Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Evaluating the presence of SARS-CoV-2 RNA in the particulate matters during the peak of COVID-19 in Padua, northern Italy

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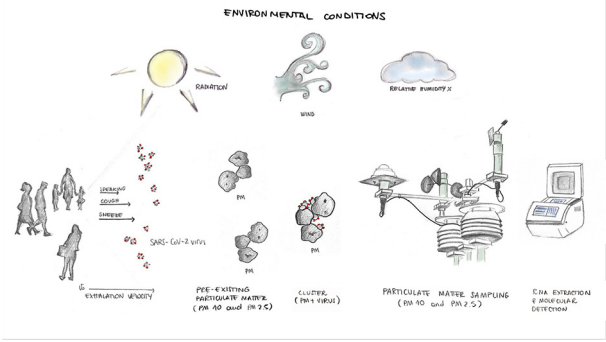
HIGHLIGHTS

- SARS-CoV-2 RNA was not detected in 44 outdoor PM samples.
- The probability of detecting SARS-CoV-2 RNA in airborne samples is considered low.
- Monitoring the presence of SARS-CoV-2 RNA on PMs does not represent an efficient early indicator of virus transmission.
- Monitoring SARS-CoV-2 RNA on PM does not represent an early indicator of the pandemic's recurrence.

GRAPHICAL ABSTRACT



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ABSTRACT

The airborne transmission of SARS-CoV-2, the etiologic agent of the current COVID-19 pandemic, has been hypothesized as one of the primary routes of transmission. Current data suggest a low probability of airborne transmission of the virus in open environments and a higher probability in closed ones, particularly in hospitals or quarantine facilities. However, the potential diffusion of the virus in open environments, especially using particulate matter (PM) as a transport carrier, generated concern in the exposed populations. Several authors found a correlation between the exceeding of the PM10 concentration limits in some Italian cities and the prevalence of Covid-19 cases detected in those areas. This study investigated the potential presence of SARS-COV-2 RNA on a representative series of PM samples collected in the province of Padua in Northeastern Italy during the first wave of COVID pandemic. Forty-four samples of PM2.5 and PM10 were collected between February 24 and March 9, 2020 and analyzed with RT-qPCR for SARS-CoV-2 RNA. The experimental results did not indicate the presence of SARS-CoV-2 RNA in the outdoor PMs, thus confirming the low probability of virus airborne transmission through PM.

1. Introduction

Airborne transmission has been recognized as one of the primary routes of conveyance of etiologic agents such as respiratory viruses, including the Severe Acute Respiratory Syndrome (SARS) and the Middle

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East Respiratory Syndrome coronaviruses (Booth et al., 2005; Yu et al., 2004; Tellier et al., 2019). SARS-CoV-2, the cause of the current COVID-19 pandemic, also falls into this category (Lewis, 2020; National Research Council, 2020; WHO, 2020; Prather et al., 2020). Recently, 239 scientists from 32 countries have written an open letter to the World Health Organization (WHO) emphasizing the importance of preventing its airborne transmission (Morawska and Milton, 2020).

Most credited SARS-CoV-2 transmission pathway is by respiratory droplets as small as 5 μm or larger, generated by sneezes, coughs, or breaths during normal speaking (Lewis, 2020; National Research Council, 2020; Yu et al., 2018; WHO, 2020). The airborne lifetime of the droplets and the range of transmission (e.g. more than 1 m) remains unclear (Anderson et al., 2020; Morawska and Cao, 2020). The mechanisms underlying the airborne transport of SARS-CoV-2 have not been fully elucidated. Also, the influence of the carrier typology (e.g., droplets and aerosols including particulate matter, PM), the role of environmental conditions (e.g., wind speed, temperature, humidity, UV radiations, seasonal allergens such as pollens and spores), and air pollutant concentrations, remain unclear.

A recent study suggests a low probability of airborne virus transmission in open environments and a higher one in closed ones, especially in hospitals or quarantine facilities (Contini and Costabile, 2020). However, the experimental evidence supporting the statement above is weak. It mainly focuses on aerosols and droplets produced by infected patients through coughing, sneezing, speaking, and breathing. The presence of SARS-CoV-2 in the aerosols sampled inside two Hospitals of Wuhan during pandemic peaks was observed by Liu et al. (2020). Santarpia et al. (2020) reported similar findings concerning 13 isolation rooms for COVID-19 patients in the Nebraska University Hospital. Frequent room ventilation and extended permanence in open spaces were also indicated as effective measures for reducing virus diffusion. Md Nor et al. (2020) assessed the presence of SARS-CoV-2 RNA on indoor PM2.5 in hospital wards with infected patients in Kuala Lumpur, Malaysia. In contrast with these findings, Faridi et al. (2020) detected the absence of SARS-CoV-2 in the air sampled in hospital rooms in a range of 2 to 5 m from the beds of symptomatic COVID-19 patients.

