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Evaluating Cleaning Services in Civil Environments: Microbiological and Life Cycle Analysis Comparing Conventional and Sustainable Methods

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Abstract: In response to the growing global concern for environmental sustainability, a Life Cycle Assessment (LCA) study was conducted to evaluate the environmental benefits of the "Formula Servizi" GREEN protocol compared to a conventional cleaning protocol, as mandated by the updated Criteria for Environmental Sustainability (CAM—Criteri Ambientali Minimi) for cleaning services. The CAM, effective on 19 June 2021, requires companies to demonstrate the environmental advantages of their cleaning protocols over traditional methods. This study aligns with the new CAM guidelines and employs UNI EN ISO 14040–14044 technical standards for a comprehensive comparative analysis. The study highlights the significance of maintaining hygiene to ensure safety in various contexts, emphasizing the importance of environmental sampling and monitoring to prevent contamination and infection transmission. Despite the complexity and expenses associated with microbiological monitoring, this research affirms its crucial role in validating cleaning procedures, particularly in healthcare facilities, food service areas, and industrial settings. The findings reveal that both the "Traditional" and "GREEN" cleaning protocols demonstrate satisfactory effectiveness in controlling microbiological contamination according to established guidelines. Moreover, the LCA results indicate that the "GREEN" protocol, while exhibiting higher water consumption and wastewater treatment, showcases a strategic use of more sustainable cleaning and laundry detergents. Despite the increased water usage in certain phases, the significantly lower environmental impact per unit of weight demonstrates the potential for optimizing both environmental sustainability and operational efficiency in future Life Cycle Sustainability Assessment (LCSA) endeavors. The comparative LCA further reveals that the "GREEN" protocol enables an annual avoidance of 260 g of CO_2-e emissions per square meter of cleaned surface. The most significant reduction in absolute terms is associated with the use of eco-labeled detergents in the laundry system, resulting in the avoidance of 654.1 kg of CO_2-e per year of service (−77% compared to traditional laundry detergents).

Keywords: Life Cycle Assessment; eco-friendly materials; disinfection; microbial pathogens; ecological impact assessment

Citation: Fontana, R.; Buratto, M.; Caproni, A.; Nordi, C.; Pappadà, M.; Bandera, B.; Vogli, L.; Buffone, C.; Marconi, P. Evaluating Cleaning Services in Civil Environments: Microbiological and Life Cycle Analysis Comparing Conventional and Sustainable Methods. *Sustainability* **2024**, *16*, 487. https://doi.org/10.3390/su16020487

Academic Editor: Alberto Quintana Gallardo

Received: 8 November 2023
Revised: 18 December 2023
Accepted: 29 December 2023
Published: 5 January 2024

Correction Statement: This article has been republished with a minor change. The change does not affect the scientific content of the article and further details are available within the backmatter of the website version of this article.

1. Introduction

Recently, there has been a growing awareness among the public regarding environmental concerns. As a result, the need to create environmentally sustainable processes has gained significant importance. These processes play a crucial role in finding a balance between societal productivity, environmental safeguarding, and natural resource preservation [1]. In the field of sustainable development, Life Cycle Assessment (LCA) stands as a

pivotal instrument for achieving and maintaining sustainability. Its primary function lies in the assessment of environmental ramifications linked to the design of specific products or processes [2]. The European Commission recognizes LCA as the foremost support currently in existence for evaluating the potential environmental effects of various products [3].

Ensuring appropriate levels of safety in various settings, such as healthcare facilities, the food industry, and different work environments, heavily relies on the maintenance of proper hygiene. Typically, performing daily cleaning procedures is sufficient to eliminate surface-bound bacteria, helping to establish a health-promoting environment. This, in turn, mitigates the potential for infection transmission [4]. The process of microbiological sampling involving air, water, and inanimate surfaces (referred to as environmental sampling) is a complex undertaking characterized by considerable expenses and time consumption. This complexity arises from numerous variables encompassing protocol definition, analysis complexities, and the interpretation of results. Nonetheless, the careful monitoring of both airborne microbial content and surface contamination remains of paramount importance. This imperative prevails, particularly in scenarios where inadequate hygiene practices could culminate in the propagation of contamination and the potential spread of infections. The implementation of such monitoring and control mechanisms assumes a crucial role in substantiating the efficacy of applied cleaning procedures, often practiced within environments such as hospitals, food service areas, and industrial facilities [5]. Over time, extensive studies have been conducted, examining the dynamics of contamination and evaluating the effectiveness of disinfection, sterilization, and cleaning methods. The formulation of appropriate cleaning, antiseptic, and disinfection protocols has been adapted to suit diverse materials and their specific applications [6,7].

