Case Report

Wind of change: Better air for microbial environmental control

G. Messina [a,b,*], D. Amodeo [b], F. Taddeini [a], I. De Palma [b], A. Puccio [c], G. Cevenini [c]

[a] Post Graduate School of Public Health, University of Siena, Italy
[b] Department of Molecular and Developmental Medicine, University of Siena, Italy
[c] Department of Medical Biotechnologies, Bioengineering Lab, University of Siena, Italy

ABSTRACT

Background: The COVID19 epidemic highlighted the importance of air in the transmission of pathogens. Air disinfection is one of the key points to reduce the risk of transmission both in the health sector and in public, civil and industrial environments. All bacteria and viruses tested to date can be inactivated by UV-C rays. Laboratory tested UV-C systems are increasingly popular and proposed as effective technologies for air purification; few studies have evaluated their performance in populated indoor environments. The aim of this investigation was to evaluate the effectiveness of a UV-C disinfection system for air in a real working context.

Methods: This experimental study was conducted between December 2020 and February 2021 in an office of the Department of Molecular and Developmental Medicine of the University of Siena, Italy. A pre-final version air purifier (Cleaning Air T12), capable of treating 210 m^3/h of air, was first tested for its ability to filter particulates and reduce microbial air contamination in the absence of people. Subsequently, the experiments were conducted in the presence of 3–5 subjects who worked for several hours in an office. During the tests, microbiological samples of air were collected in real time, switching the system on and off periodically. Air samples were collected and incubated on Petri dishes at 36 °C and 22 °C. Statistical analysis was performed with Stata 16 software assuming a significance level of 95%. An interpolating model was identified to describe the dynamics of contamination reduction when the device operates.

Results: Preliminary tests showed a significant 62.5% reduction in Colony-Forming Units (CFUs) with 36 °C incubation. Reductions in the particulate component were also observed. In the main test, comparison of CFU data, between the device-on phase (90 min) and the subsequent device-off phase (60 min), showed statistically significant increase (p = 0.001) of environmental contamination passing from a mean of 86.6 (65.8–107.4) to 171.1 (143.9–198.3) CFU/m^3, that is a rise of about 100%. The interpolating model exhibited a good fit of CFU reduction trend with the device on.

Conclusions: The system, which mainly uses UV-C lamps for disinfection, was able to significantly reduce environmental and human contamination in real time. Experimental tests have shown that as soon as the device is switched off, after at least half an hour of operation, the healthiness of the air decreases drastically within 10 minutes, bringing the airborne microbial contamination (induced by the presence of operators in the environment) to levels even higher than 150% of the last value with the device on. Re-engineering strategies for system improvement were also discussed.

1. Introduction

The Sars-Cov2 pandemic has endorsed how essential air disinfection is, especially, in closed and crowded environments where airborne pathogen transmission is facilitated. Commonly, airborne diseases are transmitted by inhalation of small airborne particles, typically 1 to 5 μm in diameter, which can be generated by coughing or sneezing [1]. Several guidelines and standards have been published by various organisations with expertise in air and surface disinfection, such as the AIA and ASHRAE, to assist in the design and construction of healthcare facilities. These standards also regulate microbiological monitoring, defining the limits of surface and air concentrations and the types of species detected in different areas of the hospital. These criteria are valuable tools in identifying potential problems and determining the success of decontamination efforts in hospital environments [2]. The situation is different for non-hospital indoor environments, where

current legislation is not very specific on monitoring actions and up-to-date on the definition of the microbiological risk of bioaerosols, the most recent references to which date back to the late 1990s. At present, the limit still in force for bacteria and moulds in non-hospital indoor environments is in the range of 500–1000 CFU/m^3 [3]. While this approximation is justified by the fact that the presence of bacteria, moulds and viruses in indoor environments does not necessarily imply the onset of human infections (except for the immunocompromised), it also clashes with what has become an issue of growing public interest, driven also by the emergence of new indoor air pollutants and the current pandemic period. In air-conditioned buildings, several studies have shown how water or condensation in ventilation systems can act as a breeding ground for harmful bacteria that are then dispersed through the ventilation process. The effects on health are different. When high counts of environmental bacteria, such as *Staphylococcus epidermidis*, Micrococcus and Flavobacterium, are found scattered on skin flakes, these can be considered indicators of inadequate ventilation [4]. Researchers have suggested that gram-negative bacteria, such as *Pseudomonas aeruginosa*, or other bacteria such as thermophilic actinomycetes, *Streptomyces albus*, *Bacillus subtilis* and complex communities of microorganisms in biofilms are aetiological agents of hypersensitivity pulmonary disease [5,6]. However, it should again be emphasised that their presence does not directly imply the occurrence of human infections. In closed environments, the bacterial load is naturally higher in crowded places and should not always be understood as a direct indicator of health risk, but aids as a screening test for further investigations to prevent health risks for users and limit the possible spread of infections.

