MEASUREMENTS OF SARS-COV-2 RNA CONCENTRATIONS IN INDOOR AND OUTDOOR AIR IN ITALY: IMPLICATIONS FOR THE ROLE OF AIRBORNE TRANSMISSION

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DEFINITION OF THE PROBLEM

The recent pandemic due to the spread of the SARS-CoV-2 virus has shown significant differences in the mortality and spread of the virus when comparing different geographic areas or different regions of the same Country.

This has led to some questions about **the role and weight of coronavirus airborne transmission** and **whether this transmission mechanism can explain the differences observed, for example, in the different Italian regions**.

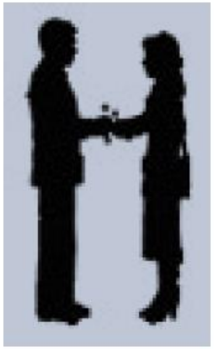
The role of airborne transmission is the subject of debate in the scientific community and the parameters that mainly influence it are still little known:

- **the actual concentrations of virus-laden particles in air;**
- the size distribution of virus-laden particles in air;
- the fraction of virus in air that is viable and its life-time;
- the minimum threshold of viral copies necessary to infect an individual;
- effects of meteorological parameters (temperature, humidity, solar radiation).

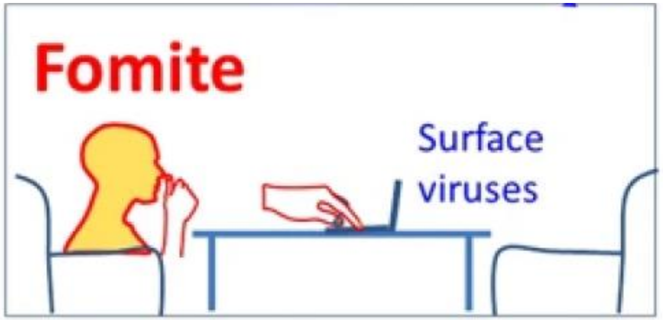
It is also necessary to distinguish between outdoor and indoor as the dynamics of the bioaerosol can be very different.

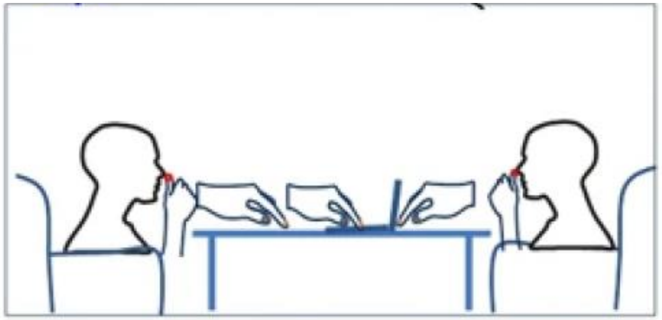
MECHANISMS OF TRANSMISSION

Once an individual has been infected it becomes extremely difficult, if not impossible, to determine the exact mechanism of contagion.



direct contact



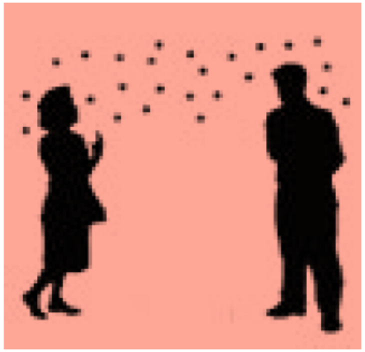


Indirect contact (via fomites)

