2020, 2013, 2005; Sabatini et al., 2006; van der Steen, 2016) by the analysis of honey bee bodies and their products, in particular pollen and bee-bread.

This trial was designed to evaluate whether forager bees can capture during the activity outside the hive and release on return detectable amounts of SARS-CoV-2. That might lead to the extension of honey bee monitoring to pathogens responsible for human diseases. For this aim, a trial was conducted during the third Italian wave of the COVID-19 pandemic in an apiary located in a densely populated metropolitan area of the Emilia-Romagna region.

2. Material and methods

2.1. Sampling

The trial was conducted in the apiary of CREA – Centro di Ricerca Agricoltura e Ambiente, located in Bologna, Italy (44°31′27.1″N 11°21′03.6″E) and consisting of approximately sixty honey bee colonies. On 18 March 2021, ten colonies housed in Dadant-Blatt (DB) hives were randomly selected from the ones showing the expected phenology for the location and season.

Under clean laboratory conditions, seventeen sterile swabs for use in microbiology were lined up alternated in two arrays on each of ten wooden bars, fixed with adhesive tape, and nailed in the front side of movable flight entrances. Spacing within and between the arrays was sufficient to allow free bee movement, but forcing leaving and returning foragers to rub against the swabs. Before putting the system in place, the swabs were drenched with pure glycerol. The substance was used to promote particulate adhesion and viral RNA preservation due to its viscosity and hygroscopicity, thus increasing the probability to capture measurable amounts of SARS-COV-2 brought in by the foragers (Fig. 1).

The flight entrances provided with swabs were installed in front of the openings of the selected hives from early morning to late afternoon of 18 March, so covering most of the foragers' flight time. After some disorientation due to the rearranged hive opening, soon the bees resumed regular flight activity. Pollen-coloured spots on the swabs gave evidence of effective rubbing.

After swab removal, each colony was inspected to evaluate the number of both combs covered with bees and containing brood. To rule out a possible in-hive viral accumulation, samples were taken to assess whether internal SARS-CoV-2 contamination had occurred, possibly carried by the foragers to the hive by contact or through pollen collection. That was accomplished respectively by i) brushing with new sterile swabs the surface of both sides of the two external combs, where pollen foragers were seen to concentrate and ii) sampling with a Beebread Collector® approximately 2 g of visibly fresh bee bread from three combs, three cells per comb, into 15 mL centrifuge tubes (Fig. 2).

Upon termination of sampling, the swabs from hive entrances and the ones used to brush the comb surface had their stems cut and were plunged separately by colony into two different 50 mL centrifuge tubes containing 10 mL lysis buffer from the RNA extraction kit (GeneJET RNA Purification Kit, ThermoFisher Waltham, MA, U.S.) and 10 mL of TRIzol™ Reagent (ThermoFisher). Each tube containing the bee bread was added 5 mL lysis buffer and 5 mL TRIzol™ Reagent and vortexed until homogenisation.

As the lysis buffer/TRIzol™ Reagent mixture does inactivate the SARS-CoV-2, the activity above was made in the apiary as a safety measure to prevent viral laboratory contamination, so undertaking the procedure prescribed for handling samples from definitely positive individuals (Bain et al., 2020; Risson, 2020; Xia et al., 2021). The samples were stored at −80 °C until analysis.

2.2. Molecular analysis

The total viral RNA was extracted under a laminar flow hood, with a GeneJET RNA Purification Kit (ThermoFisher) used according to the manufacturer's instructions.

The RT-Real Time PCR was conducted with a SARS-CoV-2 (2019-nCoV) CDC RUO Primers and Probes kit (Integrated DNA Technologies, Coralville, Iowa, U.S.), thus adopting the setup recognized by the Centers for Disease Control and Prevention (CDC), intended for the detection of two conserved regions of N gene (N1 and N2) [54]. Accordingly, a total reaction volume of

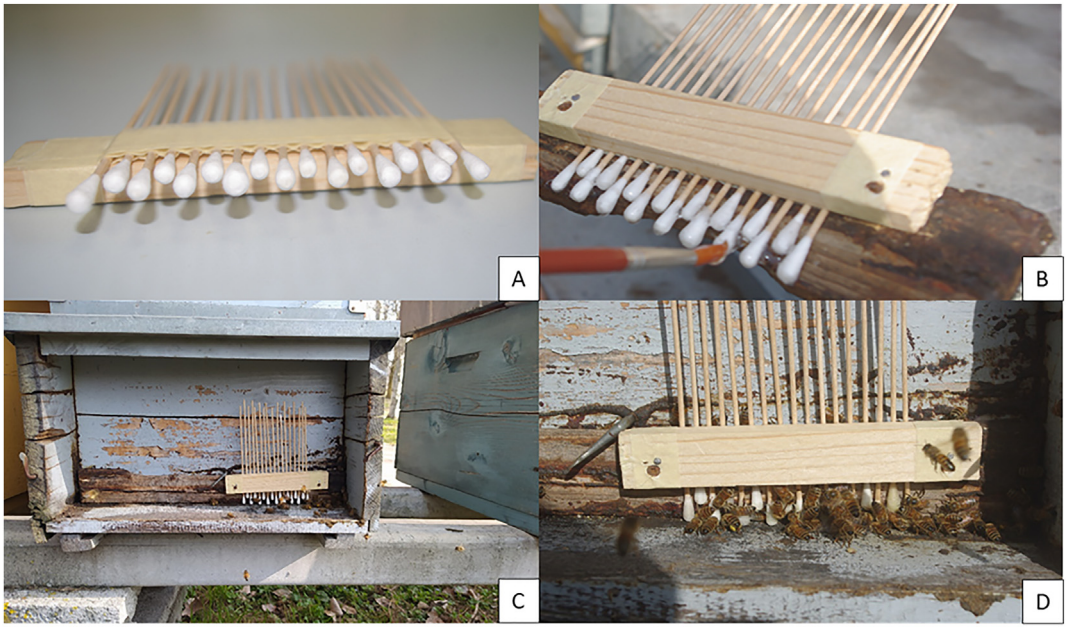

Fig. 1. Upon installation, the array of swabs used for sampling (A) was drenched with glycerol (B) and arranged on the flight entrance of the testing colonies (C). Normal flight activity was resumed within a few hours (D).

15 μL was prepared as follows: 5.0 μL TaqPath™ 1-Step RT-qPCR Master Mix2x (ThermoFisher), 1.5 μL combined primers probes mentioned above, 6.5 μL DNase/RNase-Free distilled water, 2 μL RNA extract from samples. The same analytical procedure was used for all samples, irrespective of their nature.

The reference honey bee gene *β-actin* was selected to control the successful amplification and confirm sample integrity throughout the analytical procedure (Chen et al., 2005). The 2019-nCoV_N_Positive Control (Integrated DNA Technologies) was used as the positive control containing the complete SARS-CoV-2 nucleocapsid gene. All RT-Real Time assays were made in two technical replicates on an Applied Biosystems 7300 Real-Time PCR System (ThermoFisher).

