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Short Communication

Citizen volunteers detect SARS-CoV-2 RNA from outdoor urban fomites

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HIGHLIGHTS

• Researchers and citizen volunteers found SARS-CoV-2 RNA on frequently touched urban surfaces.
• A portable, user-friendly machine for SARS-CoV-2 eRNA detection was employed.
• Citizen volunteers gained knowledge and felt more skilled to fight the pandemic.
• The positivity rate obtained was 10%, positives being obtained on a sunny day.
• Citizens volunteers empowered after their involvement in COVID-19 research.

GRAPHICAL ABSTRACT

CO-CREATED SCIENCE TO CHECK SARS-CoV-2 CONTAMINATION IN MIERES, SPAIN

POSITIVE FOR SARS-CoV-2 RNA

Sanitizer dispenser

Warning! More disinfection needed

Slide

ABSTRACT

In COVID-19 pandemics ordinary citizens are overwhelmed by the often contradictory information about transmission of SARS-CoV-2 through surfaces, especially outdoors. Citizen volunteers (N = 39) and researchers, working together for the first time on SARS-CoV-2 detection, searched this virus’ RNA on outdoors urban furniture of Mieres (Asturias, Spain) during the summer of 2020. RNA extraction and RT-PCR were conducted using point-of-care technology. A wooden slide and a sanitizer dispenser gave positive amplification of Spike gene. Contrary to expectations of higher virus survival in cold humid conditions, positivity rate was significantly higher in sunny sampling days, perhaps reflecting a higher frequentation of public outdoors spaces. All the participants considered the experience totally satisfactory and declared to have acquired new useful knowledge to face the pandemic. Significant increases of self-declared knowledge about virus transmission and protection measures, and confidence in hands hygiene for COVID-19 prevention, were found in the citizen volunteers following this experience. Results suggest the need for more control of playgrounds and public sanitizer dispensers. They also show how citizen volunteers can help to detect potential environmental reservoirs of disease agents from RNA analysis. Finally, ordinary citizens involved in COVID-19 research in small groups, following adequate training and safety protocols, feel empowered while valuably co-creating knowledge with researchers.

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1. Introduction

The emergence of an invisible threat, SARS-CoV-2 coronavirus, responsible for the new infectious disease COVID-19, is shaking the social use and enjoyment of public spaces. On the 30th of January 2020, the World Health Organization (WHO) declared the COVID-19
epidemic as a Public Health Emergency of International Interest. Since that upgrade to pandemic, actions to prevent contagions are focused on personal protection measures and the disinfection of public spaces. Although the main SARS-CoV-2 transmission route is aerial, via exposure to droplets and aerosols contaminated with the virus that are dispersed over short distances (e.g. Lu et al., 2020), coronaviruses can also be deposited directly on solid surfaces where droplets fall (Orenes-Piñero et al., 2021). It is well known that fomites of different materials contribute variably to the transmission of infectious diseases depending on their capacity of retention of microorganisms (Julian et al., 2011; Rönnvist et al., 2013; Ibiefi et al., 2016). SARS-CoV-2 can survive for a short time out of the human body and in anthropogenic surfaces (Carrato et al., 2020). Fomites carrying SARS-CoV-2 may contribute in some way to the propagation of COVID-19 (Ong et al., 2020; Pastorino et al., 2020). For example, Razzini et al. (2020) found SARS-CoV-2 virus in hospital surfaces in Italy and highlighted the potential of solid surfaces as coronavirus reservoirs. van Doremalen et al. (2020) confirmed that the stability of SARS-CoV-2 is similar to that of SARS-CoV-1 under experimental circumstances tested in aerosols and surfaces. Moreover, although all surfaces are not equal in contact transmission of SARS-CoV-2, previous studies have found it on inanimate surfaces up to 28 days after discharge of patients with COVID-19 (Zhou et al., 2020). Therefore, and from the review by Eslami and Jalili (2020), reducing the frequency of touching surfaces with our hands and increasing the disinfection of surfaces can reduce the amount of coronavirus load on them and the rate of transmission.

According to research data like those exposed above, in the World Health Organization newsroom webpage (https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-how-is-it-transmitted), one of the eight recommendations to reduce the risk of getting COVID-19 is to avoid touching surfaces in public settings. However, the role of fomites as a route of COVID-19 transmission is controversial. For some authors, it has been exaggerated (Goldman, 2020), being less frequent in real life than recognized (Mondelli et al., 2020). It seems that, although the SARS-CoV-2 can survive on a surface for at least several hours, high temperature and sunlight facilitate its destruction (Eslami and Jalili, 2020).