The concern about the diffusion of the virus in open environments, particularly using PMs as carriers, is still widespread in the population. Some studies (Cascetta et al., 2021; Coccia, 2020; Bontempi, 2020; Setti et al., 2020a) found a correlation between the exceeding of the PM10 concentration limits in some Italian cities and the number of Covid-19 cases. Despite this limited evidence and bearing in mind that

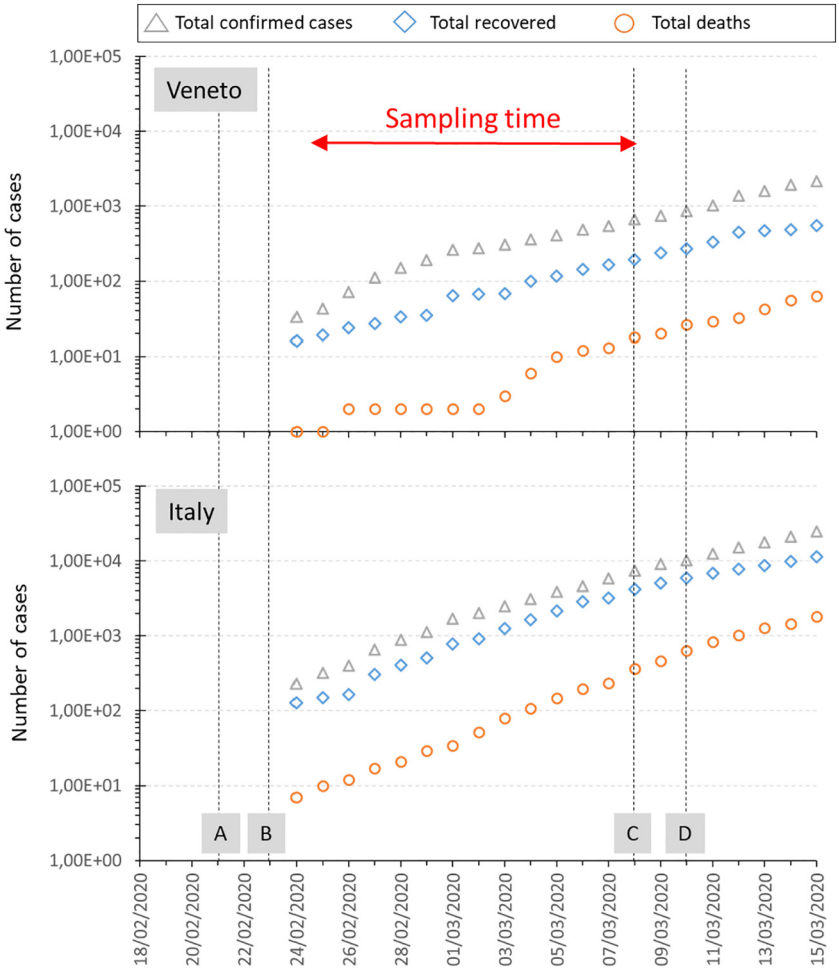

Fig. 1. The development of COVID-19 in Italy and the Veneto Region when the PM samples were collected. The graph was based on Gatto et al. (2020). Time marks (A, B, C, and D) represent the most critical epidemiological events and measures for both mobility and contact restrictions at each time point: A) On February 21, 2020 (day 1), "patient one" was officially confirmed as a case of COVID-19 by the "Ospedale Sacco" in Milan; by the end of the day, other 14 cases in Lombardy and 2 cases in Veneto were confirmed. B) On February 23, 2020 (day 3), evidence for local transmission from "patient one" increased and new cases of infections was discovered in the municipality of Vo' (Province of Padua). Ten municipalities in Lombardy and one in the Providence of Padua, identified as hotspots, were maintained under strict lockdown (i.e., labeled as critical red areas), while some preventive restrictions (e.g., temporary closure of schools and universities) were enforced in some regions. C) On March 8, 2020 (day 17), the whole of Lombardy and 14 Italian provinces (including the Province of Padua) were set under lockdown by the application of the Prime Ministerial Decree (DPCM) of August 03, 2020. Social distancing measures were implemented in the whole country. D) On March 10, 2020 (day 19), the lockdown area was extended to the whole nation by the application of the Prime Ministerial Decree (DPCM) of March 09, 2020; progressive restrictive limitations on mobility and social distance were also instituted.

correlation is not causation (Altman and Krzywinski, 2015), the cause-and-effect relationship between PM concentration and COVID-19 prevalence and symptom severity remains controversial (Anand et al., 2021).

In this context, PMs may act as physical carriers of the virus, as possible infection boosting factors (Comunian et al., 2020; Paital and Agrawal, 2020), or as a combination of both. These possibilities require further investigation and proper experimental studies.

Preliminary research on the relationship between PMs and virus transmission was carried out by Setti et al. (2020b). It reported a first preliminary detection of the presence of SARS-COV-2 RNA on the PM from examining 34 PM10 samples collected from an industrial site in the province of Bergamo in Northern Italy. On the other hand, other outdoor air samples were simultaneously collected in Venice in Northeastern Italy and Lecce in Southern Italy in May 2020 and they were tested negative for SARS-CoV-2 RNA (Chirizzi et al., 2021). In these works, the hypothesized mechanism is that virus-laden aerosol could interact with the pre-existing atmospheric particles creating clusters of carriers (Belosi et al., 2021).

Due to the contradictory results previously mentioned and the lack of studies on this topic, this project aims to further investigate the potential presence of SARS-CoV-2 RNA on a representative series of PM collected in the Province of Padua in Northeastern Italy, an area severely affected by the first wave of the COVID-19 pandemic. The methodological issues related to the extraction and detection of viral RNA are also analyzed and discussed.