2.3. Environmental and epidemiological characterization

The landscape of the study area was described with the open-source geographic information system Q-GIS v 3.10. A circular buffer with a radius of 1.5 km around the apiary site was deemed a representative range for the forager bee activity (Porrini et al., 2014, 2002).

The minimal spatial resolution was consistent with the regional cartography data "2017-Coperture vettoriali uso del suolo in dettaglio", which relies on a surface details of 0,16 Ha for polygonal areas and a length of 7 m for linear components (https://geoportale.regione.emilia-romagna.it/catalogo/dati-cartografici/pianificazione-e-catasto/uso-del-suolo/layer-9). The map legend bases on the third CORINE Land Cover level, which was further detailed according to the fourth level. Those data describe the landscape of the area of interest with remarkably better details than the newer CORINE Land Cover Map 2018, that applies at national level (http://groupware.sinanet.isprambiente.it/uso-copertura-e-consumo-di-suolo/library/copertura-del-suolo/corine-land-cover/clc2018_shapefile).

The open-source system Dext3r (https://simc.arpae.it/dext3r/), implemented by the Agency for Prevention, Environment and Energy of the Emilia-Romagna region (Arpae), was used to retrieve environmental data for the period 7-23 March 2021. In detail, meteorological data (precipitation, air temperature, scalar wind speed, relative humidity) and pollen grain concentration refer to the automatic weather monitoring station located in Dozza (44°32′02.3″N 11°22′05.4″E). The closest automatic environmental monitoring station to obtain concentration data of PM_{10} and $PM_{2.5}$ was the one located in San Felice (44°30′00.1″ N 11°19′42.6″E) (Fig. 3).

The searchable dashboard based on the open-source system ArcGIS provided by the Italian Ministry of Health (https://opendatadpc.maps.arcgis.com/apps/dashboards/b0c68bce2cce478eaac82fe38d4138b1)

Fig. 2. Sampling of bee bread from a brood comb (left) with a Beebread Collector® (right).

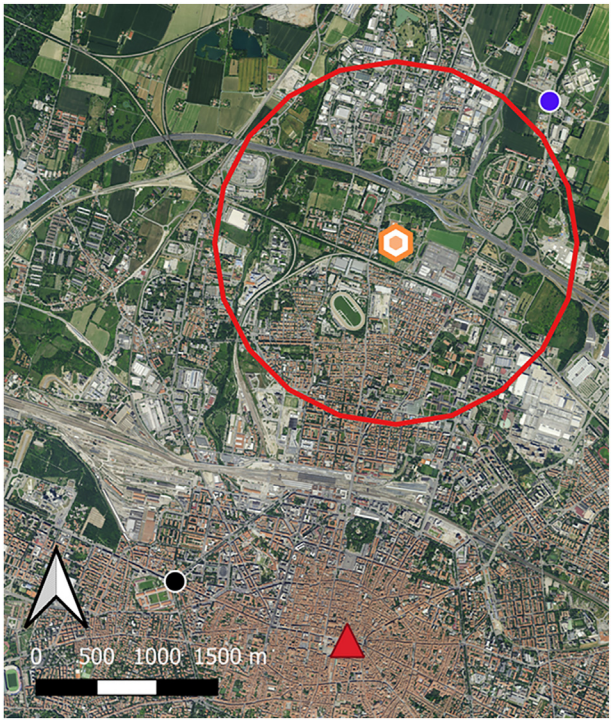

Fig. 3. Aerial view of the city of Bologna (WMS: http://servizigis.regione.emilia-romagna.it/wms/CGR2018). Triangle: city centre; hexagon: apiary site; red circle: buffer of 1.5 km indicating the bee flight range; blue dot: weather station (Dozza); black dot: environmental monitoring station (San Felice). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was accessed to download epidemiological data of new daily cases, total positives and deceased at national level and for the metropolitan city of Bologna.

3. Results

The colonies selected for the trial measured 4.2 ± 0.4 combs with adult bees and 3.4 ± 0.4 combs containing brood (average ± standard error).

The target genes of viral SARS-CoV-2 RNA were amplified from all extracts from the swabs collecting dusty material from returning foragers. None of the internal samples, either swabs or bee bread, scored positive for the *N* gene of SARS-CoV-2.

The leftmost column of Table 1 reports the surface coverage characterization according to the four I Corine levels that were present within the 1.5 km buffer from the apiary site. Consistently with the highly populated nature of the territory, artificial surfaces occupied almost 90% of the buffer, whereas agriculture, forest/seminatural coverage, and water bodies were restricted to minor areas. The other columns and Fig. 4 provide better details of land use. Categories corresponding to limited surfaces (≤2.5%) in II-IV CORINE Land Cover levels are not shown. Urban, industrial, commercial, and transport elements covered altogether more than 70% of the whole buffer surface, and transport infrastructures alone almost 20%.

Figs. 5 and 6 show respectively the SARS-CoV-2 incidence at national level and the local environmental and epidemiologic situation occurred in the period of the trial.

The days before the sampling, the concentration of airborne particulate showed peaks consisting in i) increased concentration (8–11 March) of airborne pollen from plants belonging to the botanical families of Cupressaceae, Taxaceae, and Salicaceae (especially poplar), and ii) $PM_{2.5}$ and PM_{10} exceeding the legal thresholds of respectively 25 μg/m^3 and 40 μg/m^3 (8–12 March) eliciting local restrictions to vehicle circulation (Fig. 6A). Subsequently, the relatively steady air temperature and relative humidity, moderate winds and insignificant rainfalls (14 March: 0.5 kg/m^2) (Fig. 6B–C) were not compatible with substantial particulate renovation and/or washing away.

Official data show that the province of Bologna was hit by increasing COVID-19 positive cases in the second and third week of March 2021, with new daily infected people approximately ranging between 400 and 1000 individuals (Fig. 6D).

4. Discussion

This study was not intended to assess a relationship between the SARS-CoV-2 infection and the presence of honey bees, the contact with them, or the human consumption of honey bee products. Nothing in it supports speculations on this articulate subject.

The trial was conducted during a COVID-19 pandemic peak in a densely urbanized area of northern Italy to investigate the possibility to spot out the viral RNA in the particulate left by returning forager

Table 1
Land use analysis of the 1.5 km buffer area around the apiary site, reporting different levels of detail.

