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Investigation of relative humidity distribution and its impact on disinfection using a combination of robotic fogger and hydrogen peroxide

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SUMMARY

Background: Relative humidity is a key factor in the disinfection process.
Aim: To examine the distribution of relative humidity, and the time required to reach its mean value in the target area when using a robotic fogger with 7.4% hydrogen peroxide.
Methods: The study evaluated the device in both stationary and mobile operation modes. In each mode, relative humidity sensors, along with chemical, biological, and enzyme indicators, were employed to assess the disinfection's effectiveness and consistency.
Results: The device dispersed disinfectant at a rate of 30 mL/min over 45 min in both modes. A shorter time to reach the mean relative humidity is desirable for effective disinfection. It was observed that the mobile mode reached the mean relative humidity 50% faster, maintained this level for an additional 30 min, and achieved an 11% higher relative humidity compared to the stationary mode.
Conclusion: These advancements could assist pharmaceutical manufacturing and healthcare facilities in minimizing downtime during periodic disinfection.

Introduction

The recent COVID-19 pandemic has highlighted the need to reassess and redefine disinfection practices. Disinfection involves eliminating or neutralizing hazardous substances or contaminants from surfaces, equipment, and objects to ensure they are safe for handling or reuse [1]. This is a critical step in industries such as healthcare, pharmaceuticals, food processing, and environmental remediation. Automated and semi-automated decontamination technologies are designed to enhance the efficiency of these processes while minimizing workers' exposure to harmful substances. Various technologies (see Table I) are currently in use, each with distinct features, capabilities, and applications, offering specific advantages and disadvantages.

While all of these techniques and devices are effective, each comes with its own drawbacks, such as cost of ownership and operation, the time required for effective disinfection, the level of skill needed to operate, and suitability for different target locations. Among them, H_2O_2-based devices are the most commonly used commercially for various targeted areas.

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Factors influencing performance of H_2O_2 foggers

H_2O_2-based fogging is an effective method for disinfecting both surfaces and air in enclosed spaces, as hydrogen peroxide is a powerful oxidizing agent capable of killing a broad spectrum of micro-organisms, including bacteria, viruses, fungi, and spores. However, several factors, shown in Table II, can impact the effectiveness of H_2O_2-based fogging [13,10,14–17]. The vapour or mist produced by a fogging device must travel greater distances depending on the complexity of the target area, which can increase the time required for effective coverage unless aided by fans or blowers. However, using blowers can quickly reduce the chemical concentration, necessitating longer fogging times to maintain the relative humidity (RH), a critical factor for successful disinfection. This study aims to examine the time patterns of RH distribution in a simpler environment, ensuring disinfection efficiency is not compromised.

Research on robotic fogging

A substantial amount of research has been conducted on various types of robots, but further studies are needed to evaluate their performance and effectiveness [18]. Research on delivery robots has primarily concentrated on creating robots capable of navigating their environment and delivering payloads. However, there are very few instances of robots being deployed specifically for floor cleaning [19] and decontamination using targeted disinfecting sources, such as ultraviolet light [20]. One area of research focuses on developing robots that can operate autonomously and navigate complex environments using sensors and artificial intelligence (AI) systems. These robots are typically equipped with cameras, lasers, and other sensors to collect data about their surroundings, allowing them to avoid obstacles and potential hazards [20,21]. Another research area has concentrated on optimizing disinfectant solutions and delivery systems to ensure effectiveness against a wide range of micro-organisms while being applied evenly and consistently to all surfaces and objects. This research has examined the influence of factors such as concentration, contact time, and environmental conditions on the disinfection process's efficacy.

The integration of robotic platforms with disinfectant delivery mechanisms or fogging devices presents a new frontier in research and development, focusing on disinfection and contamination control in healthcare, pharmaceuticals, biotechnology, and public facilities. Research on robotic fogging aims to create effective and efficient systems that help minimize the spread of germs and bacteria in various settings, including hospitals, schools, and other public spaces.