2. Material and methods

2.1. Experimental design and sampling strategy

Since the initial spreading of the pandemic wave (February–March 2020), Italy has been recognized as one of the most affected countries. In response to the uncontrolled increase of COVID-19 cases (Fig. 1), the Italian government imposed several restrictions (lockdown, compulsory usage of sanitary masks, etc.). Finally, on May 17, 2020, the nationwide lockdown ended, and less strict measures were adopted locally.

PM sampling was performed in the Province of Padua (Fig. 2) with the frequency reported in Table 1 between February 24 and March 9, 2020, i.e. the two weeks before lockdown. The sampling sites are described in Table 2 and classified according to the European Directive 2008/50/EC. During the sampling period (14 days), the meteorological conditions were registered from dedicated stations installed directly in the PM samplers or from the closest stations (Table 1). Considering the collected samples, the average daily temperature was 7.9 °C (Standard Deviation, SD = 1.0); the average daily irradiation was 99.7 W/m^2 (SD = 62.6); the average daily wind density was 1.2 m/s (SD = 0.5). Precipitations were observed only for 14 samples.

PM (PM10, PM2.5) samples were collected on quartz fiber filters (47 mm Ø, Whatman QMA, GE Healthcare, USA) using the low-volume sampling setting according to the European standard EN 12341:2014 at a nominal flow of 2.3 m^3 h^{-1} for 24 h, starting at midnight. The filters have a retention efficiency higher than 99.95% for particles with an aerodynamic diameter of 0.3 μm. Before reaching the laboratory, the samples remained at the sampling station from 3 to 4 days in containers kept in the dark and at 20 °C. Then, the filters were conditioned for gravimetric analysis for 48 h in a chamber with constant temperature 20 ± 1 °C and relative humidity 50 ± 5% (Emerson S05KA Emerson Network Power, Italy). The filters were then weighed twice with an analytical balance with a sensitivity of 0.0001 mg (Sartorius series Genius, mod. SE2, Germany). The final weight was calculated as the average of the two measurements. Finally, the samples were frozen in clean Petri slides at −20 °C for the subsequent analysis. Laboratory testing was performed by the BSL-2 Research Laboratory of Hygiene and Applied Microbiology of the Padua University (Italy). The laboratory implements updated OECD Good Laboratory Practices and adopted fundamental precautions for the correct handling of RNA samples.