Close contact

large droplets > 5 µm

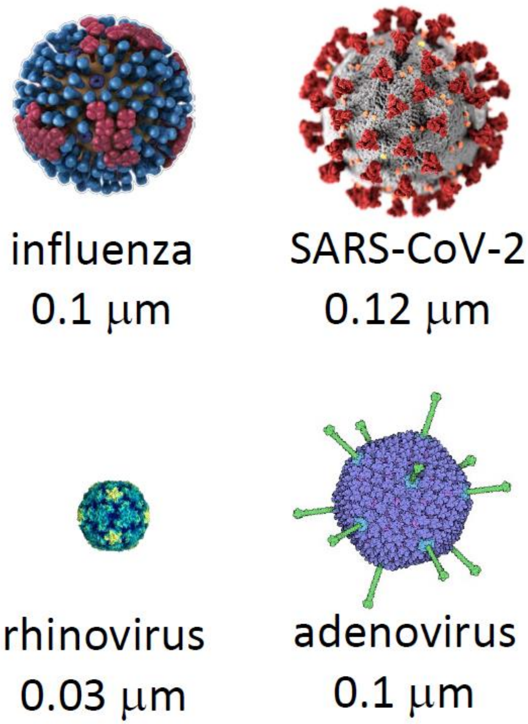
Distance transmission



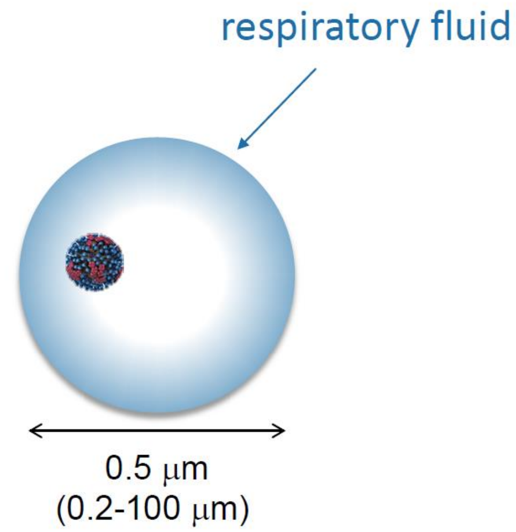
aerosols < 5 µm

Two potential airborne transmission mechanisms. The distinction at 5 µm is conventional.

VIRUS SARS-COV-2 (I)



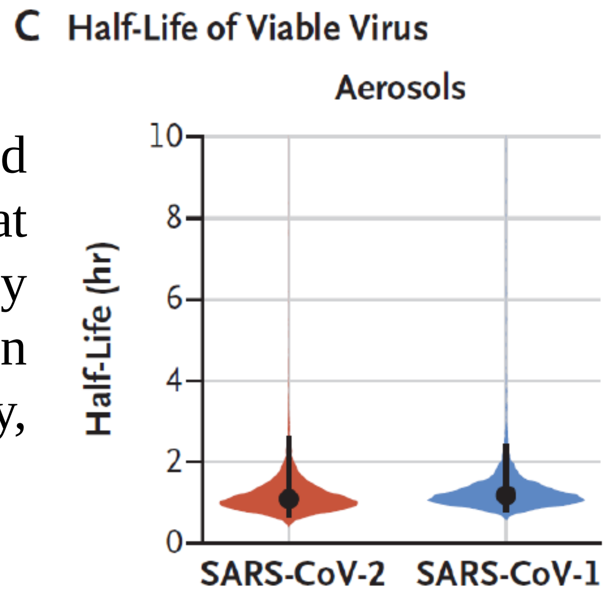
Virus «nude» and virus contained in a droplet of respiratory fluid

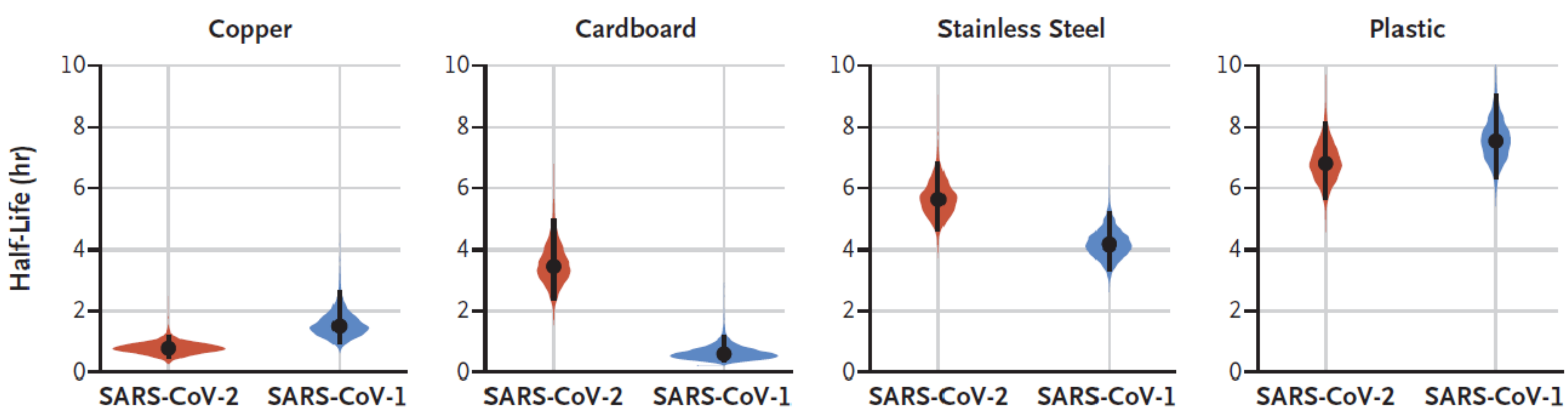


The virus is accompanied by the respiratory fluid and the droplets, once emitted, will undergo deposition, transport and transformation processes including **the total or partial evaporation of the respiratory fluid leaving residues (also called droplet nuclei)** of virions, salts and proteins of very different sizes compared to the original droplets.

VIRUS SARS-COV-2 (II)

Detections in controlled laboratory conditions, that can be significantly different from the ones in indoor and, especially, outdoor environments.

