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The Safe Return of Face-to-Face Teaching in the Post-COVID-19 Era at a University in Southern Italy: Surface Monitoring as an Early Warning System

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Abstract: Environmental monitoring for SARS-CoV-2 has become a useful adjunct to clinical testing because it is widely available and relatively inexpensive. During the period May–December 2022 (spring–summer: May–September–autumn: October–December), we assessed the presence and viability of the virus on surfaces in university settings in the Apulia region (Southern Italy) after the resumption of face-to-face teaching activities and evaluated surface monitoring as an early warning system. The sampling plan provided for the selection of 75% of the surface types (e.g., student and teacher desks, computer, handrail) in different materials (plasticized wood, wood, metal, plastic) present in different environments. Overall, 5.4% of surfaces (all students' desks) resulted in positive with RT-PCR and negative with viral culture. Greater contamination was found in the spring–summer period than in the autumn (χ^2 test with Yates correction = 7.6003; p-value = 0.006). The Poisson regression model showed a direct association between the average number of COVID-19 cases among university students in the seven days following sampling and the percentage of SARS-CoV-2 positive swabs on sampling day and (Intercept = 5.32498; β = 0.01847; $p < 0.001$). Our results show that environmental monitoring for SARS-CoV-2, especially in crowded settings such as universities, could be a useful tool for early warning, even after the end of the COVID-19 emergency.

Keywords: monitoring surface; SARS-CoV-2; university setting

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Citation: De Giglio, O.; Triggiano, F.; Apollonio, F.; Buonavoglia, C.; Capozzi, L.; Camero, M.; Colafemmina, G.; Del Prete, R.; Fasano, F.; Lanave, G.; et al. The Safe Return of Face-to-Face Teaching in the Post-COVID-19 Era at a University in Southern Italy: Surface Monitoring as an Early Warning System. *Appl. Sci.* **2023**, *13*, 13214. https://doi.org/10.3390/app132413214

Academic Editors: Vardan Galstyan and Gang Wei

Received: 20 September 2023
Revised: 1 December 2023
Accepted: 9 December 2023
Published: 13 December 2023

1. Introduction

The coronavirus SARS-CoV-2 was first detected in the city of Wuhan, China, in December 2019, and a few months later (11 March 2020), WHO proclaimed it responsible for the COVID-19 pandemic.

SARS-CoV-2 is transmitted by direct (person-to-person) or indirect contact (e.g., inhalation of respiratory droplets, aerosols produced by coughing and sneezing of infected persons) [1,2].

Indirect transmission can also occur when a person comes into contact with contaminated surfaces [2]. However, environmental spread of SARS-CoV-2 to humans via contaminated surfaces remains controversial. [3]. Some studies on the viability and resistance of the virus on surfaces [4–6] demonstrated that SARS-CoV-2 can remain infectious for days, while other authors reported that the risk of infection is relatively low [7–9]. Environmental surveillance for SARS-CoV-2 using surface swabs could demonstrate the stability of the virus in the environment and thus counteract the spread of COVID-19. Testing of environmental samples, such as wastewater and surface swabs, provides indirect evidence of the number of infected individuals shedding the virus [10–13].

Environmental monitoring has become a useful adjunct to clinical trials, in part because the molecular tests commonly used to detect SARS-CoV-2 RNA (RT-qPCR) are widely available and relatively inexpensive [14,15]. Furthermore, environmental monitoring also avoids issues of informed consent, operational logistics, and fairness that can slow down or limit clinical trial programs [16,17].

In Italy, the start of the COVID-19 epidemic was in February 2020. A lockdown was immediately imposed, ordering the closure of all social and cultural centers, including schools and universities. [18]. Throughout the lockdown period, until the fourth wave of the epidemic (autumn 2021–spring 2022), university and scientific activities (e.g., lectures, seminars, exams, conferences) were carried out remotely in several Italian regions, such as Apulia.

From March 2022, following an improvement in the pandemic situation, university teaching activities resumed in person, with the exception of people affected by chronic diseases and immunodepression and subjects with SARS-CoV-2 infection. Several authors have studied surface contamination in hospital and community settings, including cultural centers, schools, and universities [3,9,19], but few studies refer to the post pandemic period and the resumption of face-to-face activities. Similarly, the resistance and viability of SARS-CoV-2 on surfaces and fomites via cell culture have been poorly assessed due to the limitations of low sensitivity and the availability of biosafety level 3 laboratories [3].