With these lots of information, sometimes contradictory, it is normal that anxiety, depression, and indignation are significantly increasing in the population as a consequence of the pandemic (e.g. Li et al., 2020). At least, a part of the distress is caused by concerns about the exposure to the virus in daily life activities. Touching public surfaces was the most salient worry about being contaminated (0.68 loading) when measuring COVID-19-related distress in large North American population samples (Taylor et al., 2020). Knowing the conditions in which the SARS-CoV-2 survives outdoors is indeed very important for citizens and scientists concerned about the use of public spaces.

However, scientific advances in COVID-19 pandemic are not often communicated in the best way to the public. Although unintentionally, communication often transmits the uncertainty of scientists and health professionals that face the pandemic, creating confusion among nonscientists. To control COVID-19, the different measures proposed by governments will not be enough when lockdowns and movement restrictions are relaxed. It is necessary to empower citizens with tools to visualize the benefits of a healthy behavior (Eichler et al., 2020) and promote effective public messaging. This and health education programs to protect human health, are paramount during infectious disease outbreaks (Wilder-Smith and Freedman, 2020). Bavel et al. (2020) highlighted the importance of messages that appeal to scientific norms to help citizens to respond to the COVID-19 pandemic. Although people will not take action to protect their health just motivated by scientific facts (Tibbetts, 2020), during public health crises the interaction between citizens and experts (and politicians) could be improved through citizen science exercises (Pearse, 2020).

Today, citizen science adopts multiple forms, from enrolling thousands of individuals in massive data collection to organizing small groups of volunteers in order to address specific local problems (Bonney et al., 2014). Indeed citizen science initiatives were affected during COVID-19 pandemic around the world, but not all of them in the same way. For example, in southern Africa citizen science bird projects were able to continue and be even enhanced (Rose et al., 2020), while in Japan initiatives of recording city biodiversity experienced a noticeable decrease of participants in the same circumstances (Kishimoto and Kobori, 2021). Lower sampling efforts and species records were registered in Colombia biodiversity projects despite higher participation in online platforms (Sanchez-Clavijo et al., 2021), in contrast with Kishimoto and Kobori (2021) experience in Tokyo where fewer but more enthusiastic participants were able to obtain even more diversity records than in pre-pandemic years.

Directly related with COVID-19, in Kerala (India) a citizen project helped to follow the pandemic in real time using online tools (Ulahannan et al., 2020). Emotional reactions along five weeks during COVID-19 emergency were followed in 444 volunteers in Serbia (Sadiković et al., 2020). Here we put in practice Pearse’s (2020) idea to increase the interaction between citizens and experts, involving ordinary citizens in the detection of SARS-CoV-2 without putting their health at risk. The objective was, to investigate the presence of the virus on surfaces in public settings, where citizens are exposed anyway. Strict measures to prevent the direct contact with the virus were taken, as we will see below. Given the nature of this project where a close supervision of experts was needed, we opted for a citizen science project based on small groups of volunteers (Bonney et al., 2014).

We have employed an easy procedure based on environmental RNA DNA (e.g. Ibabe et al., 2020) and RNA (e.g. Palich et al., 2017 for Ebola, Razzini et al., 2020 for SARS-CoV-2) molecules are efficiently employed to detect species from different surfaces. Like biopollutant eukaryotes from environmental DNA (Thomas et al., 2019), viruses can be quantified from environmental samples using quantitative PCR (qPCR). We have used a portable, user-friendly machine that allows a rapid detection of viruses in situ (Tomaszewicz-Brown et al., 2020 for canine disemper virus). We recruited local volunteers to work in a mixed group of citizen volunteers and researchers analyzing urban surfaces. Departure hypotheses were: a) From its lower survival in open spaces (Eslami and Jalili, 2020), the SARS-CoV-2 RNA positivity rate will be lower from the urban furniture in our study than that published from indoor fomites; and b) From the importance of messages appealing to scientific norms to face COVID-19 (Bavel et al., 2020), citizen volunteers involved in this study will gain knowledge and feel more skilled to fight the pandemic (thus empowered), especially regarding touching public surfaces.

2. Material & methods

2.1. Ethical statement

This study was approved by the competent Ethical Committee in Research of the University of Oviedo with the reference number 2-RR1-20. It was carried out during the Spanish summer holidays in late July–early August of 2020, while the COVID-19 pandemic was active in Spain, with outbreaks in all the regions. A written informed consent was obtained from all participants and approved by the Ethical Committee in Research of the University of Oviedo. In this informed consent, volunteers were informed about the nature and purposes of the project and they declared that the personal data were offered voluntarily, and consequently they authorized their use exclusively for scientific dissemination purposes within this project. Volunteers did not receive any economic compensation for their contribution.