In indoor environments, air conditioning systems must ensure a low relative humidity compare to the outdoor, to minimise and prevent the microbial growth. ASHRAE Standard 62.1–2016 recommends maintaining the relative humidity of occupied spaces at 65% or less [7]. Meanwile, for indoor air disinfection, several procedures depend on the needs of each specific context in which they must be implemented [8]. The most common methods used to reduce airborne contamination in enclosed spaces are dilution and removal of infected air particles through ventilation, which can be natural or mechanical [9,10]. However mechanical ventilation are not designed for airborne infection control, except in hospital with airborne isolation rooms built for that purpose [11]. The search for new unconventional approaches, which can reduce airborne contamination in crowded indoor environments, should be encouraged and considered as a top priority for research and development projects [12]. Among different approaches, UV-C generated by lamps, LEDs and recently also by innovative Chips [13], can easily inactivate almost all microorganisms, causing irreversible DNA damage [14–17]. On the other hand, it is well known that UV-C rays can cause severe damage to human health and that it is absolutely necessary to avoid exposing eyes and skin to them [18–20]. Several researchers have studied the mechanism of action and susceptibility to UV-C radiations of microorganisms, defining dose thresholds and strategies to make the best use of this type of radiation [21–25].

UV-C light has been used extensively as a disinfection approach in healthcare and to maintain ultra-clean environments [26–28]. We can divide instruments that operate on the basis of UV-C radiation into direct-acting devices, in which UV-C light is guided directly to the surface, and flow devices, in which UV-C emitters are hidden inside the casing, through which the air is conveyed [29].

The UV-C approach to air disinfection is not new in the modern purification device market. However, as already mentioned, the SARS-CoV-2 pandemic has significantly increased the demand for these systems even for non-hospital indoor environments. Studies on the microbicidal performance of UV-C radiation on bioaerosols (by SARS-CoV-2 and others) have strongly catalysed interest in disinfection devices equipped with this type of radiation [30]. Indeed, the potential of indoor air to be a vehicle for the spread of infectious pathogens has led to the development of new and efficient decontamination technologies and methods, including germicidal UV-C irradiation devices [31]. UV-C rays

have the considerable advantage of not polluting and being effective on all microorganisms, even those resistant to antibiotics and chemicals [32]. On the other hand, the direct impact of UV radiation can damage and degrade materials [33]. In addition, UV-C air purifiers can release ozone and oxygen free radicals into the air, the higher indoor concentrations of which can cause dangerous irritation to the human conjunctiva and the mucous membranes of the respiratory tract [34].

The effectiveness of air purifier with UV-C in real-life contexts is missing. This study aims to evaluate the effectiveness of a high-flow (210 m^3/h) UV-C air disinfection system in an office with a volume of about 65 m^3. Investigation of the performance of the system, which can be classified as an inactivation device with a predominantly UV-C disinfectant effect, will allow discussion of its pros and cons in relation to mechanical filtration devices and combined devices (filtration plus UV-C), in real indoor populated areas.

2. Materials and methods

This experimental study, with analytical content, was conducted between December 2020 and February 2021 in the Department of Molecular and Developmental Medicine at the University of Siena, Italy.

2.1. The UV-C air disinfection system

The device used for the tests was a pre-final version of the Cleaning Air T12 air purifier, by ISO ITALIA GROUP S.R.L. It was made up of 3 parts: a) the lower base, which allows air to enter; b) a central germicidal chamber, with 12 UV-C lamps (254 nm); c) 2 grids, in the upper part, for the outlet of the air treated by UV-C rays. The central chamber is divided into two independent channels that allow air to flow from the lower inlets to the upper outlet grids after UV-C irradiation. A coarse anti-dust pre-filter was placed on the air inlet, while on the outlet grid there was no filtering system. The volume of air treated was 210 m^3/h. For preliminary tests, ad-hoc canalisations were placed on one of the air inlets and the outlets of the device to effectively direct the airflow, so that the air samplers were affected as little as possible by turbulence and external pollution (Fig. 1).

The UV-C lamps are arranged in pairs, within three compartments (A,B,C), into two irradiation chambers, on sides 1 and 2, as shown in Fig. 2. The two chambers, containing six UV-C lamps each, are not communicating and acts in parallel. The incoming air (from below) flows through the three compartments in a coil, exiting through the top grid.

2.2. Equipment and studied parameters

The following testing devices, software and consumables were used for the study: Plate Count Agar (PCA), SAS Microflow α Aquaria (Aquaria, Lacchiarella, Italy), Climet CI-550 particle counter (Climet, Redlands, USA), Spectrophotometer Avantes model AvaSpec-ULS2048CL-EVO-USB3 (Avantes, Apeldoorn, Netherlands), Microsoft Excel 2016 and STATA SE/16.0 (StataCorp. 2015. Stata Statistical Software: Release 16. College Station, TX: StataCorp LP), Matlab software, version R2021 for the estimation of parameters of polynomial functions.

2.3. Study design

The experimental study was organised in two stages: a preliminary stage and a main stage.