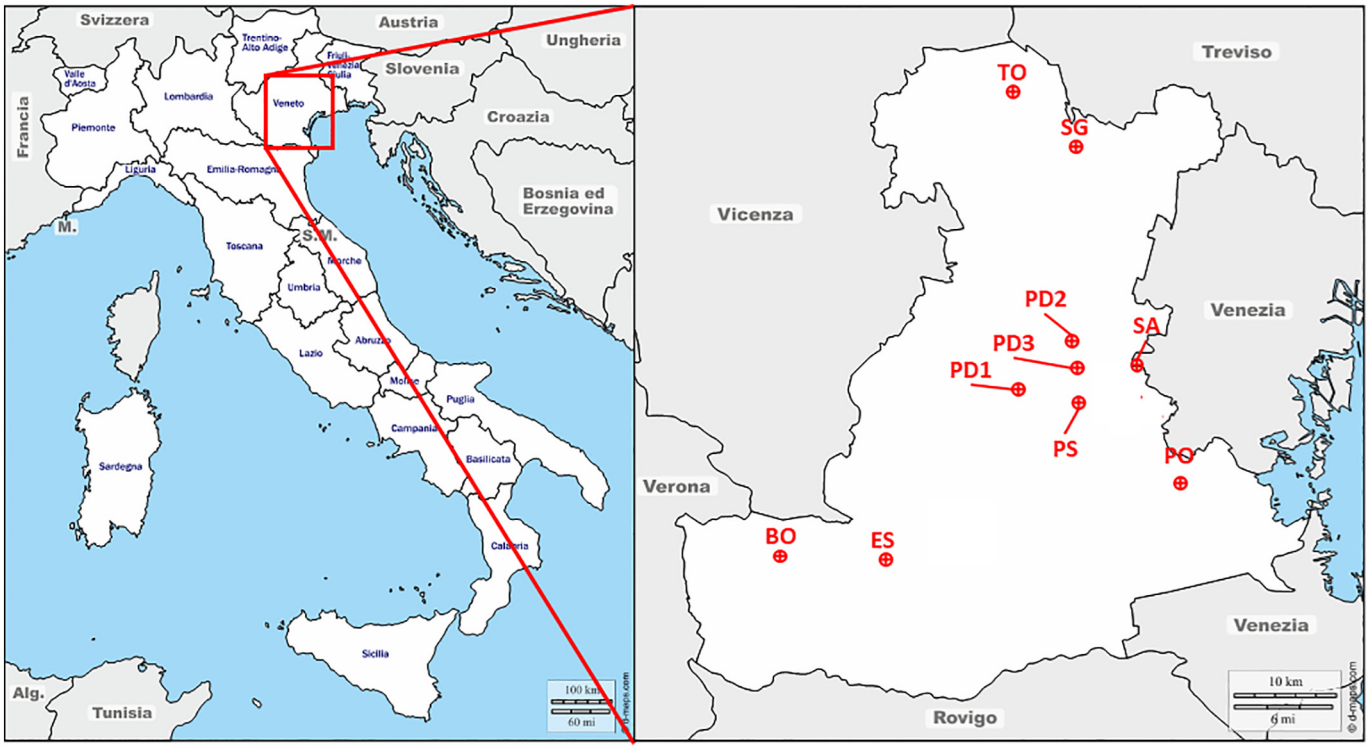

Fig. 2. Map of the area investigated in this study. The red dots indicate the locations of PM sampling in Padova Province. BO: Borgo Veneto-Piazza Della Vittoria; ES: Este-Via Stazie Bragadine; PD1: Padova-Mandria; PD2: Padova-Via Carli; PD3: Padova-Internato Ignoto; PS: Ponte San Nicolò-Via Garibaldi; SG: S. Giustina In Colle; SA: Saonara-Via Villanova; and TO: Tombolo (maps from: http://d-maps.com).

Table 1
Information of the PM samples: date of sampling; sample code (sample code used in the regional monitoring network); sampling site, including the referred codes used in Fig. 2; meteorological conditions; PM typology (PM2.5 or PM10); PM concentration.