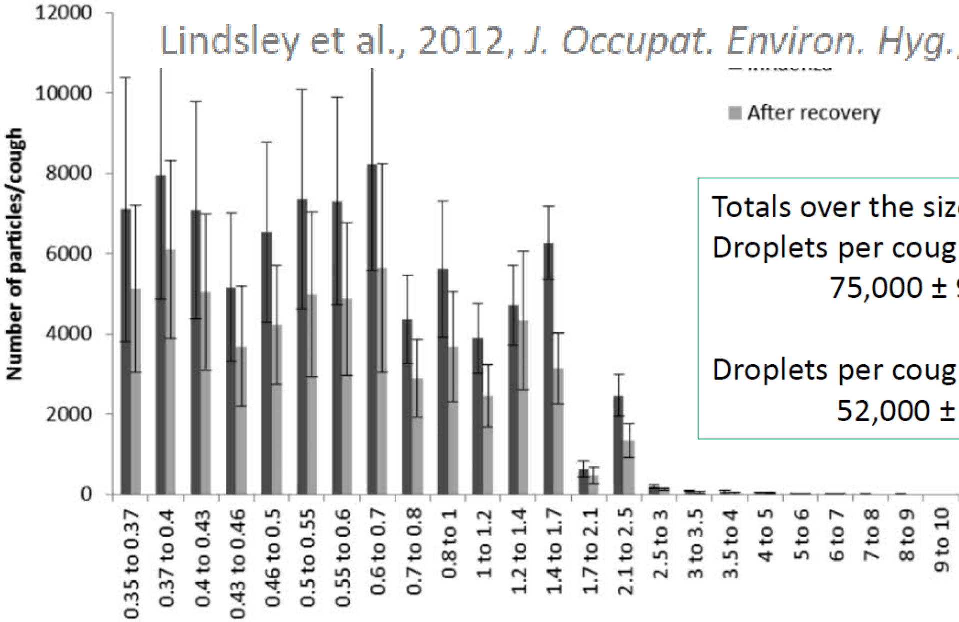




van Doremalen et al., 2020, *NEJM*, https://www.nejm.org/doi/full/10.1056/NEJMc2004973

EMISSIONS (I)

Lindsley et al., 2012, *J. Occupat. Environ. Hyg.*, https://www.ncbi.nlm.nih.gov/pubmed/22651099

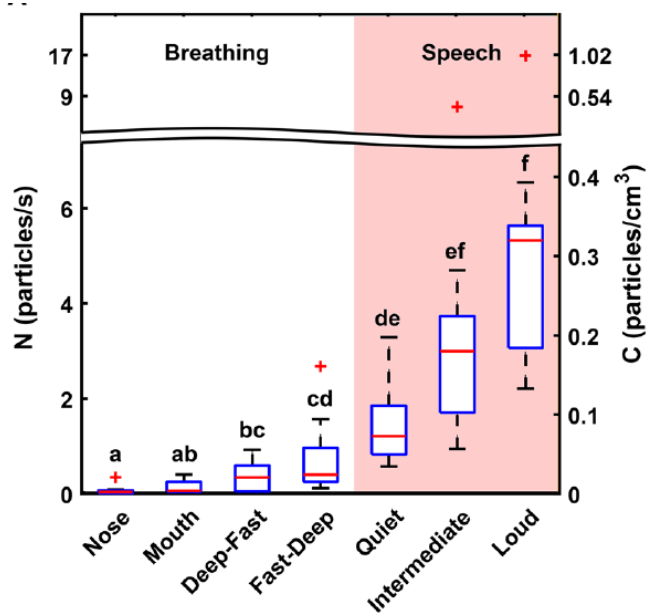


Totals over the size range 0.35-10 µm:
Droplets per cough when sick
75,000 ± 97,000

Droplets per cough after recovery
52,000 ± 99,000

Droplets are released during respiration, speaking, coughing, and sneezing.

Coughing and sneezing mainly concern the symptomatic infected.