The preliminary stage was conducted in the Environmental Hygiene Laboratory of the Department of Molecular Medicine and Development of the University of Siena, in the absence of working subjects in the room, to evaluate the percentage of reduction in air contamination due to generic microorganisms (bacteria and fungi), mainly human commensals and/or environmental species. The Cleaning Air T12 was placed

Fig. 1. Ad hoc setting of the preliminary stage: the microbiological air samplers were placed next to the inlet and on the outlet grid of the air disinfection system.
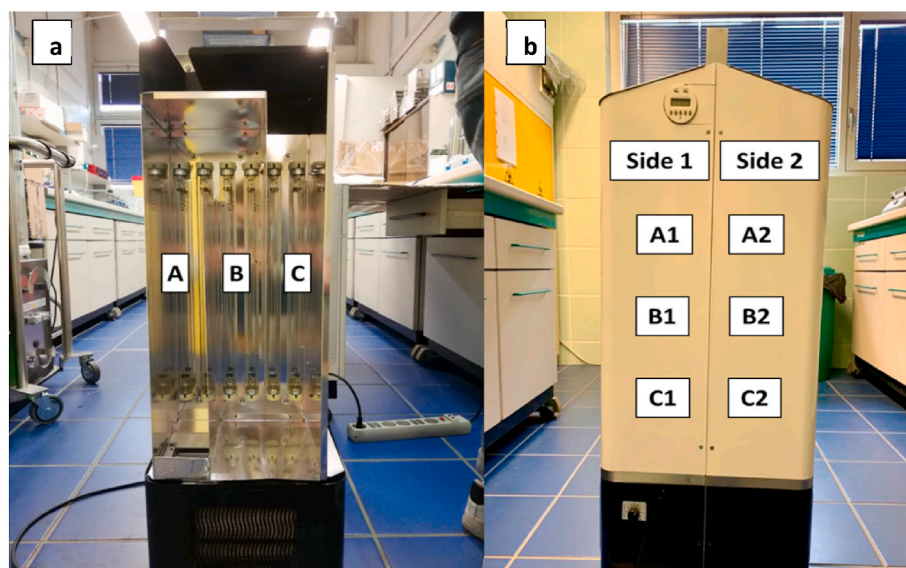

Fig. 2. (a) Front view of the device without the metal cover of the irradiation chamber. **(b)** Lateral view of the device, showing the two separated irradiation chambers.

in the center of the laboratory and switched on during working hours for two days.

The incoming and outgoing air was sampled simultaneously with two microbiological air samplers SAS Microflow α Aquaria. We sampled the untreated air close to the inlet grid and the UV-C treated air from the outlet grids (Fig. 1). For each sample, 1 m^3 of air were aspirated with SAS at a flow rate of 120 l/min and collected in 60 mm Ø Petri plates (Fischer Scientific, Waltham, United States), with PCA medium (Oxoid, Basingstoke, United Kingdom). All samples were incubated aerobically at 36 °C, to allow the growth of mesophilic microorganisms (mostly bacteria), and at 22 °C, for the growth of psychrophilic microorganisms (fungi and bacteria). Colony Forming Unit (CFU) count was performed using a manual colony counter.

In addition, the particulate matter (>0.3, >0.5, >1.0, >3.0, >5.0 and > 10 μm) was measured with a Climet CI-550 by placing the detection probes close to both the inlet and the outlet grid of the device. The particulate sampling device performed 20 air samplings of 1 minute each (28.3 l/min), from the inlet and outlet grid of the disinfection system. The particulate matter measurements were carried out to characterise the anti-dust prefilter installed on the device. As a preliminary step, we wanted to estimate the filter effect on the reduction of airborne particles.

Finally, photometric measurements, as radiant flux, R (mW), and

irradiance, I (mW/cm^2), of the internal irradiation chamber of the device were taken at six different positions, three on each side, at the maximum distance of 20 cm from the UV-C lamps and approximately halfway along their longitudinal axes (see Fig. 2b).

Assuming that each particle or microbe moves in laminar motion at a constant speed along the radiant duct, for each compartment of the irradiation chamber, its average dwell time in the air, t_d (sec), can be estimated as:

$$t_d = l_l S / Q \tag{1}$$

where l_l (cm), S (cm^2) and Q (cm^3/s) are the lamp length, the cross section and the air flow, respectively.

Therefore, the average UV-C dose, D (mJ/cm^2), that reaches each particle is:

$$D = t_d I_m \tag{2}$$

where I_m is the mean spherical irradiance in the duct, which can be calculated as the average of the doubling of the irradiance distribution over various cross sections of the duct. The assumption of the spherical irradiance as the double of the surface irradiance was made to account for the volumetric size of the particles (modeled as spherical) on which the UV-C energy has impact on at least two sides (front-back).

The main experimental stage was to evaluate the device performance in a office with a volume of about 65 m^3, in presence of people, for four days. The Cleaning Air T12 was placed in an office located in our Department. Air samples were collected during the daily work routine of the personnel. For the first 3 days, to assess the level of air contamination in the office and obtain a reliable baseline, air samples were collected for 100 min inside the office with the device switched off. Also, for each days, we changed the number of people inside to evaluate the impact of this variable on the contamination level of the office. At the beginning of each sampling, one of the operators noted the actions and behaviors of the people inside the office, so that any fluctuations in air contamination could be related to them. In particular, the separate contribution of the following main sources of variability on the level of microbial contamination in the real environment was assessed: i) different number of people present in the office, ii) changes in the state of isolation of the environment; iii) free actions and behavior of the internal staff of the office.

On the fourth day (the last one) the most complete final experiment was performed, with the device initially switched off for 16 minutes (phase 1), then switched on for about 100 minutes (phase 2) and, finally, again switched off for about 60 minutes (phase 3).