The primary objective of this work is to conduct a comparative Life Cycle Assessment (LCA) study, aimed at highlighting the environmental benefits associated with the "Formula Servizi" GREEN protocol in alignment with the updated Criteria for Environmental Sustainability (CAM—Criteri Ambientali Minimi) for cleaning services. This comparison is made against a conventional cleaning protocol. The CAM for the cleaning company services was officially published in the Italian Official Journal on 19 February 2021 and has been effective since 19 June 2021. Under these guidelines, the CAM requires competing companies to showcase the environmental and quality benefits embedded in the "GREEN" protocol procedure as opposed to traditional methods. Specifically, the endorsed LCA study conducted by Formula Servizi aligns with the newly introduced CAM, which was defined in the decree of 29 January 2021. The current study serves the purpose of demonstrating the protocol's ability to diminish environmental effects in comparison to conventional cleaning and sanitization approaches. This was accomplished through the presentation of a comprehensive comparative analysis adhering to the standards outlined in UNI EN ISO 14040–14044.

2. Materials and Methods

2.1. Cleaning Plan

The subject of analysis in this study pertains to the cleaning service offered by Formula Servizi within the premises of the M.A.R.R. Directional Centre situated in Santarcangelo di Romagna, Italy.

The focal point of the investigation is a working area encompassing various spaces relevant to civil and private cleaning. This area comprises an entrance hall, waiting rooms, an acceptance desk, elevators, stairs, offices, restrooms, and a coffee room. The rationale behind selecting this particular protocol for analysis stems from its proper representation of surface types, levels of soiling, cleaning frequencies, and methodologies commonly encountered in civil site cleaning.

Following the principles established by international reference standards for Life Cycle Assessment (LCA) evaluations, the initial step in developing the analytical and computational framework involved identifying the distinctive elements characterizing the Formula Servizi cleaning service and subsequently structuring them into a coherent system.

The designated sampling areas encompassed the entire facility, of which the surface amounted to a total of 3561.74 square meters (sqm).

2.2. Microbiological Assessment: Sampling Plan

RODAC contact plates (Liofilchem, Roseto degli Abruzzi, TE, Italy) were deployed, alongside the utilization of swabs containing a neutralizing agent (Dey Engley) (Liofilchem, Roseto degli Abruzzi, TE, Italy), for result standardization. The initial assessment encompassed a visual appraisal of the surface condition, cleanliness, and moisture levels. The microbiological evaluation, expressed as aerobic colony counts (ACCs), was predicated on growth observations after a 48 h incubation at 37 °C. This growth was facilitated on RODAC (Replicate Organism Detection And Counting) plates coated with plate count agar along with a neutralizer. Furthermore, MacConkey agar (MCA) was employed for quantifying enterobacteria, Mannitol salt agar (MSA) was employed for *Staphylococcus* spp. Enumeration and Sabouraud dextrose agar (SDA) were employed for yeast and mold quantification. RODAC plates were directly inoculated through the application of pressure onto flat surfaces utilizing a contact plate weight applicator (500 g) for a 30 s interval (VWR collection, International, Milano, Italy). In cases involving irregularly shaped surfaces, an entirely sterile, pre-moistened cotton–wool swab was employed to encompass the entire hand contact area. This swab was subsequently used to inoculate the agar plates. Swab sampling was conducted with a sterile 10 cm × 10 cm template, targeting a 100 cm^2 sampling area [8,9]. The sampled places are listed in Table 1.

Colony enumeration was performed after 24–48 h. The categorization entailed minimal growth (6–39 colonies) ≅ 10 CFU/25 cm^2 and barely detectable growth (<6 colonies) ≅ < 10 CFU/25 cm^2 as compliant with the INAIL (Istituto Nazionale Assicurazione Infortuni sul Lavoro) standard protocols, tailored to civil environments.