I CORINE level		%	II CORINE level		%	III CORINE level		%	IV Regional soil cover level		%
1	Artificial surfaces	89.15	1.1	Urban fabric	25.39	1.1.1	Continuous urban fabric	16.89	1.1.1.27	Continuous urban built-up areas	16.75
						1.1.2	Discontinuous urban fabric	8.50	1.1.2.1	Discontinuous multifamily built-up areas	6.94
			1.2	Industrial, commercial and transport unit	45.83	1.2.1	Industrial or commercial units	26.02	1.2.1.1	Industrial-storehouse areas	12.69
									1.2.1.3.	Specialized technical infrastructures areas	2.53
									1.2.1.4	Commercial-service areas	10.39
						1.2.2	Road and rail networks and associated land	19.81	1.2.2.1	Highways	4.19
									1.2.2.2	Street network	9.93
									1.2.2.4	Rail network	2.57
			1.3	Mine, dump and construction sites	3.93	1.3.3	Construction sites	3.34			
			1.4	Artificial non-agricultural vegetated areas	14.0	1.4.1	Green urban areas	7.16	1.4.1.1	Urban parks	6.10
						1.4.2	Sport and leisure facilities	6.84	1.4.2.3	Recreation and camping areas	3.12
2	Agricultural areas	7.31	2.1	Arable lands	6.34	2.1.2	Permanently irrigated land	6.34	2.1.2.1	Permanently irrigated land	6.34
3	Forest and seminatural areas	2.35									
5	Water bodies	1.19									
	Total 1.5 km radius area	100		Other cumulate classes	4.51		Other cumulate classes	5.10		Other cumulate classes	15.50

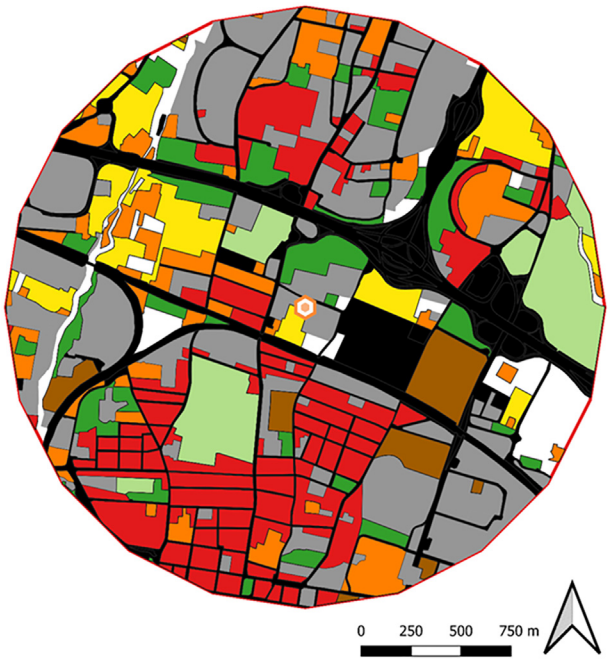

Fig. 4. Land use within the buffer with radius of 1.5 km from the apiary site according to the third level of CORINE Land Cover. Legend - red: continuous urban fabric; orange: discontinuous urban fabric; grey: industrial and commercial units; black: road and rail networks; brown: construction sites; dark green: green and urban areas; light green: sport and leisure facilities; yellow: permanently irrigated areas. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

bees at the entrance of their hive. The study was conducted on one single day of sampling. Nonetheless, it clearly showed the possible use of honey bee colonies in the monitoring of outbreaks of airborne pathogens.

Indeed, honey bees are often used as bioindicators to monitor the environmental occurrence of pesticides and other harmful compounds (Barišić et al., 1994; Celli and Maccagnani, 2003; Skorbiłowicz et al., 2018; van der Steen et al., 2012), but rarely their strict interaction with natural and artificial elements of their habitat has been exploited in pathogen detection. To the best of the Authors' knowledge, only few such cases have been reported so far (Ghini et al., 2002; van der Steen, 2016), when the 1990s witnessed an epidemic of fire blight caused by the Gram-negative bacterium *Erwinia amylovora* and mainly hitting pomaceous fruit trees in several countries (Norelli et al., 2003; Oh and Beer, 2005; Vanneste, 2009). At that time, the bees were suspected as potential vectors of the infection, but one of the Authors (SG) contributed to assess the positive aspects of honey bee-based monitoring plans in the early prediction of new outbreaks (Ghini et al., 2002; Girotti et al., 2005; Sabatini et al., 2006). The present study is well in line with those previous outcomes and represents the first known attempt to extend the honey bee monitoring to the detection of human infections sustained by airborne viruses.

This study was inspired by the previous detection of measurable concentrations of SARS-CoV-2 in airborne PMs recovered from artificial air samplers (Belosi et al., 2021; Borro et al., 2020; Chirizzi et al., 2021; Fattorini and Regoli, 2020) and by the peculiar morphology and behaviour of honey bees. External traits of the bee body bestow them the ability to intercept small particles, namely pollen grains, making them effective in capturing PMs. Besides, in one colony, the forager bees can systematically inspect wide areas of the territory around the apiary, which may be roughly estimated at around 7 km^2 (Porrini et al., 2014, 2002). This all made honey bees promising indicators of airborne SARS-CoV-2 during a COVID-19 outbreak in an area characterized by high PM concentration.

In the specific case here considered, the putative flight range of forager bees included busy streets and motorways, residential districts, shopping areas, factories, *etc.*, as shown by the GIS analysis of the area surrounding the apiary site. Those are regular sources of PMs, which may be aggravated by the heating systems that, at the time of the study, were still functioning.

All swab arrays fixed in front of the hive entrances were found positive to SARS-CoV-2. As in principle direct contact between bees and infective sources can be excluded, the environmental contamination of the foragers must be assumed. This is compatible with

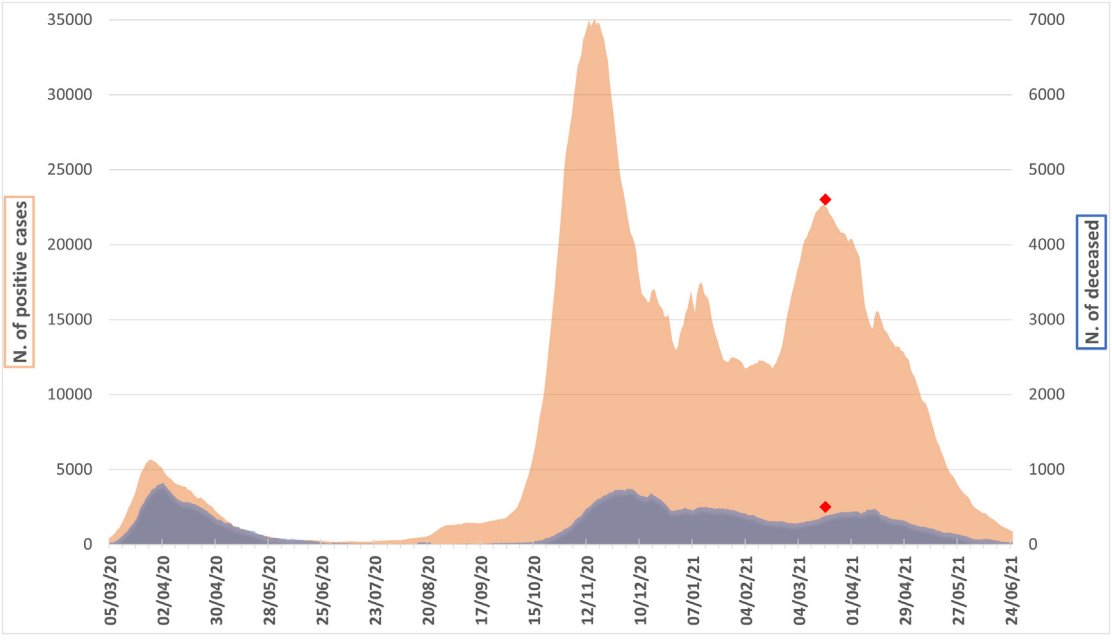

Fig. 5. Rolling 7-day average of new infected and fatalities during the three waves of the Italian pandemic. The sampling date is highlighted with a red mark on each curve. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Local environmental (A–C) and regional epidemiological (D) data retrieved respectively from the open-source searchable databases of Arpae and Italian Ministry of Health for the period 7–23 March 2021. The day of sampling is highlighted in the red box. The error bars in B indicate the min/MAX daily temperatures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