N.	Date	Sample code	Sampling site	Meteorological conditions[a]	PM typology	Concentration ($\mu g/m^3$)
1	24/02/2020	734202	PONTE SAN NICOLO' - VIA GARIBALDI (PS)	P = 0.0 mm; W = 76.1 W/m^2; *T* = 6.6 °C; *I* = 1.0 m/s	PM 10	86
2	24/02/2020	734209	PIOVE DI SACCO - VIA LONGHENA (PO)	P = 0.0 mm; 76.1 W = W/m^2; *T* = 7.1 °C; *I* = 0.8 m/s	PM 10	94
3	24/02/2020	734218	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 65.0 W/m^2; *T* = 8.4 °C; *I* = 1.4 m/s	PM 10	68
4	24/02/2020	735504	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	*P* = 0.0 mm; W = 116.6 W/m^2; *T* = 8.7 °C; *I* = 1.2 m/s	PM2.5	74
5	25/02/2020	735430	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 55.0 W/m^2; *T* = 9.8 °C; *I* = 0.9 m/s	PM 10	91
6	26/02/2020	735506	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 139.5 W/m^2; *T* = 9.7 °C; *I* = 1.9 m/s	PM2.5	24
7	27/02/2020	735415	PIOVE DI SACCO - VIA LONGHENA (PO)	*P* = 10 mm; W = 130 W/m^2; *T* = 6.8 °C; *I* = 1.1 m/s	PM 10	21
8	27/02/2020	735432	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 132.3 W/m^2; *T* = 7.5 °C; *I* = 1.6 m/s	PM 10	24
9	27/02/2020	735700	PONTE SAN NICOLO' - VIA GARIBALDI (PS)	P = 0.0 mm; W = 130.0 W/m^2; *T* = 7.3 °C; I = 1.2 m/s	PM 10	17
10	27/02/2020	735507	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 143.1 W/m^2; T = 7.3 °C; *I* = 1.0 m/s	PM2.5	18
11	28/02/2020	735508	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 185.0 W/m^2; *T* = 9.4 °C; *I* = 1.6 m/s	PM2.5	13
12	29/02/2020	735434	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 87.8 W/m^2; T = 7.3 °C; I = 1.0 m/s	PM 10	37
13	29/02/2020	735509	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 84.3 W/m^2; *T* = 8.2 °C; *I* = 1.3 m/s	PM2.5	29
14	01/03/2020	735411	SAONARA - VIA VILLANOVA (SA)	*P* = 15 mm; W = 17.3 W/m^2; *T* = 7.6 °C; I = 1.4 m/s	PM 10	25
15	01/03/2020	735418	PIOVE DI SACCO - VIA LONGHENA (PO)	*P* = 14 mm; W = 6.0 W/m^2; *T* = 6.4 °C; I = 1.3 m/s	PM 10	29
16	01/03/2020	735445	TOMBOLO (TO)	*P* = 15.0 mm; W = 17.3 W/m^2; T = 7.6 °C; I = 1.4 m/s	PM 10	20
17	01/03/2020	735703	PONTE SAN NICOLO' - VIA GARIBALDI (PS)	*P* = 2.0 mm; W = 6.0 W/m^2; *T* = 6.7 °C; I = 1.1 m/s	PM 10	24
18	01/03/2020	735446	TOMBOLO (TO)	P = 15.0 mm; W = 17.3 W/m^2; T = 7.6 °C; I = 1.4 m/s	PM2.5	18
19	01/03/2020	735467	PADOVA - MANDRIA (PD1)	P = 2.0 mm; W = 6.0 W/m^2; T = 6.7 °C; I = 1.1 m/s	PM2.5	23
20	01/03/2020	735472	PADOVA - VIA CARLI (PD2)	P = 2.0 mm; W = 6.0 W/m^2; T = 6.7 °C; I = 1.0 m/s	PM2.5	20
21	01/03/2020	735477	PADOVA - INTERNATO IGNOTO (PD3)	P = 2.0 mm; W = 6.0 W/m^2; T = 6.7 °C; I = 1.2 m/s	PM2.5	16
22	01/03/2020	735510	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	*P* = 1.0 mm; W = 19.3 W/m^2; T = 7.3 °C; *I* = 2.0 m/s	PM2.5	19
23	02/03/2020	735436	S.GIUSTINA IN COLLE (SG)	*P* = 22.0 mm; W = 20.9 W/m^2; T = 6.8 °C; *I* = 3.3 m/s	PM 10	28
24	02/03/2020	736758	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 23.7 W/m^2; T = 6.7 °C; *I* = 2.3 m/s	PM2.5	27
25	03/03/2020	736175	S.GIUSTINA IN COLLE (SG)	*P* = 4.0 mm; W = 62.4 W/m^2; *T* = 9.0 °C; I = 1.6 m/s	PM 10	16
26	03/03/2020	736759	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 50.1 W/m^2; *T* = 8.1 °C; I = 1.1 m/s	PM2.5	13
27	04/03/2020	735706	PONTE SAN NICOLO' - VIA GARIBALDI (PS)	P = 0.0 mm; W = 127.4 W/m^2; T = 7.1 °C; *I* = 0.7 m/s	PM 10	17
28	04/03/2020	736183	PIOVE DI SACCO - VIA LONGHENA (PO)	P = 0.0 mm; W = 127.4 W/m^2; *T* = 7.9 °C; *I* = 1.1 m/s	PM 10	18
29	04/03/2020	736760	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 111.3 W/m^2; T = 8.4 °C; I = 0.9 m/s	PM2.5	12
30	05/03/2020	736177	S.GIUSTINA IN COLLE (SG)	*P* = 5.0 mm; W = 84.2 W/m^2; *T* = 6.9 °C; I = 1.1 m/s	PM 10	27
31	05/03/2020	736761	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 101.8 W/m^2; T = 6.9 °C; I = 1.3 m/s	PM2.5	21
32	06/03/2020	736178	S.GIUSTINA IN COLLE (SG)	*P* = 13.0 mm; W = 142.7 W/m^2; *T* = 8.5 °C; I = 2.0 m/s	PM 10	14
33	06/03/2020	736762	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 174.8 W/m^2; *T* = 7.8 °C; *I* = 2.2 m/s	PM2.5	18
34	07/03/2020	736186	PIOVE DI SACCO - VIA LONGHENA (PO)	P = 0.0 mm; W = 133.0 W/m^2; *T* = 9.5 °C; I = 1.1 m/s	PM10	23
35	07/03/2020	736763	BORGO VENETO - PIAZZA DELLA VITTORIA (BO)	P = 0.0 mm; W = 162.5 W/m^2; *T* = 10.2 °C; I = 1.3 m/s	PM2.5	23
36	08/03/2020	736083	ESTE - VIA STAZIE BRAGADINE (ES)	P = 0.0 mm; W = 189.3 W/m^2; T = 9.7 °C; *I* = 0.5 m/s	PM 10	13
37	08/03/2020	736108	PADOVA - MANDRIA (PD1)	P = 0.0 mm; W = 176.0 W/m^2; T = 8.4 °C; I = 0.8 m/s	PM 10	13
38	08/03/2020	736118	PADOVA - INTERNATO IGNOTO (PD3)	P = 0.0 mm; W = 176.0 W/m^2; T = 8.4 °C; *I* = 0.8 m/s	PM 10	14
39	08/03/2020	736127	PADOVA - VIA CARLI (PD2)	P = 0.0 mm; W = 176.0 W/m^2; T = 8.4 °C; I = 0.8 m/s	PM 10	13
40	08/03/2020	736180	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 170.9 W/m^2; T = 9.0 °C; I = 1.2 m/s	PM 10	15
41	08/03/2020	736109	PADOVA - MANDRIA (PD1)	P = 0.0 mm; W = 176.0 W/m^2; *T* = 8.4 °C; I = 0.8 m/s	PM2.5	11
42	08/03/2020	736119	PADOVA - INTERNATO IGNOTO (PD3)	P = 0.0 mm; W = 176.0 W/m^2; T = 8.4 °C; I = 0.8 m/s	PM2.5	12
43	08/03/2020	736128	PADOVA - VIA CARLI (PD2)	P = 0.0 mm; W = 176.0 W/m^2; T = 8.4 °C; I = 0.8 m/s	PM2.5	10
44	09/03/2020	736181	S.GIUSTINA IN COLLE (SG)	P = 0.0 mm; W = 125.0 W/m^2; *T* = 8.0 °C; I = 0.8 m/s	PM 10	39

[a] Meteorological conditions were registered from dedicated stations installed directly in the PM samplers or from the closest stations. The data are average daily measures of precipitation (P) in mm, solar irradiation (W) in W/m^2, temperature (T) in °C and wind intensity (I) in m/s.

2.2. PM recovery from filters and preliminary sample processing

Recovery of PM from quartz fiber filters reprised the procedure described by Roper et al. (2015). Filters were placed with the PM face down in 100 mL glass beakers containing 5 mL of a 9:1 methanol/sterile distilled water solvent. Beakers were then sonicated for 2 min in a water bath sonicator at 50 KHz (Labsonic LBS1, Falc Instruments, Italy). Reported PM removal efficiency following sonication is of 98.0 ± 1.4%. The solvent was then collected in a 15 mL Falcon® conical centrifuge tube and the filter was sonicated again, repeating the described step.