To simulate a real context, with the necessary safety conditions during the CoViD period, the occupants were equipped with surgical masks for the entire time of the investigations. There were two desks and five chairs, two doors and a window which were kept closed during the experiments. Each of the experiments carried out in the four days of the experimental phase lasted a total of about 3 hours. Samples of 1 m^3 of air were aspirated with two SAS Microflow α, at a flow rate of 120 l/min, and collected in Petri dishes Ø 60 mm, with PCA medium. The selected flow rate is the maximum allowed for our SAS samplers. By choosing a sampling period of 8.20 minutes, we have thus achieved a fair compromise between the two opposite requirements for a rather high volume of sampled air (about 1 m^3/h), to find more CFUs in the culture plates, and a sufficient number of sampled values throughout the experiment, for statistical purposes. After sampling, the plates were removed and incubated for 48 hours at 22 °C and 36 °C. Counting of colony forming units (CFUs) was performed using a manual colony counter.

2.4. Statistical analysis and data processing

Microsoft Excel 2016 software was used to organize the empirical air contamination data, as CFUs, into a database and for descriptive statistics. Differences between sampling values of CFUs with device off and on were evaluated with Student's parametric *t*-test or Mann-Whitney's nonparametric test, depending on whether the data distribution was normal or nonnormal, respectively. Normality was tested with the Shapiro-Wilk test. Inferential statistical analysis was carried out using Stata software, version 16, choosing a 95% significance level (p<0.05). The 95% confidence intervals were also evaluated for each statistical sample estimate.

An iterative least squares method was used to interpolate the decreasing contamination level with the device turned on. Iteratively, for each subsequent sampled CFU value, a quadratic polynomial function was identified that minimised the sum of Euclidean distances in a five-points window centered around the current point.

Specifically, at any sampling point i, we solved numerically the following minimisation problem:

$$\boldsymbol{\beta}_i = \arg \ \min_{\beta} \sum_{k=i-2}^{i+2} \left[CFU_k - f_k(\boldsymbol{\beta}_i) \right]^2 \quad \text{for } \forall i \in \mathbb{Z} \ \{ 2 \leq i \leq N \} \tag{3}$$

N is the number of sampling points;

$\boldsymbol{\beta}_i = (a_i, b_i, c_i)$ is the parameter vector of the polynomial function:

$$f_k(\boldsymbol{\beta}_i) = t_k^2 a_i + t_k b_i + c_i \quad k \in \mathbb{Z} \ \{ i-2 \leq k \leq i+2 \} \tag{4}$$

$t_k = T \cdot (k\text{-}i)$ is the discrete time at the kth instant centered around i, so that $t_k = 0$ for $k = i$;

T is the sampling time.

The "lsqnonlin" function of Matlab software, version R2021, was used to estimate the parameters of the polynomial function of eq. 4.

The root mean square error, RMSE%, as a percentage of the value estimated by the interpolating function, was calculated to assess the goodness of curve fit by the data:

$$\text{RMSE\%} = \sqrt[2]{\sum_{i=2}^{N} \frac{1}{N} \left[\frac{CFU_i - f_i(\boldsymbol{\beta}_i)}{f_i(\boldsymbol{\beta}_i)} \right]^2} \times 100 = \sqrt[2]{\sum_{i=2}^{N} \frac{1}{N} \left[\frac{CFU_i - c_i}{c_i} \right]^2} \times 100 \tag{5}$$

In the device-off phase (phase 3), the data were approximated by their mean.

3. Results

The CFU reductions have been represented in percentages. Given the low abatement values obtained, we preferred to adequately quantify the decontamination effect of the device through percentage reduction values, as reporting these abatements on a logarithmic scale would have made the real extent of the reduction less immediate to read.

3.1. Preliminary stage

36 Petri dishes were used in this stage. After treatment with UV-C radiation, the laboratory test results have shown a significant mean CFU reduction (p = 0.0023) of 51 CFU (±34.9 SD) (95% CI 24.3–78.0) corresponding to a 33%, for samples incubated at 22 °C, and a significant mean CFU reduction (p = 0.001) of 77.9 (±46.5 SD) (95% CI 42.2–113.6), corresponding to a 62.5%, for samples incubated at 36 °C.

Ten consecutive measurements of the particulate matter were made in the pre/post air treatment, using the particle counter near the lower inlet and upper outlet of the device. A statistically significant difference was always found between particle means (p<0.001). Results are shown in Table 1. These results are coherent with the performance of a class G4 filter, typical anti dust filter class.

The averages of irradiance, calculated through 10 points, sampled at the maximum distances from the UV-C lamp (worst scenario of minimum irradiance), on both sides of the device (see Fig. 2), are given in Table 2. Therefore, the minimum surface irradiance value, averaged over the six compartments, can be calculated as 6.5 mW/cm^2 and consequently the mean spherical irradiance, estimated as its doubling, is $I_m = 13.5$ mW/cm^2.

For each of the two sections of T12: the output air flow rate is $Q = 105$ m^3/h = 29,167 cm^3/s; the cross-sectional area of the air duct, excluding the part occupied by the lamp, is approximately $S = 160$ cm^2; the length of each lamp is $l_l = 45$ cm, so that for the three compartments in series, the total length of the irradiated section is $3l_l = 135$ cm. Therefore, In the worst conditions of minimum irradiance, the average air dwell time, t_d, calculated from eq. (1), is about = 0.75 seconds. From eq. (2), the minimum average spherical dose is $D = 9.75$ mJ/cm^2.