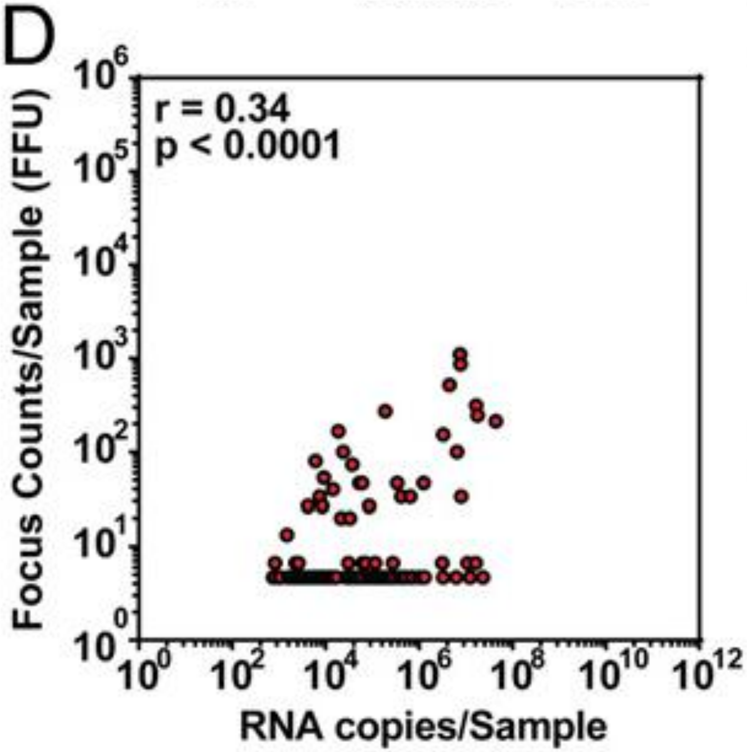


Droplet emissions were also observed during normal breathing and speech (**therefore emissions possible even from asymptomatic infected**). Asadi et al., Scientific Reports *(2019) 9:2348* | https://doi.org/10.1038/s41598-019-38808-z

Milton et al., 2013, *PLoS Pathogens*, https://www.ncbi.nlm.nih.gov/pubmed/23505369

A fraction of the respiratory particles may contain viruses, according to data available also for other viruses.

A fraction of these may be viable (i.e. infectious) as happens for other viruses.



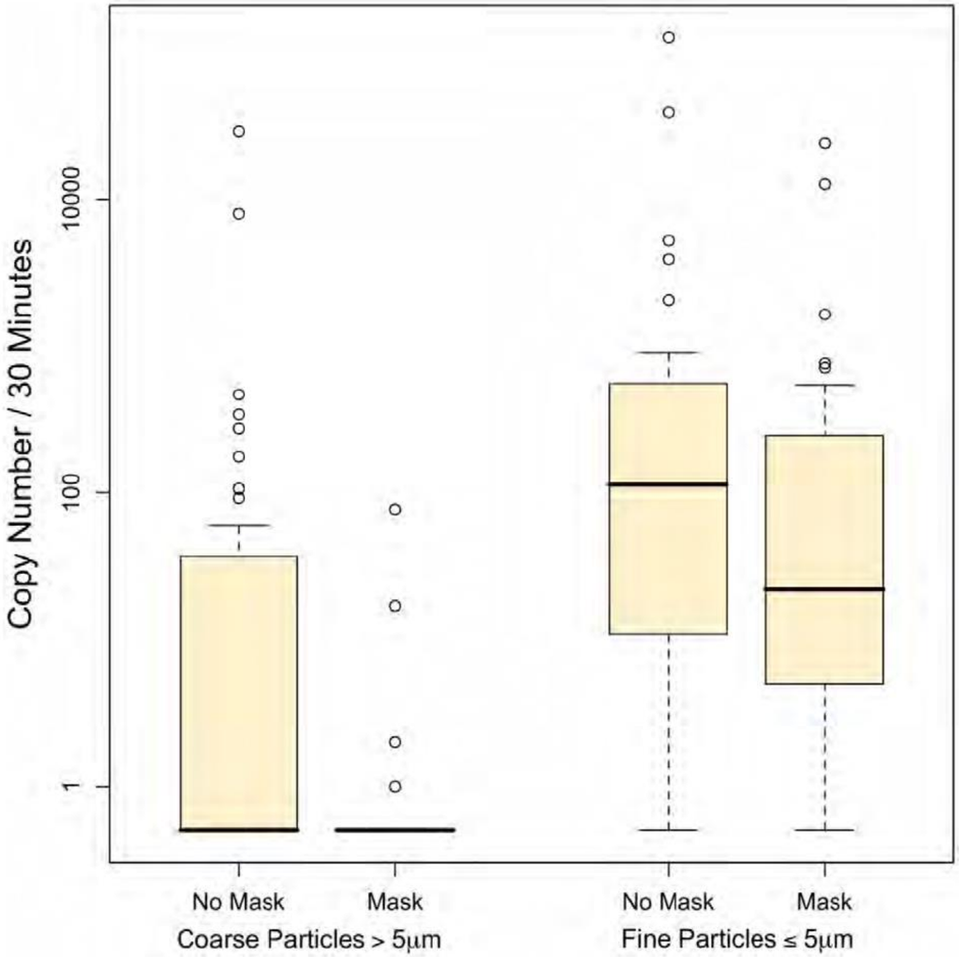


Figure 1. Influenza virus copy number in aerosol particles exhaled by patients with and without wearing of an ear-loop surgical mask. Counts below the limit of detection are represented as 0.5 on the log scale.
doi:10.1371/journal.ppat.1003205.g001

Yan et al., 2018, *PNAS*, https://www.ncbi.nlm.nih.gov/pubmed/29348203

OUTDOOR MEASUREMENTS (I)

The most important parameter to be determined, for evaluation of risk of airborne transmission,n is concentration of virus-laden particles.