Table 2
Description of the sample sites.

Code	Place	Geographical coordinates[a]	Type of station	Population density per km^2[b]
BO	Borgo Veneto - Piazza Della Vittoria	X: 1698916; Y:5011095	Urban backround	178
ES	Este - Via Stazie Bragadine	X: 1709338; Y:5011647	Industrial (suburban)	505
PD1	Padova - Mandria	X: 1722487; Y: 5028105	Urban backround	2216
PD2	Padova - Via Carli	X: 1727511; Y: 5033159	Industrial (Urban)	2216
PD3	Padova - Internato Ignoto	X: 1726463; Y: 5053899	Industrial (Urban)	2216
PO	Piove Di Sacco - Via Longhena	X: 1738075; Y: 5019476	Urban backround	534
PS	Ponte San Nicolò - Via Garibaldi	X: 1728628; Y: 5027768	Urban backround	979
SA	Saonara - Via Villanova	X: 1732825; Y: 5030147	Urban backround	742
SG	S. Giustina In Colle	X: 1726463; Y: 5053899	Rural background	397
TO	Tombolo	X: 1719591; Y: 5057855	Industrial (suburban)	747

[a] Reference coordinates: Gauss Boaga West Corner.
[b] Density of the Municipality as reported in ISTAT (2021).

After the second round of sonication, both filter and beaker were rinsed with 5 mL of clean solvent, that ultimately was also collected in the same 15 mL tube. Falcon tubes were then centrifuged at 5500*g* for 15 min (refrigerated centrifuge Allegra 21R, Beckman Coulter, California, USA) to separate solids from the liquid solvent. Subsequently, the two phases were processed in parallel to detect viral particles both complexed to the pellet PM or still suspended in the supernatant. As a matter of fact, if taking into account the hydrophilic nature of PM (Jiang et al., 2019), the liquid phases may in principle contain smaller and disaggregated PM particles and possibly non-complexed viruses. The pellet directly underwent RNA extraction, whereas the supernatant had to be concentrated prior to extraction. The supernatant was carefully removed from the tube and transferred into a concentration device (Amicon Ultra-15,100 KDa centrifugal filter, Merck-Millipore, Germany). It was then centrifuged at 2000*g* for 4 min (refrigerated swinging-bucket centrifuge PK131R, ALC, Italy), yielding a final volume of about 200 μL. Retention efficiency for particles with molecular weight similar to the SARS-CoV-2 virion is of >90%, as per manufacturer's specifications.

2.3. RNA extraction and molecular detection

RNA extraction from concentrated supernatant was carried out on a volume of 140 μL with a commercial kit (QIAamp viral RNA mini kit, Qiagen, Germany), following the manufacturer's instructions. RNA extraction from the pellet was performed with the same total RNA extraction kit (QuickRNA™ Fecal/Soil Microprep Kit R2040, Zymo Research, USA) used by Setti et al. (2020b). RNA extraction efficiency for both kits, in terms of RNA yield, is reported to be >90% by the respective manufacturers. The pellet was resuspended in 600 μL of the kit RNA lysis buffer. It was then transferred into the provided 2 mL bashing beads tube and thoroughly vortexed for 60 s. Apart from these minor modifications, extraction proceeded according to the manufacturer's protocol. An Internal Positive Control (IPC), i.e. 3 μL (9×10^4 gc/μL) of synthetic SARS-CoV-2 armored (i.e. encapsidated) RNA (2019-nCoV E gene aRNA kit cod. 001B-03886, EVAg-Protisvalor, France), was added to each sample before extraction as process indicator. IPC was also used to assess the presence of inhibitors of the quantitative reverse-transcription polymerase chain reaction (RT-qPCR).

Two WHO-shared One-step RT-qPCR assays were chosen for the molecular detection of SARS-Cov-2 RNA, targeting genes N (screening) and ORF1b-nsp14 (confirm) (Chu et al., 2020). Primers and dual-labeled probes were provided from Thermo Fisher (USA). Synthetic dsDNA fragments were used as positive controls and were also purchased by GeneArt/Thermo Fisher. In each PCR run, 2 replicates were loaded for each extract. Moreover, 2 positive and 2 negative controls were included. Amplification of the IPC was carried out with the dedicated assay (i.e. primers and dual-labeled probe), also provided with the aRNA kit, following the manufacturer's instructions. PCR runs were carried out on a StepOne-Plus™ Real-Time PCR System (Applied Biosystems, USA). Positivity was attributed only to reactions with cycle threshold (Ct) <40. The limit of detection (LOD) of the implemented assays was determined using DNA plasmids as positive standards and found to be below 10 genome copies (gc) per reaction (i.e. sample volume/well = 4 μL), that is. 2.5 gc/μL. Nevertheless, some authors suggested a possible differential performance between the N

