v. 5, n. 4, 2025

International Journal of Biological and Natural Sciences

EVALUATION OF THE EFFECTIVENESS OF REDUCING MICROORGANISMS INSIDE VEHICLES USING CHLORINE DIOXIDE DIFFUSER CARD TECHNOLOGY

Acceptance date: 06/08/2025

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Abstract: Indoor air quality, such as inside motor vehicles, has been a growing concern due to the presence of potentially pathogenic bioaerosols. This study evaluated the microbiological effectiveness of the CleansAir® system, based on the controlled release of chlorine dioxide (ClO_2), in reducing the microbial load in the air of passenger vehicles. Twenty-one vehicles were evaluated over 30 days, with microbiological collections performed at three different times: before installation of the diffuser card (T1), after 15 days (T2), and after 30 days (T3). Air samples were collected with a microbiological impactor (Mini CAPT PMS®) and seeded in selective media for fungi and bacteria. Counts were expressed in colony-forming units per cubic meter (CFU/m^3), and isolated microorganisms were identified by MALDI-TOF. A significant reduction in the average CFU of bacteria was observed between T1 (48.9 CFU/m^3) and T2 (27.0 CFU/m^3), with a slight increase in T3 (32.8 CFU/m^3), remaining below initial levels. For fungi, the load fluctuated over time, with a final reduction in T30 (15.6 CFU/m^3) compared to T0 (17.9 CFU/m^3). In addition, there was complete elimination of pathogenic genera such as Staphylococcus spp., *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. in part of the fleet. Although overall statistical significance was not achieved, the results demonstrate the potential of CleansAir® as a complementary biosafety technology in vehicular environments. Such strategies may be especially relevant in contexts of high user turnover or transportation of immunocompromised patients.

Keywords: Indoor air quality; Chlorine dioxide; Bioaerosols; Motor vehicles; Microbial contamination;

INTRODUCTION

Indoor air quality is a determining factor for human health, especially in enclosed spaces with limited ventilation, such as passenger vehicles (Escombe et al., 2007; National Center for Immunization and Respiratory Diseases & Division of Viral Diseases, 2020). These spaces accumulate suspended particles, organic compounds, and a wide variety of microorganisms (bioaerosols), including fungi and bacteria. Prolonged exposure to this contaminated air can trigger adverse health effects, such as respiratory diseases, allergies, and opportunistic infections (Aquino, de Lima, do Nascimento, & Reis, 2018; Cordeiro, Leandro, Vandesmet, Júnior, & Mendes, 2017; Cumhur et al., 2019).

Recent studies show high microbiological loads in vehicle filters and air conditioning systems, even after regular maintenance. Gołofit-Szymczak et al. (2023) identified concentrations exceeding 10^7 CFU/m3 of fungi, with a predominance of toxigenic species such as *Aspergillus fumigatus*, in addition to the presence of genes related to mycotoxin production. Similarly, Farian & Wójcik-Fatla (2024) demonstrated that automotive filters can act as reservoirs of biological contaminants, with a potential impact on indoor air quality and occupant health. The persistence of fungi such as Penicillium and Cladosporium even after conventional disinfection methods such as ozonation and the application of disinfectants was highlighted by Gołofit-Szymczak et al. (2019), indicating limitations in the protocols currently used (Farian & Wójcik, 2025; Gołofit, Angelina, Fatla, Stobnicka, & Rafał, 2023).

In this context, the validation of new vehicle sanitization strategies that combine practicality, safety, and microbiological efficacy becomes highly relevant. Technologies based on the controlled release of oxidizing compounds, such as chlorine dioxide (ClO_2), are establishing themselves as promising alternatives in various environments, including hospitals, ambulances, and public transport vehicles (Luksamijarulkul & Pipitsangjan, 2015; Ribeiro, Seabra, & Paz, 2020; Távora et al., 2003). The CleansAir® system, which uses a diffuser card impregnated with ClO_2 for gradual release of the sanitizing agent, represents one of these innovative solutions. Its practical application, combined with the absence of residues and its effectiveness, can contribute to the continuous and lasting reduction of the microbial load in motor vehicles. In view of the content presented, the present study aimed to evaluate the microbiological efficacy of the CleansAir® system, based on the controlled release of chlorine dioxide (ClO_2), in reducing fungi and bacteria present in the indoor air of motor vehicles.

METHODOLOGY

Twenty-one passenger vehicles were evaluated and sanitized using the *CleansAir* system® , as shown in Figure 1.