MASCA stands for 'Mobile Airborne Decontamination System for Cleanroom Asepsis'. This innovative system offers a cost-effective, rapid, and consistent approach to disinfection. Its effectiveness is validated using chemical, biological, and enzyme indicators (CIs, BIs, and EIs, respectively). The use of EIs represents a novel method for assessing the disinfection performance of H_2O_2-based fogging devices. EIs serve as an alternative to BIs and are designed as rapid microbial test methods specifically for evaluating the effectiveness of H_2O_2 decontamination systems. Enzyme indicators provide both quantitative and qualitative results that can be correlated to *Geobacillus stearothermophilus* (biological spore). One significant advantage of using EIs is that results are available immediately, eliminating the need to wait for the typical seven-day incubation period.

Relative humidity is a critical parameter influencing disinfection efficiency. Therefore, this research aims to study the distribution pattern of RH while the system operates both in stationary and mobile modes along a predefined path, without compromising disinfection efficiency. It is anticipated that RH will be maintained longer and that the mean RH value will be achieved more quickly in mobile mode.

Experimental setup

This experimental study seeks to analyse the distribution pattern of RH when a disinfectant is introduced into the target area using the MASCA system in both stationary and mobile modes, with each mode applied daily for three consecutive days. The variables evaluated in this research include RH along with various indicators: CIs, BIs, and EIs. Table III provides details of the experimental area and the variables considered. It is expected that all indicators will function effectively in both operational modes. The diffusion pattern will be assessed

Table I
Various technologies

Technology	Working principle
Hydrogen peroxide (H_2O_2) using	Utilizes vapourized H_2O_2 to disinfect surfaces and objects. The vapour is highly reactive and can efficiently eliminate or neutralize a broad spectrum of micro-organisms and chemical contaminants [2,3,4,5].
Ozone generators	Ozone generators use electricity to create ozone, a highly reactive form of oxygen. Ozone is effective in destroying or neutralizing a wide variety of micro-organisms and chemical contaminants [6], and it can also help eliminate unpleasant odours.
Ultraviolet (UV) light systems	UV light systems employ ultraviolet light to destroy or inactivate micro-organisms on surfaces and objects. While UV light is effective against a broad range of micro-organisms, it may not work against all contaminants and can potentially damage certain materials [7,8].
Ionizing radiation systems	Ionizing radiation systems utilize high-energy radiation, such as gamma rays or X-rays, to eliminate or neutralize micro-organisms and other contaminants [5,9].

Table II
Factors influencing performance of H_2O_2 fogger

Parameter	Impact
Hydrogen peroxide concentration	The concentration of H_2O_2 in the fogging solution can impact its effectiveness. Higher concentrations may be more effective at eliminating micro-organisms but can also be more corrosive and potentially damage certain materials. Conversely, lower concentrations may be insufficient for killing micro-organisms. The optimal concentration required depends on the D-value of the target organism. Achieving an even distribution of this concentration can be challenging in complex areas using a single device, necessitating the use of multiple devices or mechanisms to ensure proper distribution.
Contact time	The duration that H_2O_2 remains on a surface can influence its effectiveness. Longer contact times allow the generated vapour or mist to disperse throughout the target area, achieving better homogeneity. Typically, current commercial devices recommend a contact time of up to 120 min.
Surface type	H_2O_2 is more effective on non-porous surfaces like metals and plastics, as it can easily humidify and disinfect these materials. In contrast, it is less effective on porous surfaces such as wood and textiles because it struggles to penetrate these materials and may not reach all areas.
Environmental conditions	Temperature, humidity, and airflow within a space can significantly affect the effectiveness of H_2O_2-based fogging. Generally, higher temperatures and humidity levels enhance disinfection, while airflow can diminish it. The relative humidity (RH) of the air in an enclosed space impacts the effectiveness of disinfectant fogging [10,11]. Higher RH levels make the fogging process more effective, as increased moisture helps keep disinfectant droplets suspended, allowing them to reach more surfaces. However, excessively high RH can lead to droplet condensation and run-off from surfaces, which reduces the disinfectant's effectiveness. Conversely, low RH can cause droplets to evapourate too quickly, also hindering the fogging process's effectiveness. It is typically recommended to maintain a relative humidity of 40—60% before fogging for optimal effectiveness and 60—90% during the fogging process [12]. The effect of temperature on H_2O_2 disinfection varies based on the fogging device used. Devices utilizing thermal treatment are more sensitive to temperature changes, with H_2O_2 being most effective at higher temperatures as heat accelerates its breakdown into safe water and oxygen. However, excessive heat can lead to rapid decomposition, diminishing its disinfectant properties. In contrast, ultrasonic nebulizers and compressed air devices operate effectively at ambient temperatures. It is recommended to maintain a temperature of 15—30°C (60—86°F) during disinfection. Additionally, air handling units should be turned off during fogging to prevent rapid dispersion and ensure effective treatment.

by analysing the relative humidity data, which is anticipated to demonstrate a consistent increase.