2.2. Study region and town

The COVID-19 pandemic in the region of Asturias (north Spain) in July and August of 2020 could be considered active but under control,
with a prevalence of 5.2%, 60% of asymptomatic carriers and 28,443 deaths due to the disease (https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov-China/documentos/Actualizacion_174_COVID-19.pdf). The town of Mieres, in Asturias (38,000 inhabitants) was the locality chosen because of it is mid-size in the region. Located at 238 m of altitude above sea level in the middle of a former mining valley, it is representative of the region. Its climate is Atlantic humid with annual average temperature of 14.3 °C. The sampling days in this study were in the summer, with alternate rain and sunshine, and with air temperatures oscillating between 21 and 30 °C.

2.3. Recruitment and formation of citizen volunteers

Citizen volunteers were recruited with the collaboration of the city council of Mieres. A call was launched using communication channels like the official website http://www.mieres.es/areas-municipales/salud/informacion-sobre-coronavirus-covid-19/, regional radio and TV. The training was organized in three two-hour sessions: 1) training on safety measures about SARS-CoV-2; 2) learning about fomite sampling, the importance of sample traceability and introduction to RNA and PCR; 3) two-hour session to practice fomite sampling and geolocation (registering coordinates in a city map); and organization of small groups and sampling areas within the town.

The training workshop on fomite sampling was conducted during two weeks in July 2020. The volunteers worked in small groups (4–5 persons including one researcher). The first session was essential to ensure that the citizen volunteers were not unnecessarily exposed to SARS-CoV-2 infection. The volunteers learned how to put on and wear the elements of protection, that were standard equipment employed in health care settings such as facial masks, complete sterile overalls, gloves, high-protection FFP2 or surgical masks (Supplementary Fig. 1). Then they learned about the fomites as potential temporary reservoirs of viruses, how to sample fomites with total security (not exposing themselves to the virus), to store properly the samples in labeled tubes, to code and record each sample to ensure traceability. The principles of PCR and molecular analysis were also briefly explained. Finally, the volunteers organized the sampling for a good spatial coverage of the town, and chose the most touched fomites in the most frequented spots within their sampling areas. They located the preferred areas in maps.

2.4. Questionnaires

Before and after the experience, the volunteers answered a brief anonymous online questionnaire (Supplementary Annex 1, Questionnaire 1) asking for their knowledge about: a) the virus transmission; b) protection measures; and c) the importance of hand hygiene in the COVID-19 pandemic. The scale was 1–4, from none to very much.

After the experience, they were also asked online and anonymously about their satisfaction with this experience of volunteering as a citizen volunteers, and the perceived acquisition of skills to face COVID-19, will serve to test Hypothesis a.

2.5. Field work and sampling

Field sampling, RNA extraction and RT-qPCR were conducted between the 28th of July and the 4th of August of 2020, amid the summer holidays in Spain. The mixed team groups (researcher + citizen volunteers) toured Mieres town to find the urban elements previously selected on the map. The selected fomites were rubbed with sterile swabs that were immediately stored in tubes with 1 ml of preservative solution (Bioer Sample Preservative Fluid kit, Hangzhou Bioer, Technology Co., LTD). Researchers and citizen volunteers wore protective clothes and equipment – sterile overall, mask, facial shield and gloves (Supplementary Fig. 2) – that were changed or sterilized with sanitizer spray before and after collecting each sample. Strict hand hygiene protocols were followed, using sanitizer before starting dressing overalls and putting on protection equipment. After sampling all the volunteers disinfected again hands and sprayed sanitizer over their clothes and hair.

Sampling was done during the central hours in the morning or in the afternoon, when the frequentation of public spaces is maximum. Abundant public was nearby during sampling, never interfering with the collection of samples. The weather conditions were noted as Rainy, Drizzly, Cloudy, or Sunny. Differences between positivity rates for different materials and weather conditions while sampling will serve to test Hypothesis a.

2.6. Molecular work

Before starting the citizen science experience, the threshold of SARS-CoV-2 RNA detection with the method employed was estimated from different concentrations (3.6, 7.2, 9, 10.8, 18, 36, 150, 300 copies/ml) of the reference synthetic SARS-CoV-2 RNA AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control (RUC). RNA extracted from virus sampled with nasopharyngeal swabs from two patients diagnosed with COVID-19 and hospitalized in the Central University Hospital of Asturias was used as positive controls, after 1:10 dilution in distilled water. The Ct in these samples was 20, meaning an approximate virus concentration of 4 × 10^6 copies/ml. These RNA samples were kindly provided by Dr. Jose Antonio Boga, Director of the Microbiology unit in the Central University Hospital of Asturias.