both the high viral circulation and the elevated airborne PM concentration in the period of the trial. Besides, the combination between mild climate and relatively high humidity that are typical of the early bee season in the study area may have played a role in the effective sampling of viral RNA, as air temperature of 5–15 °C and RH% of 44–84% have been reported as conditions promoting the virus survival (Casanova et al., 2010; Chan et al., 2011; Marzoli et al., 2021; Mecenas et al., 2020; Riddell et al., 2020; Sajadi et al.,

2020). In the same period, minor rainfall and moderate wind speed may have caused insignificant atmospheric washout and exchange, so contributing to the persistence in the air of both PMs (Barmpadimos et al., 2012; Begum et al., 2008; Guo et al., 2016; Tiwari et al., 2012; Xu et al., 2017) and viruses.

The honey bee body may be seen as an object with a circular frontal surface with a maximum diameter of 4.5 mm (Carreck et al., 2013; Sauthier et al., 2017; Waddington and Herbst, 1987). Considering that one forager may leave the colony twenty times per day and fly each time for 500 m (Couvillon et al., 2015; Esch and Burns, 1996; Ribbands, 1951), the daily volume of air it may come in contact with reaches approximately 0.16 m^3. In a colony with 2000 foragers (He et al., 2013; Perry et al., 2015; Rodney and Purdy, 2020; Tenczar et al., 2014; Thompson et al., 2016), those last bees may intercept 318 m^3 of air per day, from which airborne PMs may be sampled with hairs and fetched to the colony.

Therefore, in terms of sampled volume, the potential of a honey bee colony is far higher than an automatic environmental monitoring station that, according to the European standards (UNI EN 12341:2001 and UNI EN 14907:2005), has a sampling capacity of 2.3 m^3/h, corresponding to 55.2 m^3 per day. This does not mean that honey bee-based monitoring stations can fully replace existing standard devices. Indeed, the colonies are sensitive to physical and biological conditions that cannot be standardized. Yet, they can be easily maintained in areas out of the reach of infrastructures needed for the functioning of automatic stations, allowing both the detection of a wide range of contaminants and sampling of environmental nucleic acids (*e*DNA and *e*RNA). A synergy between the two methods should be therefore implemented, to increase both amount and quality of the environmental information.

The absence of viral RNA in internal swabs and the bee bread collected from the combs may be due to the healthy honey bee colony environment. The hive products, bee venom included, show antibacterial, antifungal, and antiviral properties, often depending on environmental and botanical features (Cilia et al., 2020; Felicioli et al., 2019; Kolayli and Keskin, 2020; Kujumgiev et al., 1999; Kwon et al., 2020; Münstedt, 2019; Wehbe et al., 2019). Furthermore, *in vitro* and *in silico* assays demonstrated that bee products can inactivate or destroy the SARS-CoV-2 (Al Naggar et al., 2021; Ali and Kunugi, 2021; Bachevski et al., 2020; Elmahallawy et al., 2021; Lima et al., 2021; Shaldam et al., 2021; Yang et al., 2020).

5. Conclusion

The study indicates the possibility of exploiting the individual honey bee morphology and the colony foraging behaviour in the environmental detection of airborne human pathogens. So far, successful attempts in this sense have been limited to phytopathogens.

Indeed, honey bees are accurate explorers of the ecosystem, with the ability to retrieve a range of compounds from the environment and fetching them back to the hive, where they can be sampled to get them analyzed. This study assumes that SARS-CoV-2 left by the forager bees at the hive entrance is related to the airborne PM, albeit the real origin should be investigated properly. Similarly, the bee efficiency in PM trapping needs better understanding, as it may involve both the intricacy of bee hair coverage and electrostatic attractions.

Easy adaptation and handling virtually allow bee-based monitoring networks in all inhabitable areas. That makes it conceivable to extending this innovative approach to other plant, animal, and human airborne pathogens, and using it in predicting recurrent outbreaks like seasonal influenza. At this stage, no attempts to quantify SARS-CoV-2 were made but prospected epidemiological monitoring plans involving honey bees require the assessment of sensitivity to the different pathogens to be studied and the possible limitations to reliability coming from environmental factors.

Undoubtedly, instrumental air samplers provide essential data, but research efforts should be made also to synergize them with the information coming from networks of monitoring hives.

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CRediT authorship contribution statement

Giovanni Cilia: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Laura Bortolotti:** Investigation, Funding acquisition, Supervision, Writing – review & editing. **Sergio Albertazzi:** Investigation, Writing – original draft, Writing – review & editing. **Severino Ghini:** Conceptualization, Investigation, Writing – review & editing. **Antonio Nanetti:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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.

References

Al Naggar, Y., Giesy, J.P., Abdel-Daim, M.M., Javed Ansari, M., Al-Kahtani, S.N., Yahya, G., 2021. Fighting against the second wave of COVID-19: can honeybee products help protect against the pandemic? Saudi J. Biol. Sci. 28, 1519–1527. https://doi.org/10.1016/j.sjbs.2020.12.031.

Ali, A.M., Kunugi, H., 2021. Propolis, bee honey, and their components protect against coronavirus disease 2019 (COVID-19): a review of in silico, in vitro, and clinical studies. Molecules 26. https://doi.org/10.3390/molecules26051232.

Bachevski, D., Damevska, K., Simeonovski, V., Dimova, M., 2020. Back to the basics: propolis and COVID-19. Dermatol. Ther. 33, e13780. https://doi.org/10.1111/dth.13780.

Bain, W., Lee, J.S., Watson, A.M., Stitt-Fischer, M.S., 2020. Practical guidelines for collection, manipulation and inactivation of SARS-CoV-2 and COVID-19 clinical specimens. Curr. Protoc. Cytom. 93, e77. https://doi.org/10.1002/cpcy.77.

Balayiannis, G., Balayiannis, P., 2008. Bee honey as an environmental bioindicator of pesticides' occurrence in six agricultural areas of Greece. Arch. Environ. Contam. Toxicol. 55, 462–470. https://doi.org/10.1007/s00244-007-9126-x.