Experimental design

This study was conducted in two phases over three days, one with the airborne disinfection system operating in stationary mode and the other in mobile mode. In both scenarios, the airborne disinfection system was positioned at the centre of the room, with four individual sensors (for RH and temperature) and four individual indicators (chemical, biological, and enzyme) placed diagonally from one corner of the room, each separated by 1 m. Additionally, three more sensors and indicators were positioned in the other three corners of the target area, all ∼1.5 m above the floor. The positioning of the sensors, indicators, and MASCA is illustrated in Figures 1 and 2 for both stationary and mobile modes, respectively. Locations of the sensors and indicators are labelled from 1 to 7.

In the first phase, when MASCA was stationary, fogging lasted for 45 min, followed by a contact time of 60 min, resulting in a total cycle time of 105 min. During this period, a total of 1350 mL of chemical was consumed per run, with a dosing rate of 15 mL/m^3 and a diffusion rate of 30 mL/min.

In the second phase, when MASCA was in mobile mode, fogging continued for 48 min, followed by a contact time of 60 min, resulting in a total cycle time of 108 min. The total chemical consumed during this phase remained 1350 mL per run, also at a dosing rate of 15 mL/m^3 and a diffusion rate of 30 mL/min. As MASCA operated in mobile mode, it moved at a speed of 2 m/min, covering a distance of 24 m (completing three loops of 8 m each).

Phase 1 Experimental setup: MASCA is operated in stationary mode. RH sensors are positioned from MASCA to one corner of the target room, each sensor is positioned at 1 m from one another. CIs, BIs and EIs are placed in all four corners of the

Table III
Details of the experimental area, tools used for study

Items	Details
Location of study	This study was conducted in the research laboratory of VM Sciences in Hyderabad, India. The laboratory is equipped with a controlled environment, maintaining relative humidity (RH) between 45% and 65% and a temperature between 24°C and 27°C, managed by an automated air handling system. The target area has a volume of $\sim$90 m^3 (6 m length, 6 m breadth, 2.5 m height). A total of 1350 mL of disinfectant was used for the study, at a rate of 15 mL/m^3.
Device for study	The study utilized a customized airborne disinfection system integrated with a programmable robotic platform (MASCA). Ultrasonic nebulizers were employed to convert the liquid biocide into mist, with six nebulizers mounted, each generating a droplet size of 20 $\pm$ 2 μm and a diffusion rate of 10 $\pm$ 2 mL/min per nebulizer, resulting in an average output of about 60 mL/min. For this experiment, a dosing rate of 30 mL/min was applied.
Disinfectant	The disinfectant used in this study is 7.4% (v/v) H_2O_2, without any additives, manufactured by VM Sciences. The total volume consumed throughout the study was $\sim$16,200 mL, with each operational mode utilizing 1350 mL of biocide.
RH and temperature sensors	Four individual RH and temperature sensors, model BLUE-H, manufactured by MIIGO Online LLP in India, were used in the study. These sensors have functional ranges of 0—100% RH and 0—60°C, with an accuracy of $\pm$1.8% RH and $\pm$0.3°C. The units are equipped with a data logging function.
Chemical indicators	The study utilized semi-qualitative strips as chemical indicators (CIs). These strips change colour from white to blue upon exposure to H_2O_2 liquid or mist, as the study employs a mild concentration of 7.4% (v/v) H_2O_2. Manufactured by Macherey—Nagel GmbH, seven strips labelled as 1—7 were placed in all corners and other designated areas of the target location prior to the decontamination activity. After the procedure, the strips were carefully collected, and the colour change from white to blue was observed, indicating that the H_2O_2 had reached the locations of the indicators.
Biological indicators	The biological indicators (BIs) used in this study are commercially available, ready-to-use indicators manufactured by True Indicating LLC, USA, specifically for monitoring vapourized H_2O_2 disinfection. Each 6 mm stainless steel disc is inoculated with a population of 2.4 $\times$ 10^4 *Geobacillus stearothermophilus* (ATCC 12980). Seven biological indicators labelled as 1—7 are placed in all corners and other designated locations of the target area before the disinfection activity. After the indicators are exposed to H_2O_2 vapour, they are collected and aseptically transferred to individual tubes containing 15—20 mL of Soybean Casein Digest Broth. The discs are then incubated for 7 days at 55°C—65°C, with a positive control maintained for each run. No growth is observed in the exposed indicators, indicating effective bio-decontamination. In the event of a failure, cream-coloured sediment growth would be noted.
Enzyme indicators	Enzyme indicators and the luminescent assay were developed and manufactured by Protak Scientific (UK). Seven enzyme indicators (EIs) were placed in all corners and other designated locations within the target area. After the activity, the exposed indicators were carefully collected and taken to the laboratory for analysis. Following exposure to the vapour, the indicators were analysed using a photomultiplier, and the results were documented using Athena software, in accordance with the manufacturer's recommendations.