The maximum distance of the lamp from the farthest wall of the duct is 20 cm, but the irradiation surface, due to the geometry of the system, actually lies in the range of 16–20 cm, i.e. on average 18 cm. The average distance from the lamp in the remaining range of 80% of the volume of air in the duct (which lies between 0 and 16 cm) is clearly lower and can be roughly approximated to 8 cm. Therefore, taking into account the law with which the light energy is distributed around the emission source, inversely proportional to the square of the distance, in this interval, the multiplication factor of the previously calculated minimum average dose, in the absence of reflections, is approximately 5. Taking prudentially account of a moderate reflective effect of the walls, it is reasonable to assume that this multiplication factor is in any case

Table 1

Analysis of particulate matter in pre/post treatment air.

Particles	Particles mean PRE (DS)	Particles mean POST (DS)	Particles Mean Diff	95% Confidence Interval	Mean reduction %
>0.3 μm	2,959,998 (49,177)	2,190,370 (320,721)	769,628	549,190–990,066	26%
>0.5 μm	834,721 (47,050)	576,661 (35,331)	258,060	236,622–279,498	31%
>1.0 μm	180,009 (36,996)	96,556 (5,993)	83,452	57,211–109,694	46%,
>3.0 μm	5,920 (1,869)	1,708 (454)	4,212	2,789–5,635	71%
>5.0 μm	3,557 (1,210)	1,116 (345)	2,441	1,453–3,429	69%
>10 μm	1,391 (499)	500 (193)	891	457–1,326	64%

Table 2

Photometric measurements at 20 cm distance from the UV-C lamps.

Side	Compartment	Radiant flux (mW)	Irradiance (mW/cm2)
1	A1	0.707	5.92
	B1	0.897	7.50
	C1	0.785	6.60
2	A2	0.658	5.52
	B2	0.877	7.34
	C2	0.741	6.22

3–4. Therefore, 80% of the air volume is at an average irradiance of 30–40 mJ/cm^2.

In a nutshell, since the distances from the lamp have a high range of variation (0–20 cm), the range of variation of the dose distributed in the duct will also be high: it is estimated to be approximately between 10 mJ/cm^2 (at the farthest point) up to well over 100 mJ/cm^2 (in the immediate vicinity of the lamp).

3.2. Main stage

On the first, second and third day of samplings, when the device was always switched off, in plates incubated at 22 °C, we obtained an average microbial load of 186.8 (95% CI 154–219), 93.6 (69.6–117.6) and 152 (124–180.0) CFU/m^3, respectively. Samples incubated at 36 °C had an average microbial load of 161.1 (95% CI 131–191.0), 107.8 (78.7–137.0) and 181 (151.7–212.0) CFU/m^3. The lower microbial load found on the second day is consistent with the presence of only three office occupants, instead of four on the first and third days.

Table 3 shows the data collected during the 4th day in which the room was populated by 5 people and the device switched on.

On the last day, the first two sampling values at device off (phase 1), gave a CFU growth at 22 °C significantly higher (225 and 189 CFU/m^3) than the mean device-on (phase 2) value of 118.8 (95% CI 94.5–143) CFU/m^3.

After six sampling times from the device switched on, we could appreciate that the average level of microbial contamination decreased significantly with respect to the first six samples (p = 0.001), reaching a mean of 70.3 (63.5–77.1) CFU/m^3 during the last seven samples. In addition, in the device-off phase 3, we had a significant increase in CFU. The mean contamination value passed from 86.6 (65.8–107.4), in phase 2, to 171.1 (143.9–198.3) CFU/m^3 in phase 3, that is an increase of about the 100%.

A similar mean increase was experienced with samples incubated at 36 °C; we passed from 137.1 (111–162.8), in phase 2, to 259.4 (228.0–290.8) CFU/m^3 in phase 3, showing an average increase of 122.3 (83.9–160.7) CFU/m^3, equal to about the 90%.

Fig. 3 shows the CFU of the air sampled and incubated at both 22 °C and 36 °C during the three phases of the 4th day.

To model the device performance during the sampling procedures, in which the device went from off (phase 1, 2 points, 16 minutes) to on (phase 2, 13 points, 1.7 hours) and back to off (phase 3, 8 points, 1 hour), we interpolated the device-on CFU data (phase 2) with the non-parametric iterative least-squares method described by eqs. 3-5 (Fig. 4). In order to adapt the interpolating mathematical model to all

Table 3

Experimental data collected in the fourth day of the main experiment stage.

Air sample	Device status	Time (hh:mm)	Incubation at 22 °C (CFU)	Incubation at 36 °C (CFU)	People actions and/or behaviour
1	OFF	00:00	225	177	people chattering
2	OFF	00:08	189	229	people chattering
3	ON	00:16	163	240	people are silent
4	ON	00:24	101	163	people are silent
5	ON	00:32	28	121	people are silent
6	ON	00:40	106	163	people are silent
7	ON	00:48	129	120	people chattering
8	ON	00:56	107	151	people chattering
9	ON	01:04	69	165	people chattering
10	ON	01:12	83	125	people chattering
11	ON	01:20	68	139	people chattering
12	ON	01:28	74	60	people chattering
13	ON	01:36	60	124	people chattering
14	ON	01:44	65	112	people are silent
15	ON	01:52	73	99	people are silent
16	OFF	02:00	116	300	people are silent
17	OFF	02:08	204	263	people are silent
18	OFF	02:16	205	313	people are silent
19	OFF	02:24	152	245	people are silent
20	OFF	02:32	143	216	people are silent
21	OFF	02:40	171	218	people chattering
22	OFF	02:48	176	232	people chattering
23	OFF	02:56	202	288	people chattering

the N = 13 points of phase 2, it was necessary to have 2 points before the first and two points after the last. The 2 previous points were taken exactly those in phase 1, considering them properly as initial values with the device turned off. For the next 2 points it was decided to replicate the CFU value measured in the last sampling.