Microbiological samples were collected at three different times: time zero (T0), 15 days (T15), and 30 days (T30) after installation of the diffuser card. For air sampling, a microbiological air impactor (Mini CAPT PMS®) was used, with aspiration volumes between 250 and 1000 liters per point, as recommended by ABNT NBR 17037:2023 for small environments (ABNT, 2023). The samples were seeded on Sabouraud Dextrose Agar with chloramphenicol for fungi and on Tryptone Soy Agar (TSA) for bacteria, incubated at 25°C for 5 to 7 days (fungi) and at 37°C for 48 hours (bacteria). The count was expressed in colony-forming units per cubic meter (CFU/m³). Taxonomic identification of the isolates was performed by mass spectrometry (MALDI-TOF). The data were analyzed using Excel spreadsheets and SPSS®, including repeated measures ANOVA and proportion test to identify trends in microbial reduction over time.

RESULTS

Microbiological assessment of vehicle air revealed variations in bacterial and fungal loads throughout the period of exposure to *the CleansAir*® system. The average colony-forming units (CFU) for bacteria at time zero (T1) was 48.9 CFU/m³ (95% CI 19.5-78.3 CFU/m³). After 15 days of exposure (T2) to the product, there was a significant reduction to 27.0 CFU/m³ (95% CI 17.1-36.9 CFU/m³). However, at the end of 30 days (T3), a slight increase to 32.8 CFU/m³ (95% CI 21.5-43.9 CFU/m³) was observed, still remaining below the initial value. For fungi, the initial average was 17.9 CFU/m³ (95% CI 7.1-28.7 CFU/m³). After 15 days, there was an increase to 22.4 CFU/m³ (95% CI 10.6-34.2 CFU/m³), followed by a drop to 15.6 CFU/m³ (95% CI 10.3-20.8 CFU/m³) on day 30, representing a final reduction compared to the baseline value, as shown in Figure 2.

This fluctuation may reflect variability in fungal composition or the interference of environmental factors over time. Complete elimination of important microbial genera was also observed in some vehicles during the 30-day evaluation period, including *Staphylococcus* spp (5 vehicles), *Aspergillus* spp (4), *Cladosporium* spp (3), and *Penicillium* spp (2), which reinforces the qualitative effectiveness of the treatment.

DISCUSSION

Data analysis showed a positive trend toward microbial reduction, especially for bacteria, throughout exposure to the CleansAir® system. Although the statistical tests applied (Friedman ANOVA) did not identify overall statistical significance ($p > 0.05$) in comparisons between colony-forming units per cubic meter over time, the complete elimination of certain pathogenic genera in part of the fleet, such as *Staphylococcus* spp and *Aspergillus* spp, suggests relevant beneficial effects. (De Abreu,

International Journal of Biological and Natural Sciences ISSN 2764-1813 DOI https://doi.org/10.22533/at.ed.813542510077

Brand	Model	Year	Transports animals	Transports children	Cleaning in the last year	Interior trim material
Fiat	Argo	2017	No	No	3	Fabric
Ford	Fiesta	2010	Yes	Yes	3	Fabric
Honda	Fit	2014	No	Yes	1	Plastic
Honda	Fit	2018	No	No	1	Fabric
Hyundai	Tucson	2013	No	No	2	Plastic
Hyundai	HB20	2014	No	No	2	Fabric
Hyundai	Creta	2019	No	Yes	3	Fabric
Hyundai	HB20	2015	No	No	5	Mixed*
Hyundai	Creta	2019	No	Yes	3	Mixed*
JAC	T40	2018	No	No	2	Plastic
Jeep	Renegade	2020	No	No	2	Mixed
Jeep	Compass	2024	No	No	3	Mixed*
Mitsubishi	Outlander	2013	No	No	2	Plastic
Nissan	Kicks	2024	No	No	1	Plastic
Nissan	Kicks	2022	Yes	Yes	2	Fabric
Nissan	Versa	2017	No	No	1	Mixed*
Peugeot	208	2009	No	Yes	2	Fabric
Peugeot	208	2012	No	Yes	2	Fabric
Peugeot	208	2012	No	No	1	Mixed*
Volkswagen	Taos	2024	No	No	1	Plastic
Volkswagen	Polo	2025	No	No	1	Mixed

*Cars containing fabric and plastic parts were considered mixed

Figure 1. Information on the cars used in the study.