room and along with the RH sensors. All indicators and sensors are placed 1.5 m above from the floor.

Phase 2 Experimental setup: MASCA is operated in mobile mode. RH sensors are positioned from MASCA to one corner of the target room, each sensor is positioned at 1 m from one another. CIs, BIs, and EIs are placed in all four corners of the room and along with the RH sensors. All indicators and sensors are placed 1.5 m above from the floor.

In both phases operations are repeated thrice; indicators are sent for analysis as per recommendation of the manufacturer; RH and temperature sensor logged data is downloaded into Microsoft Excel 365 for analysis.

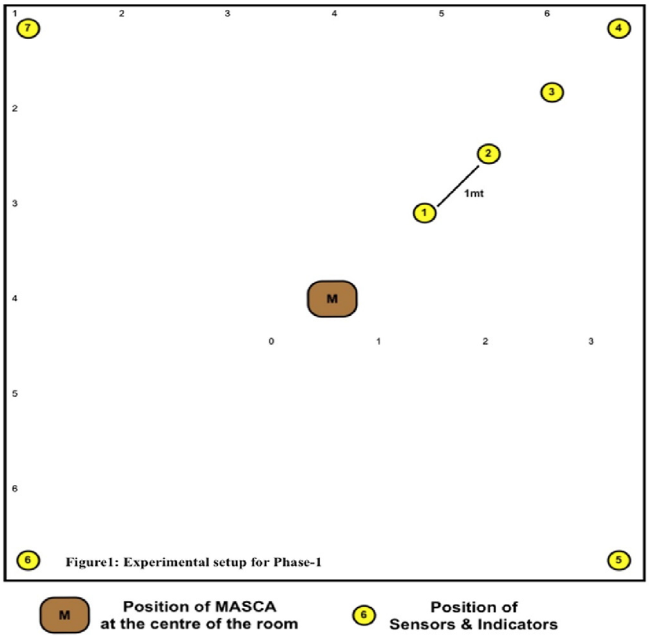

Figure 1. Experimental setup for Phase 1.

Results

The entire study was conducted in two phases: stationary and mobile mode, with each phase consisting of three runs. After collecting and analysing all the indicators according to the respective manufacturer's recommendations, the results are summarized in Table IV. The day-wise results for CIs, BIs, and EIs for both modes of MASCA operation are presented in Table IV.

For chemical indicators, '+' signifies that the indicator passed, indicating a colour change from white to blue, confirming exposure to the chemical vapour or mist. All chemical indicators passed.

For biological indicators, '+' indicates a failure, with growth observed after incubation, while '−' signifies no growth, meaning that the indicator passed or was deactivated due to the decontamination activity. All 42 biological indicators met the passing criteria.

The enzyme indicators' results are quantified as relative light units (RLU) and automated $\log_{10}$ reduction values.

The results of relative humidity are crucial for understanding its impact on disinfection efficacy. All obtained data were analysed and summarized in Table V. The initial observation indicates that RH values were highest in mobile mode of operation.