Both curves show a good fit to real data: RMSE% of 24.5% and 28.8% at 22 °C and 36 °C, respectively. The mean levels of contamination in the last eight CFU sampled values when the device was turned OFF (phase 3) were 179 and 259 at 22 °C and 36 °C, respectively. Despite the critical and continuous environmental contamination caused by the
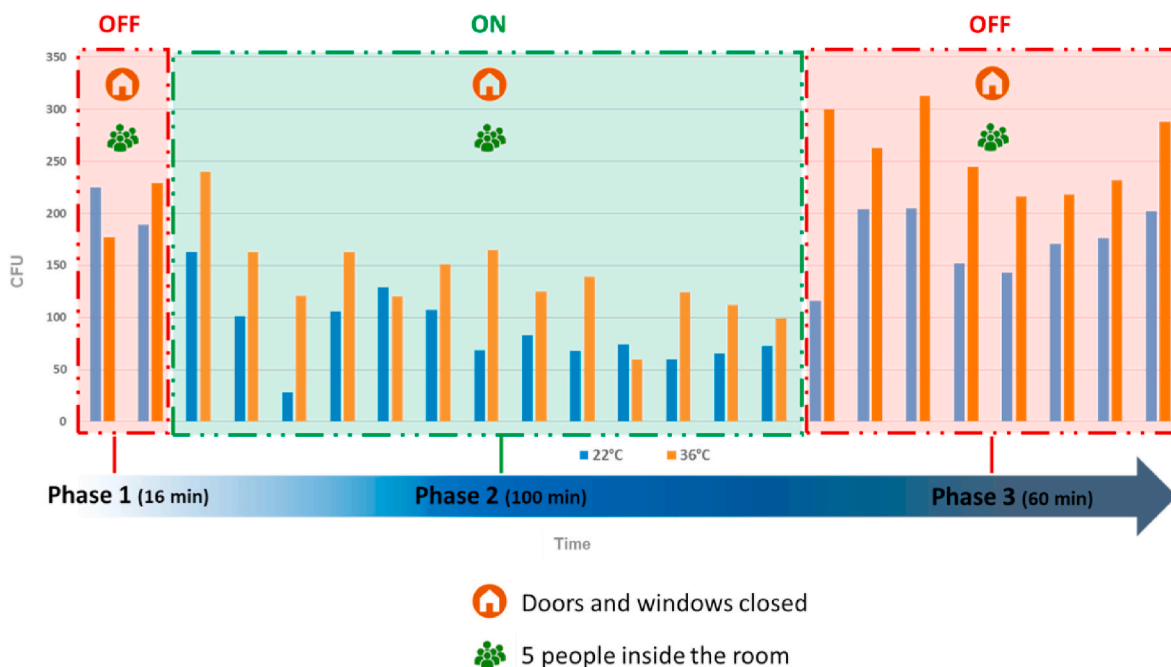
Fig. 3. Main stage: day-four graphical representation of the CFU growth on plates incubated at 22 and 36 °C during the 3 phases of the study.
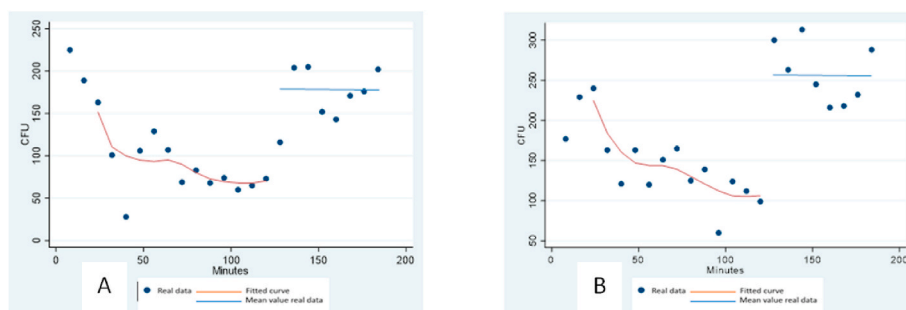

Fig. 4. CFU growth at 22 °C (A) and 36 °C (B): real data and their fitted curves during the 3 phases of day 4.

simultaneous presence of five people in a room of only 65 m³, with doors and windows closed during the whole experiment, we can note that, after just 8 min (only one sampling time) from switching on the device, the system significantly acts on the reduction of environmental contamination, reaching a drop in CFU growth of about 50% in the plates incubated at 36 °C and of about 70% at 22 °C. Finally, an increase in CFUs of about 150% is observed (for both temperatures), from the final value of the phase 2 and the average value of the CFUs during the phase 3 (Fig. 4a and b).

4. Discussion

In the current pandemic period, there is a growing need for innovative tools to limit the spread of infectious diseases in the air, especially in confined environments with large numbers of people. As a result, the market is trying to cope with a growing demand for these devices, which are now found in many everyday contexts. Furthermore, since 2008, huge economic resources have been invested in the disinfection of surfaces and air, considerably strengthening the research and development departments related to microbicidal technologies for interiors [35]. This has led to some speculation about the actual disinfection capabilities claimed by the promoters of these technologies.