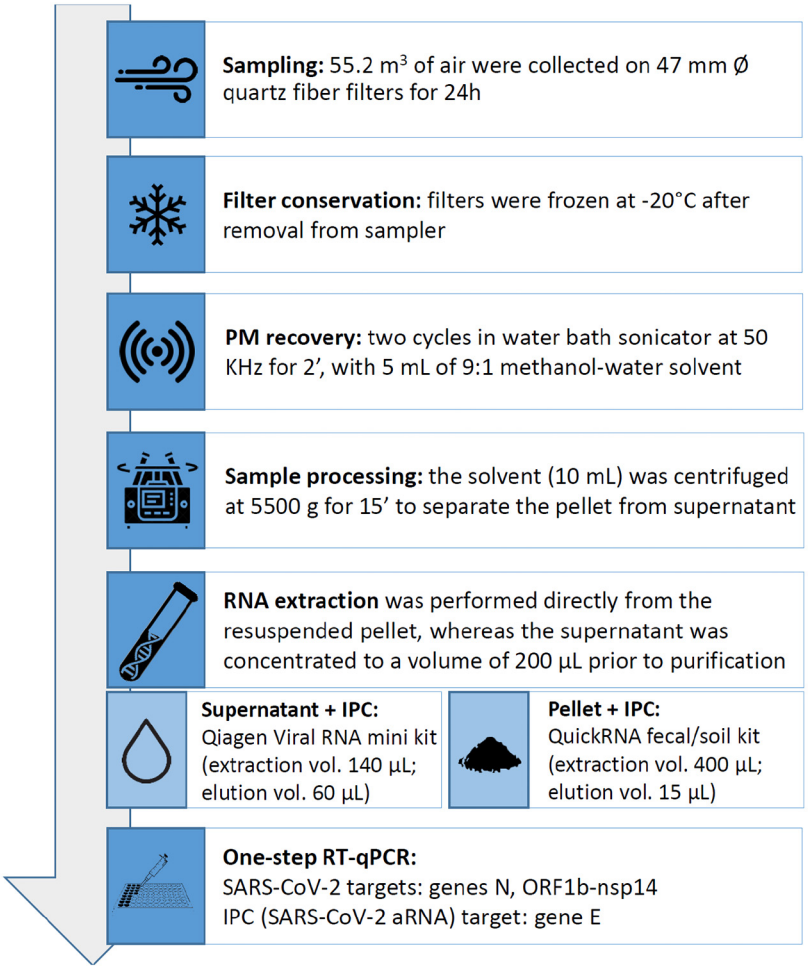

Fig. 3. Flow chart of the process of RNA extraction and molecular detection.

and the Orf1b assay, with N showing a 10× sensitivity in both clinical and environmental samples (Chu et al., 2020; Baldovin et al., 2020). The above described processes are graphically represented in the flow chart of Fig. 3.

3. Results and discussion

RNA extraction and molecular detection of SARS-CoV-2 nucleic acid was carried out on 88 individual samples (i.e. 44 paired pellets and supernatants). No successful amplification of the N gene nor the ORF1b-nsp14 was detected in any of the tested samples. However, IPC amplification was achieved for all samples, thus excluding the presence of PCR inhibitors. Moreover, an average delta of 3 cycles was observed for the Ct of IPC in supernatant vs pellet extracts, suggesting a better extraction power for the commercial kit used for the liquid phase. As reference, in a 100% efficient PCR reaction, a ten-fold dilution of the target gene should fall 3.3 cycles apart.

The experimental results suggest that SARS-CoV-2 RNA is not present, or either that the viral load falls below the detectability threshold (1.2 gc/m^3), in any of the 44 samples of PM10 and PM2.5 collected from February the 24th and March the 3rd 2020 in Padua province during the first pandemic wave. The average detectability threshold was calculated taking into account the LOD of molecular assays (i.e. 2.5 gc/μL), the recovery efficiency of each analytical procedure (i.e. PM removal from filters 98%, concentration 90% and RNA extraction 90%) and total air volume sampled over 24 h (55.2 m^3). A higher threshold (i.e. no detectable concentration < 3 gc/m^3) has been described by Belosi et al. (2021), whereas Chirizzi et al. (2021) reported a lower one (0.8 gc/m^3), but presumably the latter can be explained by a stated higher LOD for their molecular assay (i.e. 10 gc/μL = 50 gc/reaction). Setti et al. (2020b) did not report a detectability threshold, but an inferred value (1.5 gc/m^3) is proposed in Table 3.

These results agree with Chirizzi et al. (2021), who reported the absence of SARS-CoV-2 RNA in any of the 60 collected air samples. On the other hand, these results contradict the finding by Setti et al. (2020b) that reported 20 positive results for at least one of the three SARS-CoV-2 marker genes in 34 samples even if, due to the lack of enough PM materials, the simultaneous positivity for all the 3 markers was not demonstrated. A comparative analysis of the current and cited investigations is reported in Table 3.

Overall, based on the scientific evidence of this and of other studies, we are reasonably convinced of the low probability of detecting SARS-CoV-2 RNA in airborne samples. This depends on the occurrence of various events such as: a) the probability that the virus-laden aerosol

Table 3
Comparison from the current study and the ones of Chirizzi et al. (2021) and Setti et al. (2020b).