In Figure 3 a scatter plot depicts the variation of relative humidity over time, broken down by two categories: mode (stationary and mobile) and location (Location 1, Location 2, Location 3, Location 4). Here is a breakdown of the key elements:

Observations from analysis and graph (Figure 3)

- Relative humidity generally decreases over time for all modes and locations.
- Faster humidity decline in stationary mode: stationary mode leads to a quicker fall in relative humidity, possibly due to prolonged exposure to environmental factors without movement.
- More stable humidity in mobile mode: mobile mode maintains more stable relative humidity, indicating that movement may help mitigate or delay humidity loss.
- The decrease appears steeper in the stationary mode compared to the mobile mode.
- Variations in trends are evident across the different locations.
- Convergence at 150 min: at ∼150 min past 12:55, relative humidity levels converge across all locations, with values around 60% for most configurations.
- Higher humidity in mobile mode: at any given time, mobile mode consistently maintains higher relative humidity compared to stationary mode across all locations.

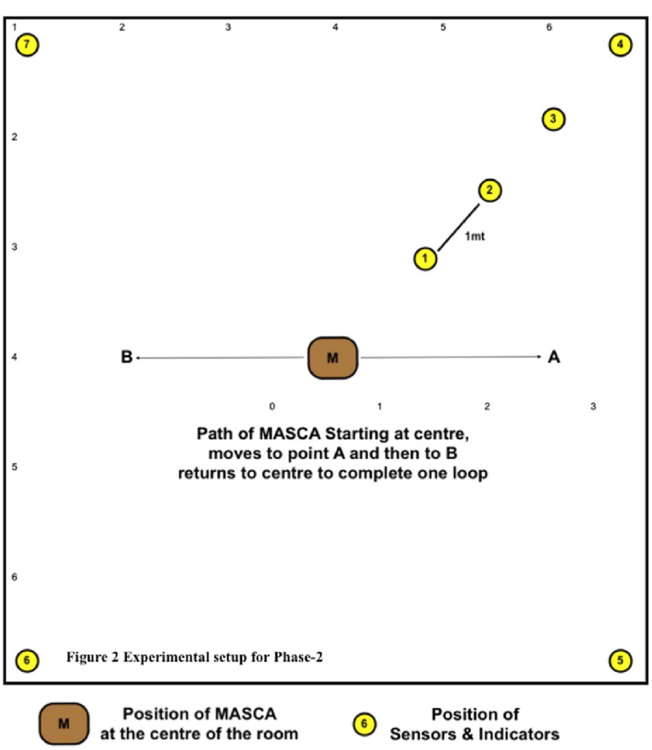

Figure 2. Experimental setup for Phase 2.

Discussion

After analysing the RH data from both stationary and mobile modes of operation, it is evident that RH distribution is more effective when the MASCA device operates in mobile mode. This improvement may be attributed to a better vapour induction rate in the mobile mode.

Observations in stationary mode

The RH increase is observed to be quicker in the sensor located near the source (1 m from the source) compared to the sensor positioned farthest away (4 m from the source). The specifics of this observation are presented in Table II. It took approximately 48 min, 48 min, and 45 min on days 1, 2, and 3, respectively, to achieve the maximum RH in each location. The

Table IV
Results of chemical, biological indicators, and enzyme indicators

Day	ID	Chemical indicator		Biological indicator		Enzyme indicator			
		S	M	S	M	S-RLU	S-R	M-RLU	M-R
Day 1	1	+	+	−	−	2.22E+06	5.4	2.32E+06	5.3
	2	+	+	−	−	2.86E+06	4.7	2.61E+06	4.9
	3	+	+	−	−	2.94E+06	4.6	2.70E+06	4.8
	4	+	+	−	−	3.40E+06	4.1	2.85E+06	4.7
	5	+	+	−	−	3.37E+06	4.1	2.82E+06	4.7
	6	+	+	−	−	3.48E+06	4.0	2.73E+06	4.8
	7	+	+	−	−	3.69E+06	3.9	2.82E+06	4.7
Day 2	1	+	+	−	−	2.22E+06	5.5	2.32E+06	5.3
	2	+	+	−	−	2.94E+06	4.6	2.67E+06	4.9
	3	+	+	−	−	2.94E+06	4.6	2.69E+06	4.8
	4	+	+	−	−	3.34E+06	4.2	2.81E+06	4.7
	5	+	+	−	−	3.40E+06	4.1	2.85E+06	4.7
	6	+	+	−	−	3.67E+06	3.9	2.70E+06	4.8
	7	+	+	−	−	3.67E+06	3.9	2.84E+06	4.7
Day 3	1	+	+	−	−	2.15E+06	5.5	2.34E+06	5.3
	2	+	+	−	−	3.03E+06	4.5	2.67E+06	4.9
	3	+	+	−	−	3.24E+06	4.3	2.86E+06	4.7
	4	+	+	−	−	3.40E+06	4.1	2.85E+06	4.7
	5	+	+	−	−	3.47E+06	4.0	2.84E+06	4.7
	6	+	+	−	−	3.58E+06	3.9	2.75E+06	4.8
	7	+	+	−	−	3.68E+06	3.9	2.80E+06	4.7

S, MASCA stationary mode; M, MASCA mobile mode; RLU, relative light units; reduction value, EI reading.