In April 2022, the University of Bari "Aldo Moro", one of the largest universities in southern Italy, attended by students from all over the Apulia region, approved the project entitled "The safe resumption of face-to-face teaching in the COVID-19 era: Precision Containment Strategies (SCOOP)", as part of the HORIZON EUROPE SEEDS projects (D.R. n. 1333, 11 April 2022). This project focused on the environmental monitoring of SARS-CoV-2 in crowded environments such as universities, where environmental contamination and the associated risk of indirect transmission between staff, students, and multitouch surfaces can easily emerge.

The aim of this study was (i) to evaluate the presence and viability of SARS-CoV-2 in university classrooms after the resumption of face-to-face teaching activities; (ii) to evaluate the influence of some parameters (e.g., type of surface, material, number of attending students, seasonality) on the viral presence; (iii) to compare positive environmental samples for virus against the trend of COVID-19 cases to identify an early warning system for disease cases.

2. Materials and Methods

During the period May–December 2022, the SCOOP project aimed to examine the surfaces of the University of Bari "Aldo Moro" (Figure 1) on a weekly basis, immediately before the start (8:00 am) and at the end (3:00 pm) of the teaching activities (i.e., lessons, script exams, graduation sessions, admission tests to the degree courses, workshops). At the end of the day, the same environments were sanitized with a solution of nebulized sodium hypochlorite. The sampling plan provided for the selection of 75% of the surface types (student and teacher desks, computer mouse, keyboard, handrail, door and win-

dow handles, others) present in university environments (classrooms, library, toilets, etc.) frequented by students (20–25 years of age) and academic staff.

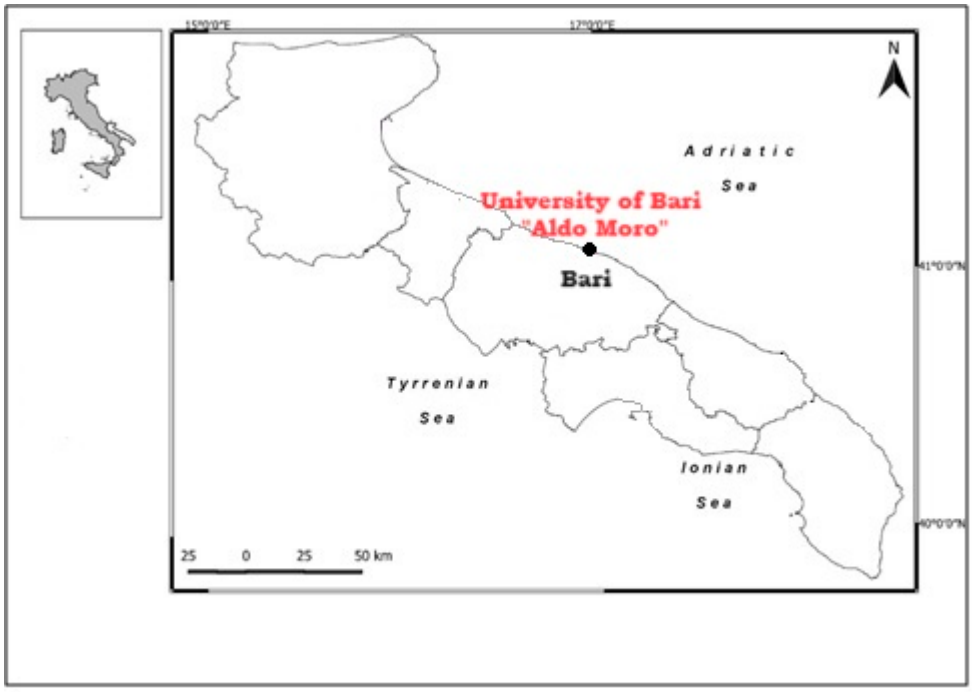

Figure 1. Location of the University of Bari "Aldo Moro" in the Apulia region (southern Italy).