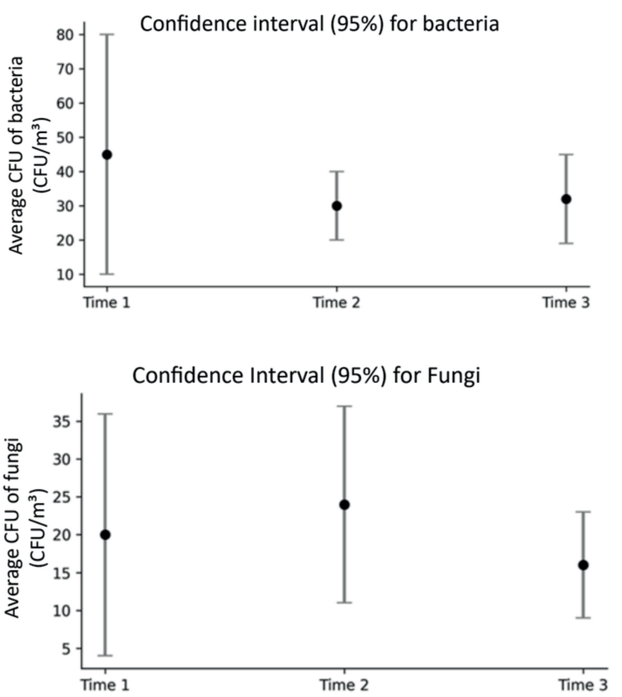

Figure 2. Effect of Chlorine Dioxide Diffusion on CFU/m^3 of Bacteria and Fungi in the Over Time Air

De Jesus Andreoli Pinto, & De Oliveira, 2003; Méheust, Le Cann, Reboux, Millon, & Gangneux, 2014; Ribeiro et al., 2020). The microbial composition inside vehicles is influenced by a variety of environmental and behavioral variables, such as the circulation of people, the presence of animals, the use of air conditioning, the quality of the outside air, and the frequency of cleaning (Aquino et al., 2018; Souza, Porcy, & Menezes, 2020). These factors are difficult to control in real-world scenarios, directly impacting the results of microbiological interventions. Passive interventions such as the use of high-efficiency filters, natural ventilation, and the choice of low-porosity interior materials should also be considered as contributing factors to improving indoor air quality (DIEL, NUNES, VIDAL, & PROCÓPIO, 2015; Lavoie & Guertin, 2001). In addition, the persistence of genera such as *Cladosporium* spp and *Penicillium* spp even after conventional cleaning procedures reinforces the need for additional strategies to ensure effective decontamination (Cumhur et al., 2019; Voorn et al., 2020). The ABNT NBR 17037:2023 standard establishes updated parameters for air-conditioned environments, while ISO 14698 provides guidelines for the control and interpretation of microbiological contamination (ABNT, 2023; ISO, 2003).

Several studies have reinforced the importance of effective strategies for microbiological control in vehicle environments. Gołofit--Szymczak et al. (2019) compared traditional methods of air conditioning system sanitation, including ozonation, chemical disinfection, and ultrasound application, and observed that, despite an immediate reduction in microbial load, some fungal genera such as *Penicillium* spp and *Cladosporium* spp remained detectable after treatment. Similarly, more recent investigations have shown the recurrent presence of toxigenic fungi, such as *Aspergillus fumigatus,* in automotive filters even after regular maintenance cycles (Gołofit-Szymczak et al., 2023; Farian & Wójcik-Fatla, 2024). These operational limitations of conventional methods point to the need for new approaches with longer-lasting mechanisms of action that are adaptable to the dynamic conditions of vehicles. Technologies based on the controlled release of oxidizing compounds, such as chlorine dioxide or hydrogen peroxide, have been explored as promising alternatives because they combine ease of application, low operating costs, and comprehensive antimicrobial efficacy. The incorporation of such methodologies may represent a significant advance in vehicular biosafety protocols, especially in contexts of continuous use, high passenger turnover, or exposure to immunocompromised populations.

CONCLUSION

The CleansAir® system has demonstrated its ability to reduce the microbiological load present in the internal air of vehicles, with a particular focus on reducing bacteria. The elimination of microbial genera with pathogenic potential indicates that the technology has value as a complementary biosafety measure in automotive environments. Given the multifactorial nature of contamination in vehicles, future research should expand sampling, include different types of vehicles, and explore combinations of sanitation methods. The integration of technological innovation, current legislation, and sustainable preventive practices is essential to ensure healthier and safer indoor environments.

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