Samples obtained from Mieres urban surfaces were analyzed by researchers together with the citizen volunteers involved in their collection (Supplementary Fig. 3). Sterility measures in the molecular work included cleaning surfaces with bleach, hydroalcoholic gel, gloves, UVA sterilization of the laboratory materials, and sterile filter pipette tips.

For its easy handling and visualization of results, we used Biomeme portable platform of qPCR. It uses a one-step approach where cDNA synthesis and amplification are performed in the same tube (Gaines, 2020), and the results can be visualized on a mobile phone. It has been employed by Thomas et al. (2019) to detect environmental DNA, and by Tomaszewicz-Brown et al. (2020) for the detection of RNA of adenovirus causing canine distemper disease in mammals. RNA was extracted with the Biomeme M1 Sample Prep Cartridge kit ©Biomeme Inc., then PCR was immediately conducted using Biomeme SARS-CoV-2 Go-Strips (©Biomeme Inc.), following the manufacturer’s instructions. It is an integrated sample preparation and RNA detection test approved as point-of-care assay with U.S. Food and Drug Administration regulatory status of Emergency Use Authorization (Parupudi et al., 2020). The kit contains lyophilized master mix, enzymes, and multiplexed primer/probes for a triplex reaction of SARS-CoV-2 Spike and Orf1ab genes and RNA of MS2 bacteriophage by ©Biomeme Inc. as RNA Process Control to ensure that qPCR is working. Results were visualized with Biomeme Go app (©Biomeme Inc.) in a smartphone where the PCR amplification curve appears in real time, and a clear color signal of “positive” or “negative” at the end. Manufacturer’s specifications for positive detection of Sars-CoV-2 using this methodology are: fluorescence over 150 RFU for Orf1ab and/or over 200 RFU for Spike gene, within 40 cycles (Ct < 40). The duration of RNA extraction process is 15 min and that of PCR is 60 min, thus the result is ready in less than one hour and a half.

2.7. Data analysis

To test Hypothesis a, samples taken on sunny versus rainy or drizzly conditions were compared, and also samples taken in this study (outdoors) with data published for indoor fomites in other studies.
Positivity rates (proportion of positive tests) were compared between groups of samples using Fisher’s exact test, taking Cramer’s V as a measure of effect size, as in Razzini et al. (2020).

The effect of the citizen science experience (Hypothesis b) on the perceived knowledge about SARS-CoV-2 transmission and protection measures, and the perceived importance of hand hygiene to prevent COVID-19, was measured comparing mean scores before and after intervention employing paired t-tests. Significance threshold was \( p < 0.05 \). Statistics was done with free PAST software version 3.1 (Hammer et al., 2001). Figs. 1 and 2 were constructed using ggplot2 library within R software version 4.0.4 (2021-02-15) (R Core Team, 2020).

### 3. Results

#### 3.1. Positive controls

The assays with synthetic SARS-CoV-2 RNA to evaluate the sensitivity of the method gave a detection threshold of 36 copies/\( \mu l \) for Spike gene and 150 copies/\( \mu l \) for ORF1ab. At that concentration, we obtained positive amplification of the two markers (Table 1). The two samples obtained from patients with COVID-19 were indeed positive for the two markers and provided lower \( Ct \) values for both ORF1ab and Spike genes than those found in the samples of 150 and 300 copies/\( \mu l \) of pure RNA (Table 1), as expected since the estimated concentration of the virus in patient samples was approx. 4000 copies/\( \mu l \) after 1:10 dilution. These results showed a higher sensitivity of Spike marker where \( Ct \) values were lower than those obtained for ORF1ab at the same concentration.

Taking into account the relative volume of the preservative fluid and the swab, we expect an approximate dilution of 1:10 of the environmental RNA copies present on a surface. Thus, from these sensitivity assays we expect to detect concentrations above 360 RNA copies/\( \mu l \) for Spike and 1500 copies/\( \mu l \) for ORF1ab from surfaces using this method. These values are much lower than the loads of SARS-CoV-2 in infected patients, with means around \( 10^4 \) virus copies/\( \mu l \) in some studies (e.g. Lescure et al., 2020).