As a control, one swab per day was collected in the morning (before any academic activities) in each enrolled environment and then analyzed for the presence of SARS-CoV-2 RNA. During the sampling, the number of individuals occupying the rooms, the type of surface, and the material surfaces were noted.

2.1. Environmental Sampling

Surface sampling was performed following Caggiano et al. [20] protocol with some modifications. In particular, sterile COPAN UTM® Universal Transport Medium swabs (COPAN Diagnostics, Inc., Murrieta, CA, USA) containing 5 mL of transport medium were used. For flat and large surfaces (such as desks), the swabs were rotated horizontally and in a vertical direction to cover an exact 10 × 10 cm surface, whereas, for small and curved surfaces, the swabs were taken from the available space. The swab samples were transported in an isothermal container to maintain a temperature of +4 °C and immediately processed at the Food and Environmental Hygiene Laboratory of the Interdisciplinary Department of Medicine of the University of Bari Aldo Moro.

2.2. Molecular Analysis

SARS-CoV-2 RNA was detected with Real-Time Reverse Transcription-PCR (RT-PCR) [21,22]. Swabs were vortexed for 20 s, and 3 mL UTM-RT® was transferred to a new 15 mL tube sterilely. Nucleic acid extraction was performed using the semi-automated NucliSENS miniMAG extraction system with magnetic silica (bioMerieux, Marcy-l'Etoile, Lyon-France) according to the manufacturer's instructions. RNA was resuspended in 100 µL elution buffer, and extracts were stored at −20 °C. To detect SARS-CoV-2, the ORF-1ab gene (nsp14) was amplified, and a 25 µL mixture was prepared containing 5 µL RNA for each sample; 12.5 µL 2× Reaction Buffer supplied with AgPath-ID ™ One-Step RT-PCR Reagents (Applied Bio-systems™, Thermo Fisher, Waltham, MA, USA); 1 µL 25× RT-PCR enzyme mix; 1 µL forward primer (12. 5 µM); 1 µL reverse primer (22.5 µM); 1 mL probe (6.25 µM); 1.83 µL nuclease-free water (not DEPC-treated); and 1.67 µL Real-Time PCR Detection Enhancer (Applied Biosystems™, Thermo Fisher, Waltham, MA, USA). The

sequences of the primers and probes used were as follows: CoV-2-F/ACA TGG CTT TGA GTT GAC ATC T; CoV-2-R/AGC AGT GGA AAA GCAT GTG G; and CoV-2-P/FAM-CAT AGA CAA CAG GTG CGC TC-MGBEQ [22]. RT-PCR experiments were performed in duplicate using the CFX96TM Touch Deep Well Real-Time PCR System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The conditions for the thermal cycling were as follows:

- Reverse transcription phase (50 °C for 30 min)
- Inactivation of the RT phase (+95 °C for 10 min)
- 45 cycles of amplification (+95 °C for 15 s and +60 °C for 45 s).

Cycle cut-offs for RT-PCR < 40 were interpreted as positive for SARS-CoV-2 RNA.

2.3. Virus Isolation

For SARS-CoV-2 isolation, 2 mL of COPAN UTM® for each sample was stored at −80 °C and then shipped in isothermal containers to a Biosafety Level 3 Laboratory at the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (Foggia, Italy), according to laboratory biosafety guidelines.

Virus isolation from swabs was performed as previously described [23]. Briefly, we used the Vero E6 cell line that was plated into 25 cm^2 cell culture flasks (Corning, Somerville, MA, USA, CLS430168) at 70–80% confluency in 10 mL EMEM containing 10% of Fetal Bovine Serum (FBS) (Life Technologies, Carisbad, CA, USA) and 100 U/mL penicillin and 100 mg/mL streptomycin (Life Technologies, Carisbad, CA, USA), incubated overnight in 5% CO_2 at 36 ± 1 °C. The next day, 1.5 mL of swab medium was incubated for 1 h at room temperature with 500 µL antibiotic broth (2000 U/mL penicillin/streptomycin and 300 U/mL neomycin). This suspension was then inoculated onto a monolayer of Vero E6 cells, and flasks were incubated in 5% CO_2 at 36 ± 1 °C for 1 h.