Indeed, many air purification devices that use HEPA (High Efficiency Particulate Air) filters or UV-C radiation are only tested in the laboratory. The ability of these devices to reduce air contamination is generally verified by experiments performed according to ISO standards [36]. We believe that these should only be considered as the beginning of a verification process, especially if the expectations from the device are those of a reduction in the risk of infection in real environments; otherwise, an illusion of security is created which can have negative or even counterproductive repercussions for the user. The microbicidal efficacy of such devices cannot be certified simply by this type of 'in vitro' test because, in a real context, several variables (such as the size of the environment, the presence or absence of people, etc.) can strongly affect the performance of such systems and reduce user safety. This should not be underestimated, also for the purpose of correct scientific and commercial communication, as it can instill false confidence and misinformation on users [37]. The strength of our study is to present the results obtained by the use of a UV-C air purifier, in an indoor context, analyzing the fluctuations of environmental contamination with respect to the variables present in the environment in which the experiments were conducted.

In both the preliminary and main stages of our study, the Cleaning Air T12 pre-final device was able to significantly reduce and control microbial contamination of the air. In fact, in the preliminary stage, the data show a significant decrease in particulate matter and a reduction in CFUs after treatment with UV-C: by 33% for plates incubated at 22 °C and by 62.5% for plates incubated at 36 °C. This is relevant because it

indicates that the system is able to control all human bacterial flora. These data were further confirmed in the main stage carried out in an office of our Department where the device has progressively reduced and controlled, over time, the contamination of a 65 m³ volume office, completely closed and isolated, inside which there were five subjects. As shown in Fig. 2, there was a significant rapid decrease in CFUs after the device was turned on (already by about 50% after 8 minutes), followed by an equally large (90–100%) increase in CFUs on shutdown. After at least half an hour of operation, as soon as the device was turned off, the healthiness of the air dropped dramatically within 10 minutes, bringing the levels of microbial contamination (induced by the presence of the operators in the room) to levels even higher than 150%.

In this work we have highlighted how, beyond the triumphal declarations that come from the various manufacturers of air purifiers in creating 'safe' environments by reducing the transmission of the infectious risk of many logs$_{10}$. Indeed, these systems allow only minor and partial reductions in the risk of contagion and contaminants in the air. It might seem reductive to be able to reduce airborne contamination by values between 50% and 70%, respectively, the microbial flora that grows at 36 °C and 22 °C. Conversely, in real environmental contexts, this represents a result of certain interest which must however be accompanied by an adequate correct communication process for users. Otherwise, such systems can paradoxically become sources of risk, mistakenly suggesting that their presence allows all other preventive defenses to be lowered.

There is no system that is 100% safe, neither able to reach 99.999% reduction in a real-world context. As an example, it is interesting to analyse the standards for operating rooms [38]. The contamination of 35 CFU/m³ is considered acceptable in operating theaters in 'at rest' conditions, that is, without active personnel, but ready to operate; the values increase to 180 CFU/m³ 'in operation' for turbulent flows and instead decrease to 20 CFU/m³ for operating rooms equipped with laminar flows. We also consider that operating theaters are already in themselves extremely controlled contamination environments due to the clothing used, the disinfection protocols, the hand washing and, most of all, the powerful ventilation systems that treat air through HEPA filters and must guarantee at least 15 air volume replacements per hour (in particular surgical contexts, even up to 40). However, even under such conditions, zero risk does not exist. Although civilian environments certainly cannot be compared to operating theaters, it still becomes difficult for far less performing systems to guarantee what some manufacturers ambiguously claim for their products.

It is becoming increasingly evident that the control of environmental contamination requires a combined approach of several types of technologies, and in any case, where these constitute only a few variables to be integrated into a more complex system that is articulated on other items, such as staff training, good hygiene practices and type of risk to which one is subject [39,40].

UV-C lamps are already in use in operating rooms to reduce air contamination [41]. Lee et al. evaluated the effectiveness of fixed ultraviolet germicidal irradiation (UVGI) air purifiers in restaurants, offices and meeting rooms: it was found that this tool can reduce bactericidal contamination in the air by 73% (p <0.0001) [42]. The study by Guimera et al. demonstrated that the in-chamber UVGI device reduces bacterial contamination by 62% [42]. This is in agreement with Ritter et al. who conducted a study comparing rooms with UV lighting and no UV lighting during total joint arthroplasty (TJA) procedures for 19 years. Their results revealed that rooms without UV radiation were 3.1 times more likely to be infected than rooms without UV radiation (p <0.001) [28]. Other studies conducted in hospitals have shown the effectiveness of UV tools to break down bacterial contamination in acute care. Others have shown a reduction in health care-associated infections through devices that use UV rays [43].

Due to its significant impact on the management of airborne infections in hospital settings, the aforementioned literature also has an important communicative and economic value on portable indoor disinfection systems. In fact, thanks to the scientific literature and the advent of SARS-CoV-2, these devices are now widespread and commonly used in crowded indoor environments. As already mentioned, this has led to considerable questions about the correct use and the actual miraculous performance of these portable devices. In our opinion, the combination of laboratory tests, which follow precise ISO standards, with experiments in real and diversified contexts could allow a more transparent and honest declaration of the real biocidal capabilities of such portable devices.