A total of 250 samples were acquired, encompassing surfaces such as floors, tables, desks, computers, telephones, chairs, escalators, toilets, sinks, and bathroom floors. The specimens were conveyed in insulated containers (2–6 °C), and the temperature conditions were supervised via a data logger. Incubation was conducted at 36° ± 1 °C for 48 h, except for SDA plates, which were incubated at 25 °C for 72–120 h. Subsequently, the colonies were counted, isolated, and subjected to identification [7,10,11].

2.3. Isolation and Characterization of Microorganisms

The swab specimens underwent vortexing to facilitate the liberation of microorganisms into the diluent solution. Following this step, the sample was transferred and evenly distributed onto 90 mm Petri dishes, each pre-loaded with 20 mL of distinct agar media (TSA, MCA, MSA, and SDA). Incubation of the Petri dishes took place at a temperature of 36 °C (25 °C for SDA plates). Over the course of a five day period, daily observations were made to monitor the growth of bacterial colonies. Distinctive colonies exhibiting unique phenotypic characteristics, including morphology, shape, and color, were meticulously selected for subsequent experimental procedures. These selected colonies were preserved through storage in a solution containing 50% (v/v) glycerol at a temperature of −80 °C. In order to ascertain the identity of the isolated bacterial colonies, API systems (Biomerieux Italia, Grassina, FI, Italy) were employed, following the instructions provided by the manufacturer [12].

Table 1. Sampling plan. The table lists the rooms and spaces sampled for microbiological contamination.

Reception	Floor
	Desk
	Turnstiles
	Waiting room table

Table 1. *Cont.*

Office area	Floor
	Desk
	Chair
	Keyboard
	Phone
Elevators (E)/Stairs (S)	Floor (E)
	Floor (S)
	Keyboard (E)
	Handrail (S)
Coffee Room	Floor
	Table
	Chairs
	Vending machines
Women's Restroom	Floor
	Toilet
	Doorknob
	Sink
Men's Restroom	Floor
	Toilet
	Doorknob
	Sink

2.4. Cleaning Modus Operandi and Protocol Selection

The trial was conducted sequentially, employing two distinct protocols. First, the "Traditional" system (referred to as TT) was used for a duration of four weeks. Subsequently, the "GREEN" experimental system (referred to as TG) was employed for another four weeks, resulting in a total study period of 8 weeks. This approach allowed for a direct comparison of the outcomes obtained through different cleaning methods in areas with identical intended use, usage patterns, and contamination characteristics. As a control measure, we conducted surface sampling on areas that were neither cleaned nor treated, denoted as NT. The protocols used are listed in Table 2. Briefly, the protocols differed as follows:

- The use of microfiber mop with fringes and cloths both in the "Traditional" and in the "GREEN" protocol; the fringes used differed in weight (heavier for TT fringes), while the cloths differed in both weight (heavier for TT clothes) and the eco-label (present only for clothes used in TG). In both protocols, the textile reconditioning cycles took place at 60 °C;
- The use of eco-labeled detergents for the cleaning of floors and surfaces with dosages in tanks ranging from 2 to 100% for the "Traditional" protocol, while for the "GREEN" protocol, more diluted dosages were used than "Traditional" protocol products. In the "GREEN" protocol, the dosages ranged from 0.08% to 100% [13,14].

2.4.1. Formula Servizi "Traditional" (TT) and "GREEN" (TG) Protocols

The execution of the TT and TG systems proceeded in the manner outlined in Table 2.

Table 2. "GREEN" and "Traditional" cleaning protocols.