After incubation, 4 mL of EMEM with 6% FBS was added, and the flask was incubated again. The EMEM with 6% FBS was replaced every 3 days in order to maintain the viability of cells. The infected cell cultures were monitored daily for up to one week, and the outcome was determined by the presence or absence of cytopathic effect with inverted microscopy (Eclipse TS2-FL, Nikon, Tokyo, Japan) in conjunction with the positive RT-PCR test in the supernatant [24].

2.4. Next-Generation Sequencing of Viral Genome

Whole Genome Sequencing (WGS) was performed at the Molecular Biology Laboratory of the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (Putignano, Apulia, Italy). The samples were handled in a Biosafety Level 2 Laboratory.

Viral RNA purification was carried out for RT-PCR positive samples using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems), according to the manufacturer's instructions. cDNA synthesis and genomic libraries preparation were performed according to Illumina COVIDSeq protocol (Illumina Inc., San Diego, CA, USA). Sequencing was performed on the Illumina MiSeq platform (Illumina) using the MiSeq Reagent Kit v2, 2 × 250 paired-end cycles.

2.5. Sequence Data and Phylogenetic Analysis

For each positive sample, the sequence data analysis was performed as previously described [25]. One consensus genome sequence passed the quality control assessment by Nextclade, whose parameters include missing data, mixed sites, private mutations, and mutation clusters, and was deposited on the GISAID database [26]. The lineage of the assembled genome was assigned using Pangolin [27].

2.6. Statistical Analysis

A chi-squared statistic with Yates correction or Fisher exact test was used to test for differences in SARS-CoV-2 swab contamination between the following groups:

1. Surface type (teacher and student desks, handrail, computer mouse, keyboard, door and window handles, other)

2. Materials of surfaces sampled (plasticized wood, wood, metal, plastic)
3. Number of students (classroom with <50 students, >50 students and environment <30 academic staff)
4. Seasonality (spring–summer vs. autumn)

The Mann–Whitney U test was used to compare mean monthly atmospheric temperatures between the spring, summer, and autumn seasons.

In order to assess which parameters may influence the surface contamination with SARS-CoV-2, a Poisson regression model was fitted between the dependent variable "% of swabs positive for SARS-CoV-2 on the day of sampling" and the independent variables:

- Seasonality: the months from May to September 2022 were considered the spring–summer period and the months from October to December 2022 as the autumn period. In particular, the spring–summer period was given a value of "1" and the autumn period a value of "2". The ordinal coding technique [28] was used to make this parameter comparable to the alphanumeric ones.
- Average number of COVID-19 cases among university students in the seven days before sampling.
- Average number of COVID-19 cases among university students in the seven days after sampling.

Seven days represent the mean incubation period of COVID-19 among patients with no severe illness [29].

To estimate which parameters could predict the disease incidence, another Poisson regression model was calculated between the dependent variable "average number of COVID-19 cases among university students in the seven days after sampling" and the independent variables:

- Seasonality: as described in the analysis above.
- Average number of COVID-19 cases among university students in the seven days before sampling.
- % of swabs positive for SARS-CoV-2 on the day of sampling.

SARS-CoV-2 cases were normalized considering the swabs performed on each day of analysis (No. cases/No. swabs performed on the day of analysis) in order to make the data comparable and to avoid bias due to the different numbers of daily swabs.

Data for the number of COVID-19 cases among university students were obtained from the national and regional databases of the epidemiological data on COVID-19 cases [30,31].

Each parameter was normalized to a single comparable unit of measurement (range 0–1) using the following formula [32]:

$$Xn = (Xnn - Min(X))/Max(X) - Min(X)$$

where Xn is the normalized value of each variable for data set n, Xnn is the non-normalized value of each variable for data set n, Max(X) is the maximum value of each variable, and Min(X) is the minimum value of each variable.

Tests were statistically significant with p-value < 0.05. Only parameters/risk factors with a significant p-value were included in the final model.

R software version 4.0.5 was used for all statistical tests (The R Project for Statistical Computing, Vienna, Austria).