#### 3.2. SARS-CoV-2 detection

Twenty samples were analyzed using the method described above: park and church benches, children playground furniture, cash/ticket vending machines, counters, outdoor railings, trash containers, door handle/knob, and a public sanitizer dispenser (Table 2). The detailed results are in Supplementary Table 1. The RNA process controls were always positive, indicating that the qPCR was working properly. Two positive qPCR results were obtained for Spike marker (>200 baseline subtracted relative fluorescence, \(<40 \text{ Ct})\), specifically in samples taken from a wooden slide of a children playground (Fig. 1-a) and from a sanitizer dispenser made of plastic (Fig. 1-b). Since strict measures of personal protection and sterility were employed during sampling and laboratory analysis, contamination can be reasonably excluded. In the rest of fomites, significant presence of virus RNA was not detected; all the samples were negative for SARS-CoV-2 RNA with ORF1ab marker (Table 2). Thus, from the specifications of the method employed in

| RNA sample | ORF1ab | Spike gene |
|------------|--------|------------|
| 3.6 copies/\( \mu l \) | −5.9 | 2.2 |
| 7.2 copies/\( \mu l \) | −8.9 | −11.5 |
| 9 copies/\( \mu l \) | −8.9 | −3.2 |
| 10.8 copies/\( \mu l \) | −14.5 | −65.2 |
| 18 copies/\( \mu l \) | −7.8 | 13.2 |
| 36 copies/\( \mu l \) | 39.3 | 382* (35.3) |
| 150 copies/\( \mu l \) | 2196.6* (32.15) | 4799.7* (29.96) |
| 300 copies/\( \mu l \) | 1731.7* (33.07) | 6127.5* (28.82) |
| Patient 1 | 1411.4* (25.62) | 1658.7* (23.58) |
| Patient 2 | 1210.5* (28.47) | 1586.9* (26.59) |

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Fig. 1. Relative fluorescence plot constructed after baseline subtraction over 45 PCR cycles is presented. A) Slide in a public playground exhibiting significant amplification of SARS-CoV-2 RNA (Spike gene). B) Public sanitizer dispenser where significant amplification of SARS-CoV-2 Spike gene was found in Mieres (Spain).

Fig. 2. Effect of this experience on the citizen volunteers involved. Mean perception of knowledge about transmission of SARS-CoV-2, protection measures and efficacy of hand hygiene for prevention, before and after the experience (scale 1–4). Standard deviation as capped bars.
Table 2
Samples analyzed, type of fomite and material, and weather conditions during sampling. The two first digits in sample codes indicate the sampling team. Baseline subtracted relative fluorescence found for the SARS-CoV-2 markers Orf1ab, Spike gene and the PCR control after 45 cycles of qPCR in each sample. Ct in parenthesis in positive amplifications. SARS-CoV-2 positive results are in bold.

| Sample | Fomite          | Material | Weather | ORF1ab | Spike gene | PCR control |
|--------|----------------|----------|---------|--------|------------|-------------|
| 1.1.7  | Church bench   | Wood     | Drizzly | 1.9    | 164.2      | 3820.3 (28.5) |
| 1.1.13 | Doorknob       | Wood     | Drizzly | 47.8   | 5.8        | 2950.3 (31.5) |
| 1.2.7  | Ticket machine | Metal    | Drizzly | 17.5   | 194.6      | 3269.1 (30.4) |
| 1.2.10 | Trash container| Metal    | Drizzly | 6.5    | 162.3      | 2919.2 (31.9) |
| 1.3.1  | Playground swing| Rope    | Drizzly | 10.4   | 1.04       | 3185.9 (31.4) |
| 1.3.10 | Vendor machine | Metal    | Drizzly | 12.5   | 29.6       | 3464.3 (29.2) |
| 2.1.2  | Vendor machine | Glass    | Rainy   | 7.7    | 5.7        | 2906.2 (33.2) |
| 2.1.3  | Door handle    | Metal    | Rainy   | 33.8   | 1.5        | 2802.9 (33.1) |
| 2.2.8  | Cash machine   | Metal    | Rainy   | 29.8   | 158.8      | 2826.0 (33.7) |
| 2.2.10 | Railing        | Metal    | Rainy   | 19.4   | 75.6       | 5.9          |
| 2.3.1  | Park bench     | Wood     | Rainy   | 5.9    | 9.1        | 2975.8 (34.2) |
| 2.3.2  | Counter        | Metal    | Rainy   | 0.27   | 8.3        | 0.02         |
| 3.1.3  | Vendor machine | Metal    | Cloudy  | 14.6   | 84.6       | 2991.6 (33.4) |
| 3.1.9  | Railing        | Metal    | Cloudy  | 30.9   | 12.03      | 2925.8 (29.8) |
| 3.2.5  | Trash container| Plastic  | Cloudy  | 18.5   | 82.5       | 2335.5 (36.4) |
| 3.2.6  | Cash machine   | Metal/Class| Cloudy | 0.4    | 15.7       | 11.7         |
| 4.1.5  | Playground slide| Wood    | Sunny   | 4.7    | 2178.8 (40.3)| 2967.9 (29.6) |
| 4.1.11 | Ticket machine | Metal    | Sunny   | 4.8    | 138.5      | 2877.7 (29.8) |
| 4.2.3  | Sanitizer dispenser| Plastic| Sunny   | 5.6    | 241.5 (39.5)| 3059.8 (30.1) |
| 4.2.10 | Railing        | Metal    | Sunny   | 4.5    | 106.5      | 2639.2 (30.3) |