The design phases of these devices are also fundamental, which must take into account a multiplicity of characteristics and components in relation to the application objectives. The optimal design must take into account the number, type and location of light sources, the geometry of the reactor, the use of UV reflective materials, the air flow rate, the desired level of disinfection and the speed to achieve it, the efficient integration of different disinfection techniques, including mechanical filters. To this end, some studies already combine photoradiometric simulation models with empirical laboratory experiments to correlate the exponential relationship of the microorganisms' response to UVGI radiation and predict the effectiveness of control devices [44,45]. Additional elements to consider for a suitable and concrete usability of air purifiers are their acoustic performance, comfort, safety in management, protection against vandalism and the limitation of harmful by-products.

The tested device also meets these criteria.

For a specific application such as the one described in our study, it is particularly important to consider the number of air volumes in the room treated in each hour. Recent recommendations with regulatory perspectives indicate how a good system that operates in a closed unventilated space should be able to reduce CFUs by at least 90% in half an hour [46].

The pre-final version of the Cleaning Air T12 air purifier is not very far from this target (see Fig. 4). However, to fully achieve the goal and/or to ensure that it can be met even in larger environments, the pros and cons of various alternative or supplementary measures can be assessed, essentially distinguished according to three different re-engineering strategies:

1) Strengthening and optimizing the current system. Use of more powerful UV-C lamps, or more in number or better positioning them more centrally in the irradiation chamber; improvement of light reflection from the walls of the UV-C reactor using technical coating materials with high reflective capacity, improvement of the internal fluid dynamics of the air by introducing geometries in the ducts to favor the laminarity of the air flow, thus increasing the flow rate. The main advantages of this strategy are an increase in flow and maybe the containment of acoustic noise. The main disadvantages are a considerable increase in cost and complexity, and major security problems.

2) Elimination of UV-C sources and their replacement with a purely mechanical high-power filtering system (HEPA filters). The advantages lie in the elimination of the risks associated with accidental exposure to UV-C radiation, the possibility of reaching high levels of disinfection in a relatively short time, but at the expense of the need to introduce significantly more powerful engines to maintain high flow rates. This involves greater energy expenditure, a considerable increase in costs, including maintenance costs, due to the need for periodic replacement of the filters, and a possible increase in acoustic noise.

3) Combined system with the addition of filters with intermediate filtering power. The advantages lie in the significant reduction of the number of UV-C lamps with consequent lowering of cost, in the greater rapidity to reach high levels of disinfection, in the disinfection of the filters through UV-C giving them a longer duration and a consequent reduction of maintenance interventions, replacements of the lamps included. The disadvantages are similar to those of point 2,

but much lower, due to the use of less performing and less expensive filters, with less need of maintenance and spare.

The third strategy seems to us the most effective, but it requires a high level of competence in the re-engineering of the system to reach a suitable compromise between filtering effect and UV-C inactivation power.

Although the engineering of portable air purifiers can be optimally conducted, we believe that further studies are necessary to better evaluate the effective cost/benefit ratio in the disinfection of civil and industrial environments of machines that safely use UV-C to disinfect air.

.1. Study limitations

The presented study has some limitations.

We tested a single scenario: changes in environmental and experimental conditions could produce different results, such as differences in the number of people, the size of the room, the operating times of the devices and the opening/closing of the door. However, the experiments are a good representation of a real context of work, study and, in general, of human attendance in a closed environment.

A second limitation of the study was that the subjects wore a disposable 3-layer surgical mask; this may have played a role in the overall level of contamination, avoiding wearing masks would likely have resulted in a higher level of contamination. However, we believe that the results remain important considering that: i) the device was nevertheless able to highlight its usefulness in reducing the risk of contamination; ii) the use of masks is representative of a real condition, in this historical moment of the SARS-CoV-2 pandemic. In fact, despite the use of air purifiers, wearing preventive devices such as masks is a condition where it must always be recommended, especially in closed environments such as that we tested.

The fact that the final experiment (on the fourth day of the main experimental stage) was repeated only once could represent an important limitation of the study. However, before the final experiment, various experiments were performed, in different conditions of human presence, in order to estimate the level of environmental contamination with the device turned off and thus to fine-tune the final experiment. The sampled contamination values were compared to test the statistical difference between the two on and off stages. Contaminations with the device off showed values consistent with the aforementioned preliminary calibration experiments, indicating the repeatability of the procedure. Therefore, the single execution of the final experiment does not seem to us to invalidate the results, also taking into account the fact that we performed a statistical significance test (95% significance level) to evaluate the non-randomness of the difference in CFUs between device turned on and off.

5. Conclusions

The reduction of the microbial load by the pre-final version of the Cleaning Air T12 air purifier, in an office with no air exchange with the outside, has reached levels of over 60% compared to the initial environmental contamination values, despite the presence of a number of people between three and five. The effectiveness of the device is even more evident when it is switched off and a noticeable rapid increase in the levels of microbial contamination of the air is observed, up to 150% compared to the start-up time.

The device has significant potential to be re-engineered for further improvements in its disinfectant performance.

It is known that the most effective way to reduce the microbial load is to ventilate the environment and dilute the air, such as in operating theaters. However, devices equipped with this type of UV-C technology, if properly designed, can help to significantly reduce the risk of contracting diseases from air, in crowded indoor environments with little air exchange.

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