Bargańska, Z., ˜lebioda, M., Namieśnik, J., 2016. Honey bees and their products: bioindicators of environmental contamination. Crit. Rev. Environ. Sci. Technol. 46, 235–248. https://doi.org/10.1080/10643389.2015.1078220.

Barišić, D., Lulić, S., Kezić, N., Vertačnik, A., 1992. 137 Cs in flowers, pollen and honey from the Republic of Croatia four years after the Chernobyl accident. Apidologie 23, 71–78. https://doi.org/10.1051/apido:19920108.

Barišić, D., Lazarić, K., Lulić, S., Vertačnik, A., Dražić, M., Kezić, N., 1994. 40 K, 134 Cs and 137 Cs in pollen, honey and soil surface layer in Croatia. Apidologie 25, 585–595. https://doi.org/10.1051/apido:19940609.

Barmpadimos, I., Keller, J., Oderbolz, D., Hueglin, C., Prévôt, A.S.H., 2012. One decade of parallel fine (PM 2.5) and coarse (PM 10-PM 2.5) particulate matter measurements in Europe: trends and variability. Atmos. Chem. Phys. 12, 3189–3203. https://doi.org/10.5194/acp-12-3189-2012.

Begum, B.A., Biswas, S.K., Hopke, P.K., 2008. Assessment of trends and present ambient concentrations of PM2.2 and PM10 in Dhaka, Bangladesh. Air Qual. Atmos. Health 1, 125–133. https://doi.org/10.1007/s11869-008-0018-7.

Belosi, F., Conte, M., Gianelle, V., Santachiara, G., Contini, D., 2021. On the concentration of SARS-CoV-2 in outdoor air and the interaction with pre-existing atmospheric particles. Environ. Res. 193, 110603. https://doi.org/10.1016/j.envres.2020.110603.

Borro, M., Di Girolamo, P., Gentile, G., De Luca, O., Preissner, R., Marcolongo, A., Ferracuti, S., Simmaco, M., 2020. Evidence-based considerations exploring relations between SARS-CoV-2 pandemic and air pollution: involvement of PM2.5-mediated up-regulation of the viral receptor ACE-2. Int. J. Environ. Res. Public Health 17, 5573. https://doi.org/10.3390/ijerph17155573.

Capitani, G., Papa, G., Pellecchia, M., Negri, I., 2021. Disentangling multiple PM emission sources in the Po Valley (Italy) using honey bees. Heliyon 7, e06194. https://doi.org/10.1016/j.heliyon.2021.e06194.

Carreck, N.L., Andree, M., Brent, C.S., Cox-Foster, D., Dade, H.A., Ellis, J.D., Hatjina, F., Van Englesdorp, D., 2013. Standard methods for Apis mellifera anatomy and dissection. J. Apic. Res. 52. https://doi.org/10.3896/IBRA.1.52.4.03.

Casanova, L.M., Jeon, S., Rutala, W.A., Weber, D.J., Sobsey, M.D., 2010. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl. Environ. Microbiol. 76, 2712–2717. https://doi.org/10.1128/AEM.02291-09.