Table 3
Comparison of studies that use eRNA for SARS-CoV-2 detection on different surfaces. Country where the study took place, rate of positivity obtained, genetic markers employed, criteria for samples to be considered positive, type of surfaces sampled and reference.

| Country | Rate of positivity | Markers used | Samples considered positive | Type of surfaces | References |
|---------|--------------------|--------------|-----------------------------|------------------|------------|
| USA     | 8.3% (29/348)      | E Sarbeco and CDC N1| At least one of the triplicates amplified with N1 or E | High-touch surfaces in a community setting- Massachusetts. | Harvey et al., 2021 |
| Brazil  | 5.25% (49/933)     | CDC N1 and N2 | When both markers are positive. | Public surfaces in a densely populated urban area. | Abrahão et al., 2020 |
| China   | 26.6% (17/64)      | ORF 1 ab and N | One of the two targets is positive. | Different surfaces from intensive care unit and a general COVID-19 ward-Wuhan | Guo et al., 2020 |
| UK      | 52.3% (114/218)    | E Sarbeco | Two replicates are positive | Healthcare setting during the peak of the COVID-19 pandemic in London. | Zhou et al., 2020 |
| Spain   | 5.56% (2/36)       | RdRP, N and E Sarbeco | Three targets are positive | COVID-19 traps placed in the rooms of 6 COVID-19 patients. | Orenes-Piñero et al., 2021 |
| Italy   | 24.3% (9/37)       | Not described (commercial kit) | Not described (commercial kit) | Different areas, contaminated, semi-contaminated and clean areas, from an Italian hospital. | Razzini et al., 2020 |
| Spain   | 10% (2/20)         | ORF 1 ab, Spike | One of the two targets is positive | Public surfaces in a medium-size village of Spain. | This study |

which one marker is sufficient for a positive result, the positivity rate in our study was 10%.

The two positive results had in common that they were obtained on a sunny day (by two different teams). Opposite to expectations (Eslami and Jalili, 2020), the positivity rate obtained in sunny conditions (50% over 4 samples from sunny days) was significantly higher than that in not sunny conditions, 0% of 16 samples taken on not sunny days (Fisher's exact test with \( P = 0.03 \)). The size effect was moderate (Cramer’s \( V = 0.66 \)). This indicates a difference between weather conditions, that could be attributed to a higher frequentation of outdoor urban spaces with good weather, thus augmenting the probabilities of SARS-CoV-2 carriers to touch urban furniture and leave virus traces on fomites.

3.3. Study results in a wider context

To put the positivity results found in this study in a more global context we have compared them with six other studies of SARS-CoV-2 detection on public surfaces (Table 3), two outdoors and the rest indoors. In these studies different markers were employed: N, CDC N1 and N2, E Sarbeco, Orf 1ab, RdRP; three studies employed two markers simultaneously as in our study, one employed one marker, another employed three markers, and one used a commercial kit where markers were not disclosed (Table 3). Criteria to consider a test SARS-CoV-2 positive were also varied: Guo et al. (2020) and Harvey et al. (2021) considered enough one marker to be significantly amplified, while other authors needed all the markers employed to be positive (Abrahão et al., 2020; Orenes-Piñero et al., 2021) (Table 3). Despite the diversity of SARS-CoV-2 genes employed as markers in the different studies, and positivity criteria, the results were generally consistent with lower positivity outdoors than indoors (healthcare settings). The two studies conducted outdoors gave similar positivity rates to that of 10% found in our study: 5.25% and 8.3% in Massachusetts (Harvey et al., 2021) and Brazil (Abrahão et al., 2020) respectively. In contrast, positivity rates indoors were generally higher than 20%, with the exception of 5.6% in rooms of COVID-19 patients in a Spanish hospital (Orenes-Piñero et al., 2021), being as high as 52% in health care facilities in London during the peak of the pandemics (Zhou et al., 2020).