3. Results

SARS-CoV-2 RNA was detected on 41/686 (6%) students' desks (Table 1), while all the other surface types were negative. Figure 2 shows the daily distribution of positive samples, restricted to the period ranging from June to November 2022. SARS-CoV-2 RNA was detected during a written exam (43.9%), followed by lessons and a medicine test (17.1% each), a graduation session (14.6%), and a workshop (7.3%). In the positive samples, the mean Ct was 38.04, and the median Ct was 38.09 (range 35.79–39.29). The

positive sample with the lowest Ct (35.79 Ct), examined by the WGS, yielded a genome sequence that passed Nextclade's quality control. In silico analysis of the data obtained with WGS data allowed us to assign this sample to lineage BA.5.1.30 (clade 22B). The genomic sequence has been deposited in the GISAID database and is available under Accession ID EPI_ISL_17716776. The sequencing data obtained from the other positive samples did not meet the quality requirements due to insufficient genomic coverage and a high number of missing nucleotides along the sequence. No cytopathic effect was observed in any of the positive samples subjected to the viral viability test. Table 2 shows the presence of SARS-CoV-2 RNA on student desks by type of material.

Table 1. Number of samples positive and negative for SARS-CoV-2 RNA by type of surface and rate of positivity.

Tested Surface (No.)	Positive (No.)	Negative (No.)	%
Students' desks (686)	41	645	6
Handles (20)	0	20	0
Handrail (11)	0	11	0
Mouse (7)	0	7	0
Keyboard (7)	0	7	0
Teachers' desks (6)	0	6	0
Others (26)	0	26	0
Total (763)	41	722	5.4

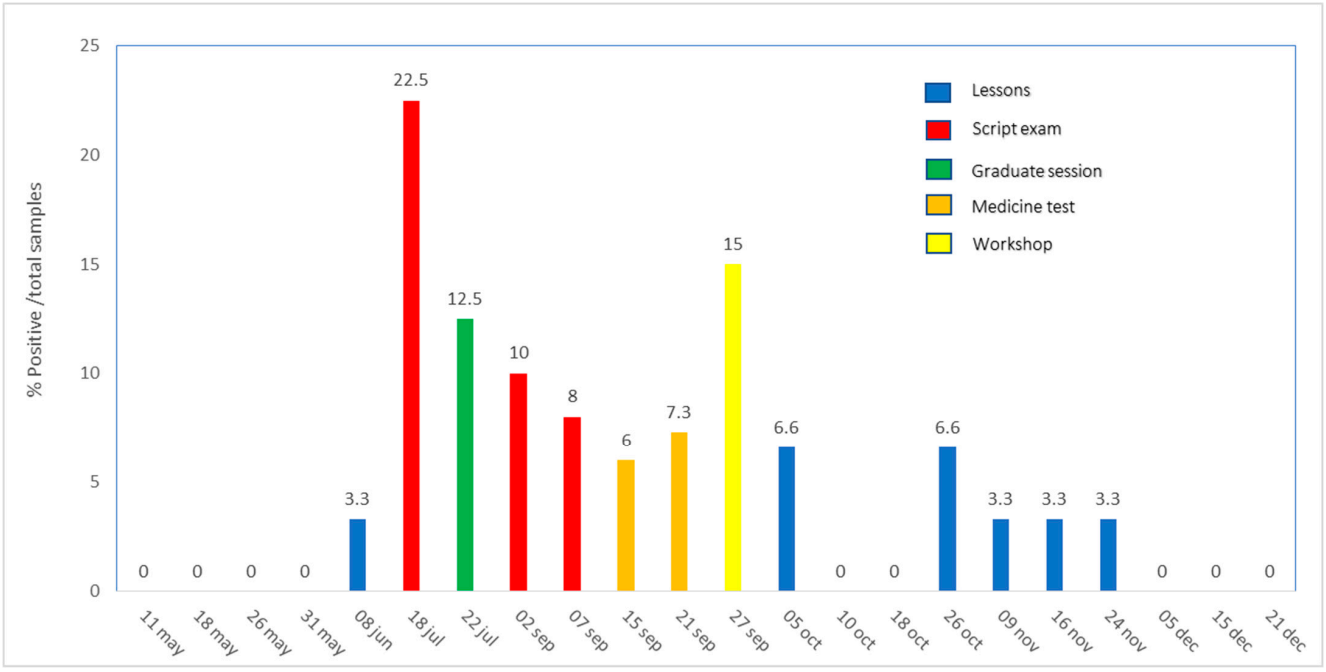

Figure 2. Temporal distribution (%) of positive swab specimens for SARS-CoV-2 RNA during academic events (May–December 2022).