Celli, G., Maccagnani, B., 2003. Honey bees as bioindicators of environmental pollution. Bull. Insectology 56, 137–139.
Chan, K.H., Peiris, J.S.M., Lam, S.Y., Poon, L.L.M., Yuen, K.Y., Seto, W.H., 2011. The effects of temperature and relative humidity on the viability of the SARS coronavirus. Adv. Virol. 2011. https://doi.org/10.1155/2011/734690.
Chen, Y.P., Higgins, J.A., Feldlaufer, M.F., 2005. Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (Apis mellifera L.). Appl. Environ. Microbiol. 71, 436–441. https://doi.org/10.1128/AEM.71.1.436-441.2005.
Chirizzi, D., Conte, M., Feltracco, M., Dinoi, A., Gregoris, E., Barbaro, E., La Bella, G., Ciccarese, G., La Salandra, G., Gambaro, A., Contini, D., 2021. SARS-CoV-2 concentrations and virus-laden aerosol size distributions in outdoor air in north and south of Italy. Environ. Int. 146, 106255. https://doi.org/10.1016/j.envint.2020.106255.
Cilia, G., Fratini, F., Marchi, M., Sagona, S., Turchi, B., Adamchuk, L., Felicioli, A., Kačániová, M., 2020. Antibacterial activity of honey samples from Ukraine. Vet. Sci. 7, 181. https://doi.org/10.3390/vetsci7040181.
Comunian, S., Dongo, D., Milani, C., Palestini, P., 2020. Air pollution and covid-19: the role of particulate matter in the spread and increase of covid-19s morbidity and mortality. Int. J. Environ. Res. Public Health https://doi.org/10.3390/ijerph17124487.
Conti, M.E., Botrè, F., 2001. Honeybees and their products as potential bioindicators of heavy metals contamination. Environ. Monit. Assess. 69, 267–282. https://doi.org/10.1023/A:1010719107006.
Contini, D., Gambaro, A., Donateo, A., Cescon, P., Cesari, D., Merico, E., Belosi, F., Citron, M., 2015. Inter-annual trend of the primary contribution of ship emissions to PM2.5 concentrations in Venice (Italy): efficiency of emissions mitigation strategies. Atmos. Environ. 102, 183–190. https://doi.org/10.1016/j.atmosenv.2014.11.065.
Couvillon, M.J., Riddell Pearce, F.C., Accleton, C., Fensome, K.A., Quah, S.K.L., Taylor, E.L., Ratnieks, F.L.W., 2015. Honey bee foraging distance depends on month and forage type. Apidologie 46, 61–70. https://doi.org/10.1007/s13592-014-0302-5.
Distante, C., Piscitelli, P., Miani, A., 2020. Covid-19 outbreak progression in italian regions: approaching the peak by the end of march in northern Italy and first week of April in southern Italy. Int. J. Environ. Res. Public Health 17, 3025. https://doi.org/10.3390/ijerph17093025.
Elmahallawy, E.K., Mohamed, Y., Abdo, W., El-Gohary, F.A., Ahmed Awad Ali, S., Yanai, T., 2021. New insights into potential benefits of bioactive compounds of bee products on COVID-19: a review and assessment of recent research. Front. Mol. Biosci. 7, 513. https://doi.org/10.3389/fmolb.2020.618318.
Esch, H.E., Burns, J., 1996. Distance estimation by foraging honeybees. J. Exp. Biol. 199, 155–162.
Fattorini, D., Regoli, F., 2020. Role of the chronic air pollution levels in the Covid-19 outbreak risk in Italy. Pollut, Environ https://doi.org/10.1016/j.envpol.2020.114732.
Felicioli, A., Cilia, G., Mancini, S., Turchi, B., Galaverna, G., Cirlini, M., Cerri, D., Fratini, F., 2019. In vitro antibacterial activity and volatile characterisation of organic Apis mellifera ligustica (Spinola, 1906) beeswax ethanol extracts. Food Biosci. 29, 102–109.
Ferrero, L., Perrone, M.G., Petraccone, S., Sangiorgi, G., Ferrini, B.S., Lo Porto, C., Lazzati, Z., Cocchi, D., Bruno, F., Greco, F., Riccio, A., Bolzacchini, E., 2010. Vertically-resolved particle size distribution within and above the mixing layer over the Milan metropolitan area. Atmos. Chem. Phys. 10, 3915–3932. https://doi.org/10.5194/acp-10-3915-2010.
Gamberini, L., Coniglio, C., Cilloni, N., Semeraro, F., Moro, F., Tartaglione, M., Chiarini, V., Lupi, C., Bua, V., Gordini, G., 2021. Remodelling of a regional emergency hub in response to the COVID-19 outbreak in Emilia-Romagna. Emerg. Med. J. 38, 308–314. https://doi.org/10.1136/emermed-2020-209671.
García, J.C.R., Rodríguez, R.I., Crecente, R.M.P., García, J.B., Martín, S.G., Latorre, C.H., 2006. Preliminary chemometric study on the use of honey as an environmental marker in Galicia (northwestern Spain). J. Agric. Food Chem. 54, 7206–7212. https://doi.org/10.1021/jf060823t.
Ghini, S., Girotti, S., Calzolari, A., Sabatini, A.G., Alessandrini, A., Zeri, L., Porrini, C., 2002. Use of honeybees (Apis mellifera L.) as indicators of the presence of the phytopathogenic bacteria Erwinia amylovora. Insect Soc. Life 4, 69–77.
Ghini, S., Fernández, M., Picó, Y., Marín, R., Fini, F., Mañes, J., Girotti, S., 2004. Occurrence and Distribution of Pesticides in the Province of Bologna, Italy, Using Honeybees as Bioindicators. https://doi.org/10.1007/s00244-003-3219-y.
Girotti, S., Bolelli, L., Fini, F., Rinaldi, D., Ghini, S., Chirila, E., 2005. Bioluminescent bacteria and honeybees: bioindicators in environmental monitoring. Environ. Eng. Manag. J. 4, 143–148.
Girotti, S., Ghini, S., Maiolini, E., Bolelli, L., Ferri, E.N., 2013. Trace analysis of pollutants by use of honeybees, immunoassays, and chemiluminescence detection. Anal. Bioanal. Chem. https://doi.org/10.1007/s00216-012-6443-3.
Girotti, S., Ghini, S., Ferri, E., Bolelli, L., Colombo, R., Serra, G., Porrini, C., Sangiorgi, S., 2020. Bioindicators and biomonitoring: honeybees and hive products as pollution impact assessment tools for the Mediterranean area. Euro-Mediterr. J. Environ. Integr. 5, 62. https://doi.org/10.1007/s41207-020-00204-9.
Grasselli, G., Pesenti, A., Cecconi, M., 2020. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy. JAMA 323, 1545. https://doi.org/10.1001/jama.2020.4031.
Guo, L.C., Zhang, Y., Lin, H., Zeng, W., Liu, T., Xiao, J., Rutherford, S., You, J., Ma, W., 2016. The washout effects of rainfall on atmospheric particulate pollution in two Chinese cities. Environ. Pollut. 215, 195–202. https://doi.org/10.1016/j.envpol.2016.05.003.
He, X., Wang, W., Qin, Q., Zeng, Z., Zhang, S., Barron, A.B., 2013. Assessment of flight activity and homing ability in asian and european honey bee species, Apis cerana and Apis mellifera, measured with radio frequency tags. Apidologie 44, 38–51. https://doi.org/10.1007/s13592-012-0156-7.