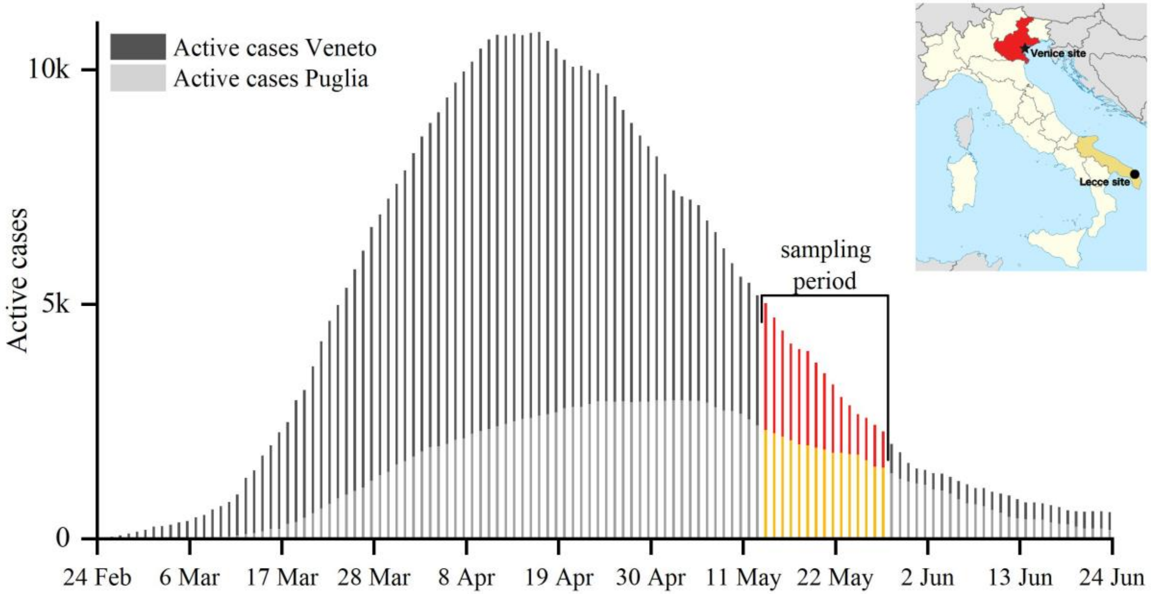
The AIR-CoV collaboration project focuses on experimental measurements of concentrations of SARS-CoV-2 RNA in air samples in outdoor and indoor environments in different towns in Italy.

A fraction of the respiratory particles may contain viruses, according to data available also for other viruses. A fraction of these may be viable (infectious). Outdoor samples were collected in May 2020 during the first wave of pandemic in simultaneously in Venice (North of Italy, strongly hit by Covid-19) and Lecce (South of Italy less hit during the first wave of pandemic).

Both sequential PM10 samplers (samples over 48h, each of about 110 m^3) and MOUDII multistage impactors capable of selecting atmospheric particulate matter in 12 dimensional classes covering from nanoparticles (D <0.056 µm) to the fraction were used in parallel in the two sites. coarse (D> 18 µm). The size-segregated samples were collected for 6 days, each approximately 250 m^3).

The collected samples were analyzed to detect and quantify the genetic material (RNA) of SARS-CoV-2 using two different approaches: **real-time RT-PCR** and **Droplet Digital dd-PCR** more sensitive to low concentrations. Details are available on Chirizzi et al. 2021 (Environment International 146, 106255).

OUTDOOR MEASUREMENTS (II)



While the virus was still circulating, the following samples were collected:

- 6 PM_{10} samples at each site;
- 24 impactor samples at each site.

All samples analyzed tested negative with viral RNA concentrations below the detection limits for both measurement sites:

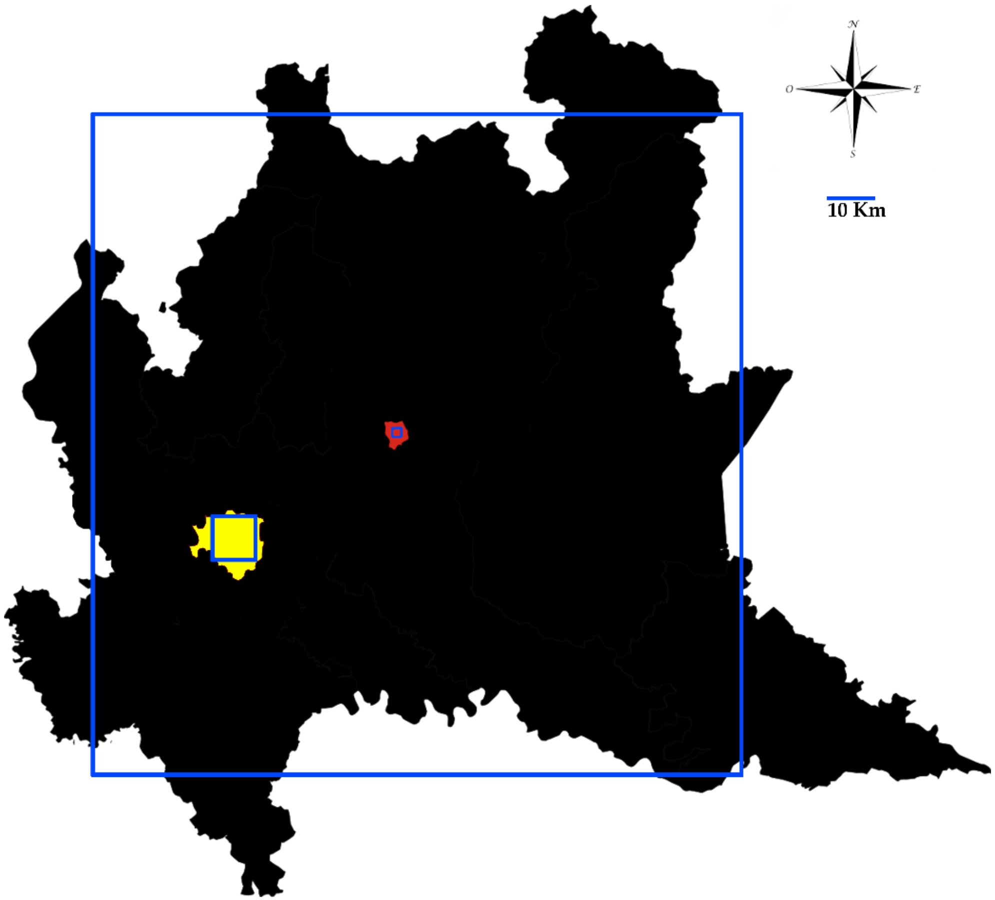
- SARS-CoV-2 **in PM_{10} < 0.8 copies m^{-3}**.
- SARS-CoV-2 < **0.4 copies m^{-3}** for each size interval from nanoparticles up to coarse particles.

Recovery was measured at about 49% and considered in evaluation of the indicated thresholds.

COMPARISON WITH OTHER MEASUREMENTS

Reference	Sites	Sampling	Method	Results	Notes
Liu et al. (2020) Wuhan (China) Nature 582, 557–560	Different sites near hospital, community check point, department stores and supermarket and residential buildings.	TSP sampled on gelatine substrate at 5 L min-1. Volumes 1.5-5 m^3.	dd-PCR targeting Orf1ab and N genes.	3/8 (37%) of samples positive collected near hospital and near the door of a busy department store.	Outdoor concentration non-detectable or very low (<3 copies m^{-3}) with the exclusion of crowded sites that arrived at 11 copies m^{-3}. Recovery not reported.
Setti et al. (2020) Italy Environ. Res. 188, 109754	Industrial area of Bergamo over a continuous 3-week period 21/02/2020-13/03/2020.	34 PM_{10} samples on quartz fiber filters (24h at 38.3 L min^{-1}). Volume 55.2 m^3.	RT-PCR targeting E, RdRp, and N genes.	58.8% of samples (20/34) positive for one gene. 11.8% (4/34) positive for 2 genes. None positive for 3 genes.	Concentrations not reported. LOD not reported. Recovery not reported.
Chirizzi et al. (2021) Italy – this study **Environ. Int. 146, 106255**	**Two urban background sites: Veneto (Venice, North Italy) and Apulia (Lecce, South Italy) simultaneously studied, period 13/05/2020-27/05/2020.**	**At each site, 6 PM_{10} samples on quartz fibre filters (48h at 38.3 L min^{-1}) and 24 multi-stage impactor samples (6d at 30 L min^{-1}, size range from D<0.056 µm up to D>18 µm. Volumes 110 m^3 or 250 m^3.**	**RT-PCR targeting E and RdRp genes.** **dd-PCR targeting N1 and N2 genes.**	**100% of samples negative with both methods RT-PCR and dd-PCR.**	**LOD - PM_{10} 0.8 copies m^{-3}.** **LOD - impactor 0.4 copies m^{-3}.** **Recovery 49%.**
Dunker et al. (2021) Germany Sci. Total Environ. 755, 142881	University of Leipzig Medical centre. Samples collected between 11/03/2020 and 28/05/2020.	7 weekly air samples and one 14 days sample. Sampling at 15 L min^{-1} with a cyclone trap directly into 1.5 mL micro centrifuge tube.	RT-PCR targeting E gene or N and RdRp genes.	100% of samples negative	LOD not reported. Recovery not reported. Fresh pollen samples were also collected finding no presence of SARS-CoV-2.