Cleaning Operation	Frequency	Working Modality
Dusting desks and office furniture, meeting rooms, and entrance desks; removing fingerprints from glass surfaces with more frequent contact	Daily: 5/7	Cloth and cleaning spray
Cleaning and sanitizing of the refreshment and relaxation areas, with a second cleaning step in the afternoon; entrance turnstiles with the elimination of footprints; internal elevators with the sanitization of contact points	Daily: 5/7	Cloth and cleaning spray
Wet trash from office floors and meeting rooms, including those in common areas	Daily: 5/7	Sweeping with a disposable cloth
Manual cleaning of office floors and meeting rooms, including those in public areas	Every other day: 3/7	Flat fringe-mop wash
Mechanized floor washing using a scrubbing machine	Weekly: 1/7	Scrubbing
Cleaning and sanitizing of sanitary ware, with an afternoon review	Daily: 5/7	Cloth and bottle with drip guard
Sanitary descaling	Weekly: 1/7	Cloth and cleaning spray
Afternoon restroom refresher	Daily: 5/7	Cloth and cleaning spray
Textile reconditioning—Deterging	Daily: 5/7	
Textile reconditioning—Alkalizing	Daily: 5/7	
Textile reconditioning—Bleaching	Daily: 5/7	
Textile reconditioning—Disinfecting	Daily: 5/7	

2.4.2. Standard/GREEN Protocol Active Ingredients

Phenylphenol

A widely recognized antimicrobial compound, 2-phenylphenol, forms the primary constituent of disinfectant products. When combined with surfactants, it demonstrates effectiveness against a broad spectrum of microorganisms, encompassing Gram-positive and Gram-negative bacteria and fungi, as well as lipophilic viruses. Its antimicrobial action derives from the reactivity of its hydroxyl groups, which interact with various macromolecules and microbial structures. This interaction leads to the transformation of integral membrane proteins into a colloidal state. Additionally, in specific instances, it has been observed to inhibit fatty acid synthesis by targeting the activity of enoyl reductases. Importantly, the safety data sheet (SDS) indicates its suitability for use on diverse surfaces and across different application scenarios, including domestic, healthcare, and food industry settings [15].

Sodium Hypochlorite

When maintained in an aqueous solution, sodium hypochlorite gives rise to hypochlorous acid (HOCl), which serves as a potent chlorine reservoir with high oxidizing capabilities against microbial cells. Disinfectants derived from sodium hypochlorite exhibit a broad spectrum of activity, making them highly efficient in combating fungi, spores, bacteria, and viruses. The product is used in both protocols for toilet disinfection (100% D.U.—chlorine 26.000 ppm) and restroom sanitation (5% D.U.—chlorine 1.300 ppm) [16].

Hydrogen Peroxide

Hydrogen peroxide, a chemical compound with the molecular formula H_2O_2, has garnered significant attention as a potent disinfection agent in various scientific and medical contexts. Its disinfection activity stems from its ability to release highly reactive oxygen species, including hydroxyl radicals, which exhibit strong oxidative properties. These radicals can effectively target and damage a wide range of microorganisms, including fungi, spores, bacteria, and viruses. Hydrogen peroxide's disinfection efficacy is influenced by factors such as concentration, exposure time, and environmental conditions. At higher concentrations, it can achieve rapid and broad spectrum microbial inactivation, while lower concentrations may be suitable for more delicate materials and surfaces. Moreover, its decomposition into harmless water and oxygen makes it an environmentally friendly choice for disinfection. Hydrogen peroxide, being a versatile disinfectant agent, finds applications in healthcare environments, pharmaceutical manufacturing, food processing, and numerous other fields, contributing to the maintenance of hygienic conditions and the prevention of the transmission of infectious diseases. Ongoing research continues to explore its potential for optimizing disinfection protocols and enhancing our understanding of its mechanisms of action [17,18].

Peracetic Acid

Peracetic acid combines the disinfection and germicidal properties of hydrogen peroxide while exhibiting improved lipid solubility and resistance to decomposition mediated by peroxidases and catalases. Notably, it maintains its effectiveness even in the presence of organic matter, and its degradation by-products, including acetic acid, oxygen, hydrogen peroxide, and water, pose no significant hazards and can be easily disposed of. Its biocidal mechanism is primarily associated with its oxidizing impact on lipid membranes, DNA, and other essential cellular components. The sporicidal effect can be attributed to its capacity to degrade peptides [17].