To test Hypothesis a, we compared our results with data obtained from indoor fomites in those articles. The positivity rate found in our study was indeed not lower than that found by Orenes-Piñero et al. (2021) indoors; it was higher but not significantly different (Fisher’s exact test with \( P = 0.611 \); Cramer’s \( V = 0.083 \), not significant effect size). Razzini et al. (2020) found 24.3% positivity rate in 37 tests in an Italian hospital, which was not significantly different from the positivity rate of 10% found in our study in 20 tests (Fisher’s \( P = 0.29 \); Cramer’s \( V = 0.17 \)). With Guo et al. (2020) results the difference was also not significant (Fisher’s \( P = 0.22 \); Cramer’s \( V = 0.169 \)). These results would not allow to accept Hypothesis a of a significantly lower
positivity rate outdoors. However, the comparison with London data of Zhou et al. (2020) was highly significant (Fisher’s P = 0.0003, Cramer’s V = 0.235).

The positivity rate in our study was not significantly different of those found in similar studies about high-touch public surfaces with much larger sample sizes. Although the rate found in Brazil by Abrahão et al. (2020) was lower, the difference was not significantly different (Fisher’s test P = 0.291, not significant; Cramer’s V = 0.03). Compared with Harvey et al. (2021) study in Massachusetts, the positivity rate of our study was also not significantly different (Fisher’s test P = 0.681, Cramer’s V = 0.014).

3.4. Citizen science

Of the 141 citizens who expressed interest in the project, the 27.7% (39 persons aged 18–70, 59% women, mean age 46.7 with standard deviation SD = 15.4) followed the training sessions programmed and were actively involved in the project until the end. Only five of them (12.8%) had any previous experience in biology or health sciences. For the 78% of the volunteers, this was the first time they participated in volunteering, and it was the first time working in a citizen science project for all of them.

The mixed research group was completed with three professional researchers. In teams of 3-5 persons (one researcher and 2–4 citizen volunteers), they sampled with swabs the surfaces selected in Mieres; put the swabs in preservative fluid; maintained a rigorous traceability of samples using unique codes; extracted RNA from the preserved samples; and conducted RT PCR, as explained above.

The raw results of the survey are reported in Supplementary Table 2. One participant did not fill in any questionnaire, and two participants did not answer Questionnaire 1 before the experience (7.6% of missing data in Questionnaire 1 and 2.5% in Questionnaire 2).

Results of Questionnaire 1 showed that the perceived knowledge gains (maximum score of 4) augmented significantly after this citizen science experience (Fig. 2): from a mean of 2.92 (SD 0.77) before the experience to 3.5 (SD 0.72) for the knowledge about SARS-CoV-2 virus transmission ways (t = 3.35 with p = 0.001); from 3.1 (SD 0.64) to 3.8 (SD 0.49) for protection measures (t = 4.73 with p ≪ 0.001); and from 3.78 (SD 0.48) to 3.97 (SD 0.16) for the perceived importance and adherence to hand hygiene (t = 2.36 with p = 0.02). These results confirmed the expectations of Hypothesis b about the increase of skills against the pandemic following exposure to real scientific facts.

Regarding volunteers’ satisfaction with this citizen science experience, results of Questionnaire 2 showed that even though most participants (87%) had no previous experience in molecular biology, all of them considered the experience totally satisfactory (mean = 5 for a maximum of 5, SD = 0), and felt that they had acquired new useful knowledge to face the pandemic (mean = 4.8, SD = 0.4) (Supplementary Table 2).

4. Discussion

The results obtained in this study, originated from a joint work of citizen volunteers and researchers, demonstrate the presence of SARS-CoV-2 RNA on urban surfaces outdoors in sunny days. Similar positivity rates were found outdoors in other countries using much larger samples (Abrahão et al., 2020; Harvey et al., 2021). Perhaps the main novelty of our study in this regard is the occurrence of positives only in sunny sum-mer days, something not expected given the fragility of this virus at high temperatures and when exposed to sunlight (Esami and Jalili, 2020).

Indeed, positive virus tests are also found in indoor surfaces that are frequently touched (e.g. Guo et al., 2020) and located in areas more frequently by patients with COVID-19 (Zhou et al., 2020; Orenes-Piñero et al., 2021), like a corridor for patients, the intensive care unit, and an undressing room, including a public sanitizer dispenser (Razzini et al., 2020). However, some of those rates (Guo et al., 2020; Orenes-Piñero et al., 2021; Razzini et al., 2020) were not significantly higher than those found in our study, not allowing a full support of our Hypothesis a; only was supported when our results were compared with those of Zhou et al. (2020) in the middle of the pandemic peak. The small number of fomite samples analyzed in this citizen science initiative is a limitation of our study. However, although our sample size was small, taking into account that it was collected from outdoor spaces, in summer and in a moment of controlled pandemic, our results should be taken seriously as a signal of public urban furniture as potential, likely ephemeral but not negligible, virus carriers.