Herrero-Latorre, C., Barciela-García, J., García-Martín, S., Peña-Crecente, R.M., 2017. The use of honeybees and honey as environmental bioindicators for metals and radionuclides: a review. Environ. Rev. https://doi.org/10.1139/er-2017-0029.
Kolayli, S., Keskin, M., 2020. Natural bee products and their apitherapeutic applications. Studies in Natural Products Chemistry. Elsevier B.V., pp. 175–196 https://doi.org/10.1016/B978-0-12-817907-9.00007-6.
Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., Popov, S., 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. J. Ethnopharmacol. 64, 235–240. https://doi.org/10.1016/S0378-8741(98)00131-7.
Kwon, M.J., Shin, H.M., Perumalsamy, H., Wang, X., Ahn, Y.J., 2020. Antiviral effects and possible mechanisms of action of constituents from Brazilian propolis and related compounds. J. Apic. Res. 59, 413–425. https://doi.org/10.1080/00218839.2019.1695715.
Lai, C.C., Wang, C.Y., Wang, Y.H., Hsueh, S.C., Ko, W.C., Hsueh, P.R., 2020. Global epidemiology of coronavirus disease 2019 (COVID-19): disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. Int. J. Antimicrob. Agents 55, 105946. https://doi.org/10.1016/j.ijantimicag.2020.105946.
Leung, N.H.L., Chu, D.K.W., Shiu, E.Y.C., Chan, K.H., McDevitt, J.J., Hau, B.J.P., Yen, H.L., Li, Y., Ip, D.K.M., Peiris, J.S.M., Seto, W.H., Leung, G.M., Milton, D.K., Cowling, B.J., 2020. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat. Med. 26, 676–680. https://doi.org/10.1038/s41591-020-0843-2.
Li, L., Huang, Tian, Wang, Yong-qing, Wang, Z., Liang, Y., Huang, Tao-bi, Zhang, H., Sun, W., Wang, Yuping, 2020. COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. J. Med. Virol. 92, 577–583. https://doi.org/10.1002/jmv.25757.
Lima, W.G., Brito, J.C.M., da Cruz Nizer, W.S., 2021. Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). Phyther. Res. 35, 743–750. https://doi.org/10.1002/ptr.6872.
Liu, L., Zhang, J., Du, R., Teng, X., Hu, R., Yuan, Q., Tang, S., Ren, C., Huang, X., Xu, L., Zhang, Y., Zhang, X., Song, C., Liu, B., Lu, G., Shi, Z., Li, W., 2021. Chemistry of atmospheric fine particles during the COVID-19 pandemic in a megacity of eastern China. Geophys. Res. Lett. 48. https://doi.org/10.1029/2020GL091611 (2020GL091611).
Marzoli, F., Bortolami, A., Pezzuto, A., Mazzetto, E., Piro, R., Terregino, C., Bonfante, F., Belluco, S., 2021. A systematic review of human coronaviruses survival on environmental surfaces. Sci. Total Environ. 778. https://doi.org/10.1016/j.scitotenv.2021.146191.
Mecenas, P., da Rosa Moreira Bastos, R.T., Rosário Vallinoto, A.C., Normando, D., 2020. Effects of temperature and humidity on the spread of COVID-19: a systematic review. PLoS One 15, e0238339. https://doi.org/10.1371/journal.pone.0238339.
Ministero della Salute, Istituto Superiore di Sanità, 2021. Monitoraggio Fase 2 Report settimanale 46 - Aggiornamento Epidemiologico Regione Emilia-Romagna.
Münstedt, K., 2019. Bee products and the treatment of blister-like lesions around the mouth, skin and genitalia caused by herpes viruses—a systematic review. Complement. Ther. Med. 43, 81–84. https://doi.org/10.1016/j.ctim.2019.01.014.
Negri, I., Mavris, C., Di Prisco, G., Caprio, E., Pellecchia, M., 2015. Honey bees (Apis mellifera, L.) as active samplers of airborne particulate matter. PLoS One 10, e0132491. https://doi.org/10.1371/journal.pone.0132491.
Norelli, J.L., Jones, A.L., Aldwinckle, H.S., 2003. Fire blight management in the twenty-first century: using new technologies that enhance host resistance in apple. Plant Dis. 87, 756–765. https://doi.org/10.1094/PDIS.2003.87.7.756.
Oh, C.S., Beer, S.V., 2005. Molecular genetics of Erwinia amylovora involved in the development of fire blight. FEMS Microbiol. Lett. 253, 185–192. https://doi.org/10.1016/j.femsle.2005.09.051.
Pellecchia, M., Negri, I., 2018. Particulate matter collection by honey bees (Apis mellifera, L.) near to a cement factory in Italy. PeerJ 2018. https://doi.org/10.7717/peerj.5322.
Perry, C.J., Søvik, E., Myerscough, M.R., Barron, A.B., 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proc. Natl. Acad. Sci. U. S. A. 112, 3427–3432. https://doi.org/10.1073/pnas.1422089112.
Perugini, M., Manera, M., Grotta, L., Abete, M.C., Tarasco, R., Amorena, M., 2011. Heavy metal (Hg, Cr, Cd, and Pb) contamination in urban areas and wildlife reserves: honeybees as bioindicators. Biol. Trace Elem. Res. 140, 170–176. https://doi.org/10.1007/s12011-010-8688-z.
Porrini, C., Ghini, S., Sabatini, A.G., Gattavecchia, E., Celli, G., 2002. Use of honey bees as bioindicators of environmental pollution in Italy. In: Devillers, J., Pham-Delegue, M. (Eds.), Honey Bees: The Environmental Impact of Chemical. 11. Routledge-Taylor & Francis Group, pp. 186–247.
Porrini, C., Sabatini, A.G., Girotti, S., Fini, F., Monaco, L., Celli, G., Bortolotti, L., Ghini, S., 2003. The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. Bull. Insectology 56, 147–152.
Porrini, C., Caprio, E., Tesoriero, D., Prisco, G.Di, 2014. Using honey bee as bioindicator of chemicals in Campanian agroecosystems (South Italy). Bull. Insectology 67, 137–146.
Ribbands, C.R., 1951. The flight range of the honey-bee. J. Anim. Ecol. 20, 220. https://doi.org/10.2307/1541.
Riddell, S., Goldie, S., Hill, A., Eagles, D., Drew, T.W., 2020. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol. J. 17, 145. https://doi.org/10.1186/s12985-020-01418-7.
Rissato, S.R., Galhiane, M.S., de Almeida, M.V., Gerenutti, M., Apon, B.M., 2007. Multiresidue determination of pesticides in honey samples by gas chromatography-mass spectrometry and application in environmental contamination. Food Chem. 101, 1719–1726. https://doi.org/10.1016/j.foodchem.2005.10.034.
Risson, E., 2020. Inactivated vaccine for SARS-CoV-2. Nat. Rev. Immunol. 20, 353. https://doi.org/10.1038/s41577-020-0334-1.
Rodney, S., Purdy, J., 2020. Dietary requirements of individual nectar foragers, and colony-level pollen and nectar consumption: a review to support pesticide exposure assessment for honey bees. Apidologie https://doi.org/10.1007/s13592-019-00694-9.
Ruschioni, S., Riolo, P., Minuz, R.L., Stefano, M., Cannella, M., Porrini, C., Isidoro, N., 2013. Biomonitoring with honeybees of heavy metals and pesticides in nature reserves of