Operative conditions	Current study	Chirizzi et al. (2021)	Setti et al. (2020b)
Sampling size	44 samples from 10 sites	60 samples from 2 sites	34 samples from 2 sites
Positive samples	0 samples	0 samples	20 samples
Period of sampling	From February 24th to March 9th 2020 (14 days)	From 13th to 27th of May 2020 (14 days)	From February 21st to March 13th 2020 (21 days)
Location of sampling	Padua province (NE Italy)	two different Italian regions: Veneto (NE Italy) and Apulia (in the SE Italy)	Bergamo Province (North Italy)
Typology of sampling point	Different: urban and rural background sites; traffic and industrial sites	Urban background site	Industrial site
Filter used	Quartz fiber filters (47 mm Ø, Whatman QMA, GE Healthcare, USA)	Quartz fiber filters	Quartz fiber filters
Sampler typology	Two different samplers were used: Low-volume aerosol sampler (Skypost PM-TCR Tecora) equipped with a sequential sampler (Charlie) that operates at flow rate of 38.3 L min^{-1} for 24 h; it was used for the following sites: BO, TO, ES, PO, SA. Low volume aerosol sampler (SWAM 5a Dual Channel Monitor-FAI Instruments) that operates at flow rate of 38,3 L min^{-1} for 24 h; it was used for the following sites: PD1, PD2, PD3, SG.	Two different samplers were used per site: In Veneto: low volume aerosol sampler (Skypost PM-TCR Tecora) equipped with a sequential sampler (Charlie) that operates at flow rate of 38.3 L min^{-1} for 48 h; model 110 MOUDI cascade impactor with an average flow of 30 L min^{-1} for about 6 d. In Apulia: low volume aerosol sampler (SWAM 5a Dual Channel Monitor-FAI Instruments) that operates at flow rate of 38 L min^{-1} for 48 h; a rotating model 120 MOUDI-II™ cascade impactor, operating at 30 L min^{-1} for about 6 d.	Low-volume gravimetric air sampler (38.3 L/min for 24 h)
Average air collected per sample	55.2 m^3	110 m^3 or 250 m^3	55.2 m^3
Particulate matter retention	>99.95% for particles with an aerodynamic diameter of 0.3 μm	Not reported	99.9%
Sampling procedure	EN 12341:2014	Not reported	EN 12341:2014
Meteorological conditions	Temperature; irradiation; precipitation; wind intensity	Temperature; relative humidity; precipitation	Temperature; relative humidity; irradiance
Solid phase extraction	Quick-RNA™ Fecal/Soil Microbe Microprep Kit, Zymo Research, USA	Total RNA Purification Kit, Norgen Biotek Corp.	Quick-RNA™ Fecal/Soil Microbe Microprep Kit, Zymo Research, USA
Internal Positive Control	SARS-CoV-2 (E gene) aRNA, EVA, Marseille, France.	Not reported	Not reported
RT-PCR reference protocol	Chu et al., 2020	Corman et al., 2020	Corman et al., 2020
RT-PCR oligos	Custom oligos, Thermofisher	Diatheva commercial kit	Not reported
RT-PCR molecular targets	Genes N and Orf1b-14nsp	Genes RdRp and E	Genes E, RdRP and N
Limit of Detection	2.5 gc/μL	10 gc/μL	Not reported
Detection threshold	1.2 gc m^{-3}	<0.8 gc m^{-3}	1.5 gc m^{-3}[a]

[a] Detection threshold for the method of Setti et al. (2020b) was calculated assuming a LOD for their molecular assay of 2 gc/μL (Corman et al., 2020) and that the RNA extraction protocol strictly followed the kit manufacturer's instructions, with a 90% purification efficiency.

emitted outside may impact pre-existing particulates to form a cluster or a complex; b) the probability that the RNA structure of the virus may remain intact (nucleic acid persistence) during and after the formation of the cluster, the sampling procedure, the transport and storage phase until the molecular analysis is performed.

Regarding the first aspect, Belosi et al. (2021) estimated a very low average outdoor concentration of SARS-CoV-2 RNA, at less than 1 RNA gc/m^3, in the uncrowded public areas in the Lombardy Region, even in the worst-case scenario with an infection rate of up to 25% of the local population. These results are comparable with those found experimentally in an outdoor residential area in Wuhan, China, during the COVID pandemic (Liu et al., 2020).

Regarding the second aspect, the considerable atmospheric residence time (days to weeks) of PM before sampling dominates the nucleic acid persistence because, in this period, the cluster of particulate and virus could be primarily influenced by meteorological parameters, such as UV radiation, temperature, and oxidizing agents like NO_X and ozone. This scenario is particularly relevant in the Province of Padua, which is characterized by low wind speed accompanied by long periods of stable conditions with shallow mixing layers, especially during the winter period. Therefore, it is also unlikely that the virus will stay viable in these conditions.

Moreover, considering other parameters, such as SARS-CoV-2's viability, infectivity, and infective dose, which remain unclear (Barakat et al., 2020), it can be concluded that the outdoor airborne transmission is much less probable than the indoor route.

In conclusion, based on the experimental results and the above-reported observations, we believe that monitoring for the presence of SARS-CoV-2 RNA in outdoor particulates is not suitable for an efficient early indicator of SARS-Cov-2 diffusion or/and an early indicator of a recurrence of the pandemic.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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