Passos et al. (2021) Brazil Environ. Res. 195, 110808	Metropolitan area of Belo Horizonte. Period 25/05/2020-06/08/2020.	2 $PM_{2.5}$ and 7 PM_{10} samples in total at: car parking of a COVID-19 hospital, sidewalk near hospital, busy bus station. Quartz fibre filter sampled at 1130 L min^{-1}. Volumes 7-4500 m^3.	RT-PCR targeting N1 and N2 genes.	100% of samples negative	LOD not reported. Recovery ~ 100%
Linillos-Pradillo (2021) Spain Environ. Res. 195, 110863	Madrid (district 09) university area in the period 04/05-22/05/2020.	6 PM_{10}, 6 $PM_{2.5}$, and 6 PM_1 simultaneous samples on quartz fibre filters at 30 m^3 h^{-1} for 17.5-24 h. Volumes 525-720 m^3.	RT-PCR targeting N1 and N2 genes and control of human RNase P (RP) gene.	100% of samples negative	LOD not reported. Recovery not reported.
Pivato et al. (2021) Italy Sci. Total Environ. 784, 147129.	10 sites (urban-rural background, traffic, industrial). NE Italy (Padua province) period 24/02/2020-09/03/2020.	25 PM_{10} and 19 $PM_{2.5}$ samples were collected in total over the 10 sites, on quartz fibre filters (24h at 38.3 L min^{-1}). Volume 55.2 m^3.	RT-PCR targeting N and Orf1b-14nsp genes.	100% of samples negative	LOD 1.2 copies m^{-3}. Recovery not reported.
Kayalar et al. (2021) Turkey Sci. Total Environ. 789, 147976	Samples from 13 locations in 10 towns, period 13/05/2020-14/06/2020. Hospital garden (HG) sites; urban (U) and urban background (UB) sites.	80 TSP, 19 PM10, 23 PM2.5-10, 33 PM2.5 samples with different samplers and filters (PTFE, quartz and glass fibre, polycarbonate. Volumes 7.2-360 m^3. 48 size segregated (6 sizes) samples on glass fibre filters, volume 1422 m^3.	RT-PCR targeting N1 and RdRp genes. 3D-dPCR targeting N1 gene.	HG sites 13/87 samples (14.9%) positive. U and UB sites 2/68 samples (3%) positive. U (Istanbul) 5/48 size segregated samples (10%) positive.	Near hospitals 5-23 copies m^{-3}. Urban and urban background 7-21 copies m^{-3}. Urban size segregated <0.2 copies m^{-3}. No recovery reported.