Table V
Relative humidity distribution: stationary mode and mobile mode

Day	Parameter	Stationary mode				Mobile mode			
		L1	L2	L3	L4	L1	L2	L3	L4
Day 1	Maximum RH (%)	80.51	76.91	75.96	73.35	86.53	82.37	82.52	81.09
	Time to max RH (min)	48	48	48	48	36	48	48	48
	Mean RH of location (%)	68.67	66.64	65.14	64.21	72.96	72.65	72.27	71.24
	Mean RH, day (%)	66.16	66.16	66.16	66.16	72.28	72.28	72.28	72.28
	Time to mean RH (min)	9	12	12	15	12	18	15	15
	Max RH, far point (min)	18	27	36	48	33	48	33	48
	Distance to source (m)	1	2	3	4	1	2	3	4
Day 2	Maximum RH (%)	80.44	77.3	75.93	73.69	85.85	81.91	82.14	81.12
	Time to max RH (min)	48	48	48	48	33	48	45	48
	Mean RH of location (%)	68.61	66.63	65.06	64.16	72.88	72.7	72.22	71.34
	Mean RH, day (%)	66.12	66.12	66.12	66.12	72.28	72.28	72.28	72.28
	Time to mean RH (min)	6	9	12	15	12	15	18	15
	Max RH, far point (min)	18	27	33	48	30	48	42	48
	Distance to source (m)	1	2	3	4	1	2	3	4
Day 3	Maximum RH (%)	80.2	76.83	75.67	73.28	85.96	81.98	82.41	81.1
	Time to max RH (min)	45	45	45	45	33	45	45	48
	Mean RH of location (%)	68.52	66.52	64.97	64.04	72.97	72.71	72.38	71.32
	Mean RH, day (%)	66.01	66.01	66.01	66.01	72.34	72.34	72.34	72.34
	Time to mean RH (min)	6	9	12	15	12	15	18	15
	Max RH, far point (min)	15	24	27	45	30	45	39	48
	Distance to source (m)	1	2	3	4	1	2	3	4

L1 to L4, sampling location details; each sampling location is 1 m apart from each other starting from source.