With the method here employed we have detected RNA, and we cannot say it corresponds to integer, viable viruses. Dowell et al. (2020) did not find viable virus on hospital fomites. However, Goldman (2020) found a window of 1–2 h for the transmission of viable viruses from fomites: if an infected person coughs or sneezes on a surface, and someone else touches that surface soon after, there will be a chance of virus transmission; and Zhou et al. (2020) found it on inanimate surfaces up to 28 days after discharge of patients with COVID-19. Although the presence of RNA of the virus in urban furniture does not determine the infectivity, detecting viral RNA in an item indicates the shedding of the virus (Razzini et al., 2020). Therefore, although the RNA found is likely a trace of viruses and it does not necessarily belong to integer virus particles, a precautionary approach should be adopted. Caution could be recommended with sanitizer dispensers, favoring touchless systems. On the other hand, the occurrence of virus RNA on a slide cannot be automatically interpreted as transferred from children, because childcare persons also touch the playground furniture. Modeling studies of COVID-19 predict that school closures would have much less effect in prevention of deaths than playground closure (Viner et al., 2020). Whether children or childcare persons were the carriers, our results emphasize the need of periodical disinfection of children playgrounds for a safer enjoyment of these public spaces.

Another main novelty of this study was the citizen science approach adopted, with citizen volunteers directly involved in the scientific process of sampling and in the molecular work, being the process of hypotheses verification developed by the researchers and transferred finally to the citizen volunteers. Other citizen science studies of COVID-19 employed online approaches (Sadiković et al., 2020; Ulahannan et al., 2020), in line with lockdowns in the moments of emergency, but our study was conducted when some mobility was possible after the first wave in Spain. In our study, Hypothesis b was fully supported from the data. The volunteers acquired new knowledge that they considered important to fight the pandemic, being thus empowered after the citizen science experience. A high satisfaction with the experience of volunteering for this project was another important and significant result. A strong message of co-creation is that, at the interface of science and practice, the interests of both practitioners and academics have equal weight and benefits (Skipper and Pepler, 2020). The benefit for the practitioners (in this case, citizen volunteers) here was principally the acquisition of a better preparation to face COVID-19. From the academic side, this study was conceived with volunteers, and their knowledge about the use of the most frequented urban locations was essential for this project. Thus, comparable benefits were gained by academics and volunteers.

Volunteering plays an increasingly important role in modern society, and citizen science should take into account the satisfaction of the volunteers that helps them to adhere to, feel motivated, and be retained in a project (e.g. West and Pateman, 2016). Provenzi and Barello (2020) argue that this pandemic is changing our lives in all aspects, including the way science presents its researches and advances; towards a new form of participatory and collaborative approach to research, they support that the partnership among citizens, clinicians and scientists is no longer deferrable and the year 2020 appears to be a point of no return to plan the science of the future. Our study could open new perspectives in the ways of approaching science to the general public, for example,
through the involvement of small groups in projects that benefit both professional and citizen volunteers, as in the present case.

From the technical side, the ©Biome equipment and app used in this study have been successfully employed to quantify the biopollutant New Zealand mudsnail this study have been successfully employed to quantify the biopollutant. Our results confirm its utility in SARS-CoV-2 environmental RNA, although the level of sensitivity here found was lower than that found by Thomas et al. (2019) for DNA (21 copies per reaction). Other methods are much more sensitive, being able to detect even less than one copy of SARS-CoV-2 per μl in surfaces (e.g. 0.17 copies/μl in Santarpia et al., 2020). However, the rapidity of the process and easy use in situ of this portable equipment compensates its lower sensitivity found in the present study.

5. Conclusions
In conclusion, this study demonstrates the presence of SARS-CoV-2 RNA in objects that are part of public urban furniture, located outdoors and in good weather conditions, using a user-friendly methodology and a portable qPCR machine. Results suggest that there is a not negligible risk of virus transmission in open environments through anthropogenic fomites. It also shows how citizen volunteers can help to detect potential environmental reservoirs of disease agents like SARS-CoV-2 from RNA analysis, while working in total security in collaboration with researchers. From the survey among the volunteers of the study, the final message is that ordinary citizens involved in COVID-19 research, following adequate training and safety protocols, feel empowered while valuably co-creating knowledge with researchers.

CRediT authorship contribution statement
AA, ED and EGV designed the citizen science protocol. ED led citizen recruitment. AA and EGV were in charge of the practical learning of the citizens. AA and SF developed the laboratory work. EGV wrote the initial manuscript. All authors reviewed and improved the first manuscript. All authors approve the submission and publication.

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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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147719.
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