Table 2. Number of surface swabs positive for SARS-CoV-2 RNA, distinguished by type of material.

Material of Surface	Positive/Total Samples No. (%)
Plasticized-wood	32/448 (7.1)
Non-plasticized wood	9/265 (3.4)
Metal	0/36 (0)
Plastic	0/14 (0)
Total	41/763 (5.4)

The application of the chi-square statistic with Yates correction or the Fisher exact test statistic to the sampling results by type of surface and material did not lead to statistically significant results in either case.

Analyzing the results according to the degree of crowding of university staff (students, teachers, administrators, etc.) in the environments (Table 3), a higher level of contamination was found in the larger classrooms (>50 students) than in the smaller ones (less than 50 students) (7.1% vs. 1.5%—chi-square statistic with Yates correction is 7.3304, p-value = 0.006).

Table 3. Number of positive samples for SARS-CoV-2 RNA differentiated by degree of environmental crowding.

Environmental Crowding	Positive/Total Samples No. (%)
classrooms with >50 students	38/534 (7.1)
classrooms with <50 students	3/191 (1.5)
other with <30 academic staff	0/35 (0)
Total	41/763 (5.4)

Regarding the seasonal trend of SARS-CoV-2, higher contamination was found in the spring–summer period (May–September 2022) than in the autumn (October–December 2022), with a statistically significant difference (7.3% vs. 2.4%—chi-square test with Yates correction = 7.6003; p-value = 0.006).

The mean atmospheric monthly temperatures in Apulia from May to December 2022 differed significantly between seasons (spring–summer 24.1 °C [19.3–22.3 °C] and autumn 14.1 °C [10.2–18.1 °C], Mann–Whitney U test $p < 0.05$).

The Poisson regression model (Table 4) showed that there was an inverse proportional association between the percentage of SARS-CoV-2 positive swabs on the day of sampling and seasonality (e.g., the number of positive swabs decreases from spring–summer to autumn). Instead, a direct proportional association was found between the percentage of SARS-CoV-2 positive swabs on the day of sampling and the average number of COVID-19 cases among university students in the seven days following sampling.

The regression analysis reported in Table 5 showed a directly proportional association between average COVID-19 cases among university students in the seven days postsampling (Figure 3) and % of positive swabs for SARS-CoV-2, as well as between average COVID-19 cases in the seven days after and before sampling.

Table 4. Poisson regression model of positive swabs (%) for SARS-CoV-2 RNA.

	Beta	(eβ − 1) = RR (%)	*p*-Value
		Preliminary model	
Intercept	5.622603		<0.001
Seasonality	−3.188502	−95.8	0.04
Average COVID-19 cases among university students in the seven days before sampling	−0.009186	−0.9	0.41
Average COVID-19 cases among university students in the seven days post sampling	0.020769	2.1	0.05
		Final model	
Intercept	5.622603		<0.001
Seasonality	−2.737769	−93.5	0.04
Average COVID-19 cases among university students in the seven days post sampling	0.08199	8.5	0.008

RR, relative risk.

Table 5. Poisson regression model of average COVID-19 cases among university students in the seven days post sampling.

	Beta	(eβ − 1) = RR (%)	*p*-Value
		Preliminary model	
Intercept	5.342		<0.001
Seasonality	−0.01092	−1.1	0.695
Average COVID-19 cases among university students in the seven days before sampling	0.0011	0.001	<0.001
Positive swabs for SARS-CoV-2 (%)	0.01853	0.02	<0.001
		Final model	
Intercept	5.32498		<0.001
Average COVID-19 cases among university students in the seven days before sampling	0.00111	0.11	<0.001
Positive swabs for SARS-CoV-2 (%)	0.01847	1.9	<0.001

RR, relative risk.