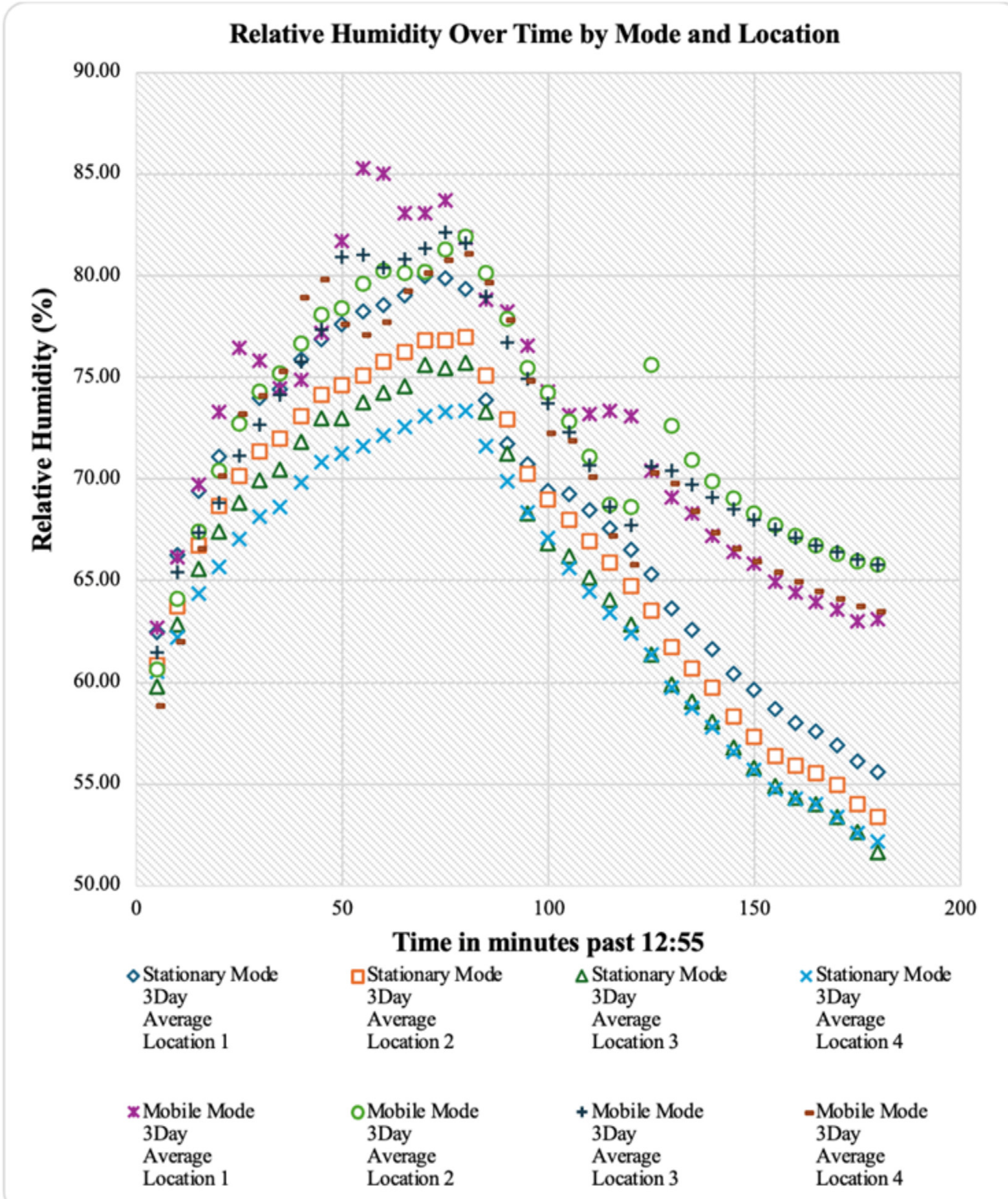

Figure 3. Scatter plot of relative humidity (%) over time (min) by mode and location.

time taken for each sampling location to reach a specific RH value varies, even though the distances between the locations are the same; the time taken does not increase incrementally and ranges from 9 to 19 min across different days and locations.

Observations in mobile mode

Similarly, an increase in RH is noted to occur faster in the sensor closest to the source (1 m away) compared to the one furthest away (4 m). The maximum RH for the nearest sensor was reached in 36, 33, and 33 min on days 1, 2, and 3, respectively, as detailed in Table II. The time required for each sampling location to reach a specific RH value varies, with the same non-incremental time difference ranging from 6 to 18 min across different days and locations.

Additionally, it was noted that the maximum RH achieved in mobile mode (81%) surpasses that in stationary mode (73%). Moreover, a mean RH of 70% was maintained for a longer duration in mobile mode, lasting 22 min longer than in stationary mode.

Conclusion

Relative humidity is a crucial parameter during disinfection processes. We examined the distribution pattern, time taken to reach peak and mean RH values, and the duration for which mean RH was maintained throughout the disinfection activity, all without compromising disinfection efficiency. CIs, BIs, and EIs were employed to assess disinfection effectiveness, while RH and temperature sensors monitored RH during the process. A mist of 7.4% H_2O_2 was introduced into the laboratory using the MASCA system at a constant diffusion rate. MASCA was operated in both stationary and mobile modes (along a predefined path) under identical conditions and operational parameters. The RH distribution was observed to be quicker and sustained for a longer duration in mobile mode. This improvement could also potentially reduce the overall disinfection cycle time. Understanding RH distribution patterns in more complex environments may further decrease disinfection cycle durations and reduce the need for multiple fogging devices. These advancements could assist pharmaceutical manufacturing and healthcare facilities in minimizing downtime during periodic disinfection.

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Ethical approval

Not required.

Credit author statement

Prasanna Kumar Sistla: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft.

P. Kanaka Raju: Validation, Formal analysis, Writing - Review & Editing.

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Conflict of interest statement

None declared.

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