the Marche region (Italy). Biol. Trace Elem. Res. 154, 226–233. https://doi.org/10.1007/s12011-013-9732-6.
Sabatini, A.G., Alexandrova, M., Carpana, E., Medrzycki, P., Bortolotti, L., Porrini, C., Bazzi, C., Ghini, S., Girotti, S., Baroni, F., Alessandrini, A., 2006. Relationships between Apis mellifera and Erwinia amylovora: bioindication, bacterium dispersal and quarantine procedures. Acta Hortic. 704, 155–162. https://doi.org/10.17660/ActaHortic.2006.704.19.
Sajadi, M.M., Sajadi, M.M., Habibzadeh, P., Vintzileos, A., Shokouhi, S., Miralles-Wilhelm, F., Miralles-Wilhelm, F., Amoroso, A., Amoroso, A., 2020. Temperature, humidity, and latitude analysis to estimate potential spread and seasonality of coronavirus disease 2019 (COVID-19). JAMA Netw. Open 3. https://doi.org/10.1001/jamanetworkopen.2020.11834.
Sauthier, R., l'Anson Price, R., Grüter, C., 2017. Worker size in honeybees and its relationship with season and foraging distance. Apidologie 48, 234–246. https://doi.org/10.1007/s13592-016-0468-0.
Seto, W.H., 2015. Airborne transmission and precautions: facts and myths. J. Hosp. Infect. 89, 225–228. https://doi.org/10.1016/j.jhin.2014.11.005.
Setti, L., Passarini, F., De Gennaro, G., Barbieri, P., Pallavicini, A., Ruscio, M., Piscitelli, P., Colao, A., Miani, A., 2020. Searching for SARS-COV-2 on particulate matter: a possible early indicator of COVID-19 epidemic recurrence. Int. J. Environ. Res. Public Health https://doi.org/10.3390/ijerph17092986.
Shaldam, M.A., Yahya, G., Mohamed, N.H., Abdel-Daim, M.M., Al Naggar, Y., 2021. In silico screening of potent bioactive compounds from honeybee products against COVID-19 target enzymes. Environ. Sci. Pollut. Res., 1–8 https://doi.org/10.1007/s11356-021-14195-9.
Skorbiłowicz, E., Skorbiłowicz, M., Ciesluk, I., 2018. Bees as bioindicators of environmental pollution with metals in an urban area. J. Ecol. Eng. 19, 229–234. https://doi.org/10.12911/22998993/85738.
van der Steen, J.J.M., 2016. Beehold: The Colony of the Honeybee (Apis mellifera L) as a Bio-sampler for Pollutants and Plant Pathogens. https://doi.org/10.18174/375348.
van der Steen, J., de Kraker, J., Grotenhuis, T., 2012. Spatial and temporal variation of metal concentrations in adult honeybees (Apis mellifera L.). Environ. Monit. Assess. 184, 4119–4126. https://doi.org/10.1007/S10661-011-2248-7.
van der Steen, J.J.M., de Kraker, J., Grotenhuis, T., 2015. Assessment of the potential of honeybees (Apis mellifera L.) in biomonitoring of air pollution by cadmium, lead and vanadium. J. Environ. Prot. 6, 96–102. https://doi.org/10.4236/JEP.2015.62011.
van der Steen, J.J.M., Cornelissen, B., Blacquière, T., Pijnenburg, J.E.M.L., Severijnen, M., 2016. Think regionally, act locally: metals in honeybee workers in the Netherlands (surveillance study 2008). Environ. Monit. Assess. 1888 (188), 1–9. https://doi.org/10.1007/S10661-016-5451-8.
Tenczar, P., Lutz, C.C., Rao, V.D., Goldenfeld, N., Robinson, G.E., 2014. Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. Anim. Behav. 95, 41–48. https://doi.org/10.1016/j.anbehav.2014.06.006.
Thompson, H., Coulson, M., Ruddle, N., Wilkins, S., Harkin, S., 2016. Thiamethoxam: assessing flight activity of honeybees foraging on treated oilseed rape using radio frequency identification technology. Environ. Toxicol. Chem. 35, 385–393. https://doi.org/10.1002/etc.3183.
Tiwari, S., Chate, D.M., Srivastava, M.K., Safai, P.D., Srivastava, A.K., Bisht, D.S., Padmanabhamurty, B., 2012. Statistical evaluation of PM10 and distribution of PM1, PM2.5, and PM10 in ambient air due to extreme fireworks episodes (Deepawali festivals) in megacity Delhi. Nat. Hazards 61, 521–531. https://doi.org/10.1007/s11069-011-9931-4.
Travaglio, M., Yu, Y., Popovic, R., Selley, L., Leal, N.S., Martins, L.M., 2021. Links between air pollution and COVID-19 in England. Environ. Pollut. 268, 115859. https://doi.org/10.1016/j.envpol.2020.115859.
Vanneste, J.L., 2009. What is fire blight? Who is Erwinia amylovora? How to control it? Fire Blight: The Disease and Its Causative Agent, Erwinia amylovora. CABI, pp. 1–6 https://doi.org/10.1079/9780851992945.0001
Vignatelli, L., Zenesini, C., Belotti, L.M.B., Baldin, E., Bonavina, G., Calandra-Buonaura, G., Cortelli, P., Descovich, C., Fabbri, G., Giannini, G., Guarino, M., Pantieri, R., Samoggia, G., Scaglione, C., Trombetti, S., D'Alessandro, R., Nonino, F., 2021. Risk of hospitalization and death for <scp>COVID</scp> -19 in people with Parkinson's disease or parkinsonism. Mov. Disord. 36, 1–10. https://doi.org/10.1002/mds.28408.
Waddington, K.D., Herbst, L.H., 1987. Body size and the functional length of the proboscis of honey bees. Florida Entomol. 70, 124. https://doi.org/10.2307/3495099.
Wang, J., Tang, K., Feng, K., Lin, X., Lv, W., Chen, K., Wang, F., 2020a. High Temperature and High Humidity Reduce the Transmission of COVID-19. arXiv https://doi.org/10.2139/ssrn.3551767.
Wang, B., Liu, J., Fu, S., Xu, X., Li, L., Ma, Y., Zhou, J., Yao, J., Liu, X., Zhang, X., He, X., Yan, J., Shi, Y., Ren, X., Niu, J., Luo, B., Zhang, K., 2020b. An Effect Assessment of Airborne Particulate Matter Pollution on COVID-19: A Multi-city Study in China. medRxiv https://doi.org/10.1101/2020.04.09.20060137.
Wehbe, R., Frangieh, J., Rima, M., Obeid, D.El, Sabatier, J.M., Fajloun, Z., 2019. Bee venom: overview of main compounds and bioactivities for therapeutic interests. Molecules 24, 2997. https://doi.org/10.3390/molecules24162997.
Wu, F.Q., Xiao, A., Zhang, J.B., Gu, X.Q., Lee, W.L., Kauffman, K., Hanage, W.P., Matus, M., Ghaeli, N., Endo, N., Duvallet, C., Moniz, K., Erickson, T.B., Chai, P.R., Thompson, J., Alm, E.J., 2020a. SARS-CoV-2 Titers in Wastewater Are Higher Than Expected From Clinically Confirmed Cases. medRxiv https://doi.org/10.1101/2020.04.05.20051540.
Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., Kuang, L., Fang, X., Mishra, N., Lu, J., Shan, H., Jiang, G., Huang, X., 2020b. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol. Hepatol. https://doi.org/10.1016/S2468-1253(20)30083-2.
Wu, X., Nethery, R.C., Sabath, B., Braun, D., Dominici, F., James, C., 2020c. Exposure to air pollution and COVID-19 mortality in the United States: a nationwide cross-sectional study. Sci. Adv. 6, eabd4049. https://doi.org/10.1126/sciadv.abd4049.
Xia, S., Zhang, Y., Wang, Y., Wang, Hui, Yang, Yunkai, Gao, G.F., Tan, W., Wu, G., Xu, M., Lou, Z., Huang, W., Xu, W., Huang, B., Wang, Huijuan, Wang, Wei, Zhang, W., Li, N., Xie, Z., Ding, L., You, W., Zhao, Y., Yang, Xuqin, Liu, Y., Wang, Q., Huang, L., Yang, Yongli, Xu, G., Luo, B., Wang, Wenling, Liu, P., Guo, W., Yang, Xiaoming, 2021. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infect. Dis. 21, 39–51. https://doi.org/10.1016/S1473-3099(20)30831-8.
Xu, X., Zhang, Z., Bao, L., Mo, L., Yu, X., Fan, D., Lun, X., 2017. Influence of rainfall duration and intensity on particulate matter removal from plant leaves. Sci. Total Environ. 609, 11–16. https://doi.org/10.1016/j.scitotenv.2017.07.141.
Yang, W., Hu, F.liang, Xu, X.feng, 2020. Bee venom and SARS-CoV-2. Toxicon 181, 69–70. https://doi.org/10.1016/j.toxicon.2020.04.105.
Yao, Y., Pan, J., Liu, Z., Meng, X., Wang, Weidong, Kan, H., Wang, Weibing, 2021. Ambient nitrogen dioxide pollution and spreadability of COVID-19 in chinese cities. Ecotoxicol. Environ. Saf. 208, 111421. https://doi.org/10.1016/j.ecoenv.2020.111421.
Zhao, Y., Richardson, B., Takle, E., Chai, L., Schmitt, D., Xin, H., 2019. Airborne transmission may have played a role in the spread of 2015 highly pathogenic avian influenza outbreaks in the United States. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-47788-z.
Zhu, Y., Xie, J., Huang, F., Cao, L., 2020. Association between short-term exposure to air pollution and COVID-19 infection: evidence from China. Sci. Total Environ. 727, 138704. https://doi.org/10.1016/j.scitotenv.2020.138704.