SIMULATIONS FOR LOMBARDIA REGION (I)

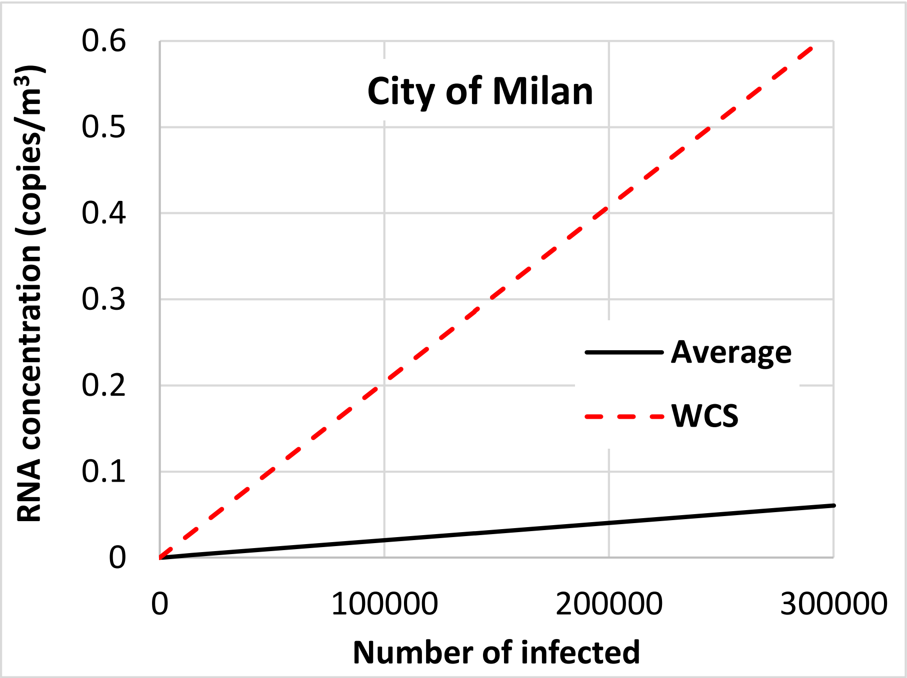


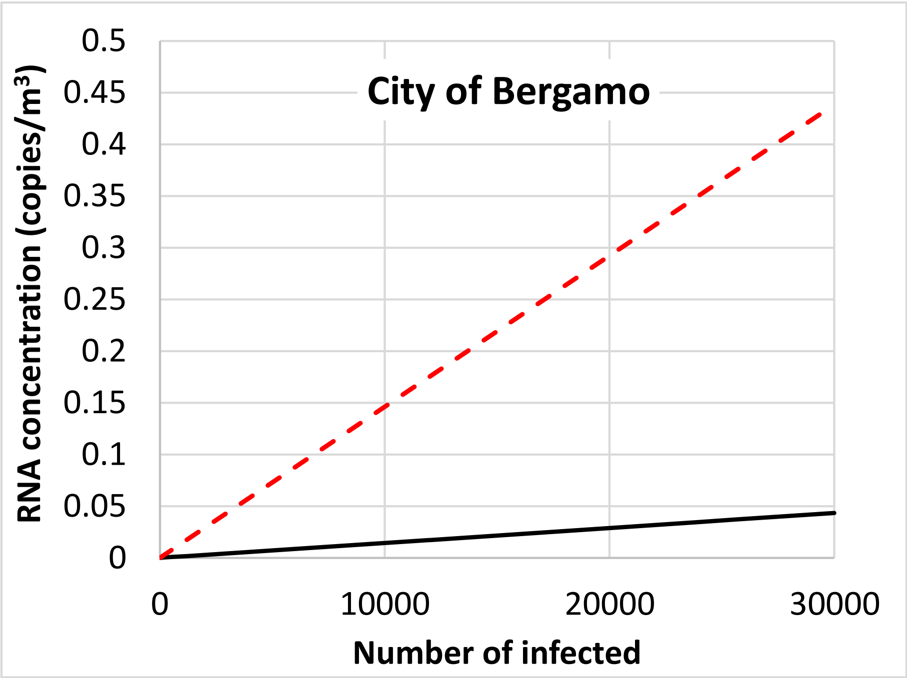
A simple box-model was used with a focus on the whole of Lombardy (boundary conditions) and two other simulations centered on the urban areas of Milano and Bergamo.

The box-model was applied by simulating a typical (average) scenario and a worst-case-scenario (WCS) based on the measurements in the winter of 2020 of the Arpa Lombardia monitoring network and from previous studies.

- Typical scenario: wind speed at 10 m from ground equal to 1.1 m/s, mixing height equal to 250 m.
- Worst-case-scenario: wind speed reduced at its 25^{th} percentile (0.9 m/s) and mixing height reduced accordingly to 60 m.

SIMULATIONS FOR LOMBARDIA REGION (II)





The **estimated concentrations are relatively low < 1 copy m^{-3}** in both urban areas even considering the worst-case-scenario and for a number of "active cases" (currently positive individuals) up to over 20% of the population.

Belosi et al. 2021 (Environmental Research 193, 110603)

INDOOR MEASUREMENTS

Measurements of indoor concentrations of virus-laden particles are **mainly available in hospitals and quarantine areas and limited studies are available for community indoors** such as commercial centres, markets, pharmacies, hair saloons, public transports and similar.

Moreno et al. (Environ. Int. 147, 106326, 2021) collected six $PM_{2.5}$ samples in subway trains in Barcelona (Spain) in June 2020. Maximum estimated concentration was 23.4 copies m^{-3}. In the same period (i.e. June 2020) also six air samples were collected in buses during normal operation and one sample was found positive for one of the three genetic targets with a concentration of 1.4 copies m^{-3}.

Hadei et al. (Atmospheric Pollution Research 12(3), 302-306, 2021) investigated community indoor environments in Iran collecting 28 air samples, in different indoors, that were found to be positive to the presence of SARS-CoV-2 in 64% of the cases but no details on viral particle concentrations were reported.

A recent study on a bus, during normal operations in Chieti (Central Italy), collected samples in the first wave of pandemic when restrictions policies were enforced and reported that all samples tested negative for the presence of SARS-CoV-2 (Di Carlo et al., PLoS ONE 15(11), e0235943, 2020).

This suggests that in indoor environments risks of airborne transmissions could be larger compared to outdoors and specific attention should be given.

CONCLUSIONS

The **concentrations of virus-laden particles in outdoor environments**, with the exception of crowded areas or areas very near intense sources, **are relatively low (<1 copy m^{-3})** based on measurements and models even in conditions of limited atmospheric dispersion.

The **probability of contagion in outdoor public areas is very low** with the exception of areas of gathering and/or close contact. **Outdoor airborne transmission does not seem to explain the differences in the development of outbreaks between Northern and Southern Italy**.

Currently, there is not a standard protocol for the study of airborne SARS-CoV-2, and this leads to limited ability to compare results from different studies. Therefore, in order to improve detection of SARS-CoV-2 genetic material and to make more comparable results obtained in different studies, it would be necessary to develop a standard measurement approach.

It is possible that **in specific indoor community environments** there are higher concentrations of airborne virus compared to outdoors. **There are therefore greater risks for airborne transmission**, especially in small and poorly ventilated environments, that should be mitigated with **specific actions such as: use of masks; disinfect surfaces exposed to the possible deposition of bioaerosol; frequently ventilate the rooms with outside air (in practice open the windows); avoid or limit the recirculation in central air conditioning systems**.

THANK YOU FOR THE ATTENTION!!!

Università Ca'Foscari Venezia

Environment International 146 (2021) 106255

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SARS-CoV-2 concentrations and virus-laden aerosol size distributions in outdoor air in north and south of Italy

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Environmental Research 193 (2021) 110603

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On the concentration of SARS-CoV-2 in outdoor air and the interaction with pre-existing atmospheric particles

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atmosphere

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Editorial

Does Air Pollution Influence COVID-19 Outbreaks?

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