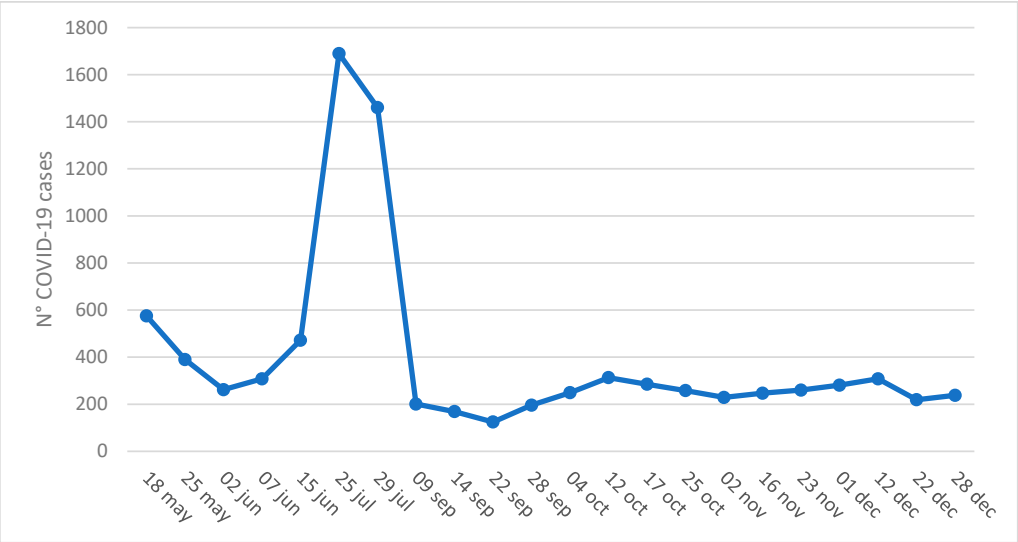

Figure 3. Average COVID-19 cases among university students during the sampling period of May–December 2022 in the seven days post sampling.

4. Discussion

During the COVID-19 pandemic, environmental surveillance was used as a complementary tool to clinical surveillance for SARS-CoV-2 infection. Since the pandemic began, the investigation of wastewater has played a crucial role in environmental surveillance through the application of wastewater-based epidemiology (WBE) [33,34]. To support this type of investigation, surface sampling has been used to increase information on the presence of the virus even in indoor environments [7,9,14,20,22].

Access to the university, which is normally visited by a significant number of students and teachers during the period of academic activities, was limited during the COVID era [35] and, therefore, few studies were carried out for the detection of the presence of the virus in the university environment [3,36].

The SCOOP project aimed to investigate the presence and viability of SARS-CoV-2 in the environment of the University of Bari Aldo Moro during the fifth wave of the pandemic, when face-to-face activities started without the restrictions imposed by the lockdown. To our knowledge, this is the first study carried out in a university setting during the fifth phase of the pandemic.

Our results show a higher viral positivity rate (5.4%) than that reported by other authors [3,36], perhaps because this study was conducted over a longer period (seven months) and included particularly crowded events (e.g., graduation sessions) without the imposition of restrictive measures. According to some authors [37], crowding may be a risk factor for the detection of SARS-CoV-2, so more control and prevention measures, including better ventilation and symptom screening, may reduce viral transmission in various settings [38].

The presence of the virus on surfaces was not viable, probably because the viral load was not sufficient to determine the in vitro infection, but the transit of infected individuals was documented. Indeed, there was a linear correlation between Ct values and the probability of isolating the virus in vitro. Previous studies have shown that at high Ct values (>35), no virus growth was observed [24,39].

Whole genome sequencing allowed the characterization of SARS-CoV-2 in a single positive sample. This characterized strain belonged to the omicron BA.5 clade, one of the lineages circulating during the sampling period [40]. In the remaining samples, sequencing was not feasible due to low RNA genome copies or possible RNA degradation in the samples, as reported in other studies [19,41]. Although sequencing success correlates with the presence of a sufficient amount of virus on the swab and the integrity of the viral genome, this study shows that surface control may reflect the trend of circulating lineages of SARS-CoV-2.

Another interesting aspect of our study relates to the nature of the surfaces tested. The presence of SARS-CoV-2 RNA was detected on both plasticized and non plasticized wood but never on plastic and metal. Some authors have demonstrated viral viability under artificial conditions for up to one day on wood and for longer periods (3/4 days) on plastic [4].

Our study is consistent with regional epidemiological data on COVID-19 cases among university students (https://covid-19.iss.it (accessed on 22 October 2023) [30], which confirm an increase in positive swabs for SARS-CoV-2 in the spring–summer period. Several authors have studied meteorological factors that could influence the spread of the virus, but uncertainties remain on the influence of humidity, temperature, rainfall, and ultraviolet radiation. These uncertainties could be due to high-risk bias related to vaccination practices [42], underestimation of notified cases, asymptomatic patients, geographical differences in the virus variant [43], climate change, and other related factors [44].

Most systematic reviews [45–47] have shown that cool and dry conditions favor the transmission of SARS-CoV-2. The same authors [48] in a previous study—in the same study area (Apulia region) but over a longer period (October 2021–December 2022) (SARI project)—found an inverse correlation between SARS-CoV-2 load in wastewater samples

and mean atmospheric temperature on the day of sampling. No effect on SARS-CoV-2 load was found for rainfall data.

The increase in swabs and positive cases for SARS-CoV-2 in the spring–summer period, particularly the peak in July, despite the higher temperatures, could be explained by the spread of the highly transmissible BA.4/BA.5 subclasses, together with the gradual relaxation or removal of epidemiological restrictions (e.g., wearing a face mask) [49]. This surveillance may be particularly useful during the spring/summer season when there is a strong influx of tourists in the Apulia region, increasing the risk of infection transmission in indoor environments highly frequented by young people such as university students. On the other hand, warm temperatures may lead people to meet in enclosed spaces, increasing the transmission of SARS-CoV-2 [50]. On the contrary, in the months following July, our study shows that there was a decrease in number of positive swabs and COVID-19 cases. In September, after the summer holidays, students return to their studies and spend less time in crowded places (pubs, cinemas, nightclubs, etc.) where the likelihood of infection increases. This phenomenon was also reflected in a decrease in the virus' transmissibility index, known as Rt (Effective Reproductive Number), which represents the number of possible new infections generated per case and can be modified by the application of effective interventions [51–53]. Moreover, the positive association between the percentage of positive swabs for SARS-CoV-2 on the day of sampling and COVID-19 cases among university students occurring in the seven days following the sampling (average incubation period of COVID-19) [29] supports the hypothesis that surface monitoring could have a predictive value for SARS-CoV-2 infection and serve as a complementary method to assess and prevent the spread in selected areas [14].

Our results highlight that good surface and indoor air hygiene practices in public spaces, especially when they are enclosed and crowded, are desirable for the control and prevention of airborne infectious diseases such as COVID-19. This pushed the National Institute of Health [54] in May 2021 to publish some recommendations and updates according to the ministerial circular [55] on the sanitation of non health environments (e.g., schools, offices, etc.) during the COVID-19 pandemic.

5. Conclusions

The surface inspection and environmental monitoring plans during outbreak and epidemic transition phases can be considered a complementary investigation to preventive measures (e.g., disinfection programs and social distancing). Since our surface swab results are directly proportional to the number of COVID-19 cases among university students, environmental monitoring could be a useful tool for predicting disease incidence. This early warning system would allow activities such as face-to-face teaching to resume safely, especially after the declaration of the end of the COVID-19 emergency (in Italy, May 2023).

Author Contributions: Conceptualization, O.D.G., C.B., G.P., S.T. and M.T.M.; methodology, O.D.G., C.B., G.P., S.T. and M.T.M.; software, F.F.; investigation, F.T., F.A., L.C., L.P., H.M. and A.P.; data curation, O.D.G. and F.F.; writing—original draft preparation, O.D.G. and M.T.M.; writing—review and editing, O.D.G., C.B., G.P., M.C., G.C., G.L., A.M., R.D.P., V.T., P.S., S.T. and M.T.M.; supervision, O.D.G. and M.T.M.; project administration, O.D.G.; funding acquisition, O.D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Horizon Europe Seeds (emanated with D.R. 1940 del 04/06/2021), Identification code Project: S52 "La ripresa in sicurezza della didattica in presenza al tempo del COVID: Strategie di COntenimentO di Precisione SCOOP" CUP: H91I21001650006(D.R. n. 1333, 11/04/2022, cofunding D.M. 737/2021).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: We greatly appreciate Carla Maria Leone for providing technical support during the experiments and Andrea Martinelli for helping to find epidemiological data.

Conflicts of Interest: The authors declare no conflict of interest.

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