Surfactants

Surfactants, or surface-active agents, play a pivotal role in cleaning processes by facilitating the removal of dirt, grease, and other contaminants from various surfaces. These compounds possess a unique structure, with hydrophilic (water-attracting) and hydrophobic (water-repelling) regions. When applied to a soiled surface, surfactants reduce the interfacial tension between water and the surface, allowing water to penetrate and dislodge the dirt and grime. The hydrophobic portion of the surfactant surrounds and encapsulates the loosened particles, forming micelles that remain suspended in the cleaning solution. This process effectively emulsifies and solubilizes the contaminants, allowing them to be rinsed away. Surfactants also aid in the dispersion and suspension of soil particles, preventing them from reattaching to the surface during cleaning. As versatile cleaning agents, surfactants find extensive use in household cleaning products, industrial cleaning processes, and personal care products, contributing to the efficient removal of unwanted substances and the maintenance of cleanliness and hygiene. Ongoing research continues to explore novel surfactant formulations and applications, enhancing their effectiveness and minimizing their environmental impact [19,20].

2.5. LCA

The LCA approach, as specified in ISO 14040:2006 and ISO 14044:2006 [21,22], was employed as the standard methodology. Furthermore, ISO 14067:2018 was used as a benchmark for quantifying the cleaning service's carbon footprint, including its greenhouse gas emissions [23].

ISO 14067:2018 establishes the requirement to refer to relevant Product Category Rules (PCR) when available. In this context, PCR 2011:03 v3.0.1, titled "Professional cleaning services for buildings", was utilized to provide specific guidance for the UN CPC 853 product [24].

PCR outlines the main analysis criteria, including functional units, system boundaries, data type and quality requirements, and applicable cutoff criteria. The functional unit employed adheres to the specifications outlined in the existing PCR, which is defined as cleaning and maintaining 1 square meter of an average representative surface for a duration of 1 year. When determining this representative surface, various types of environments within the sample area were considered.

The system boundaries applied follow a "cradle-to-grave" approach. The processes considered in the analysis, categorized into three phases—namely, "upstream", "core", and "downstream"—comprise the aspects outlined below.

In the initial "upstream" phase, several crucial activities come into play:

- The extraction and processing of raw materials;
- The transportation of raw materials and semi-finished products to suppliers;
- The manufacturing of consumer goods, specifically chemicals (such as detergents and disinfectants) and textiles (fringes and cloths), along with their primary (plastic) and secondary (cardboard) packaging;
- The production of cleaning trolleys;
- The manufacturing of machinery, such as washers, dryers, and washing machines.

Transitioning into the "core" phase, the following significant aspects are managed:

- The oversight of the supply chain for consumer goods, ensuring their smooth journey from manufacturers to the service site;
- The execution of services utilizing chemicals, textiles, cleaning equipment, and machinery;
- The production of transportation fuels;
- The generation of electricity used on-site for service execution;
- Water consumption is required for diluting chemicals and operating washing machines.

Please note that this study does not consider the transportation of personnel and maintenance workers in this phase. Additionally, the transport of durable goods, which have a shelf life exceeding 3 years, has been excluded in accordance with point 4.3.1.2 of the PCR due to the intermittent nature of the service life of goods transport.

Lastly, in the "downstream" phase, the focus is on the handling and treatment of solid waste and wastewater generated during the processes within the "core" phase. The three phases have been resumed in Figure 1.

The primary assumptions made in this study are in alignment with the principles outlined in ISO:14026 for conducting a comparative analysis. Specifically, the following assumptions have been considered:

- Both systems under comparison share an identical functional unit, possessing equivalent spatial characteristics and requiring similar interventions;
- Due to the nature of this comparative analysis, we have not taken into account equivalent processes. For instance, the transportation of personnel on-site, which is consistent in both protocols, has not been individually considered;
- The areas under investigation are consistent and remain unchanged;
- The criteria for including inputs and outputs are uniform;
- The quality requirements for the data are the same;
- The Life Cycle inventory units are uniform;
- The calculation procedures closely resemble each other;

- The allocation rules are comparable;
- The selected impact categories and characterization factors are identical, with reference to ISO:14067 and GWP100. This is the only impact category taken into consideration because global warming is the environmental theme of highest concern;
- The types of interventions and the frequency of operations are indistinguishable between the two cases;
- The surfaces are comparable in terms of floor type, property usage, and overall dimensions;
- The extent of usage in the sampled areas and the level of dirt are similar;
- Regarding electricity consumption, a precautionary approach has been taken due to the absence of specific data for the site-specific supply contract. Therefore, the national energy mix has been used as an approximation;
- The total property area measures 3561.74 square meters.

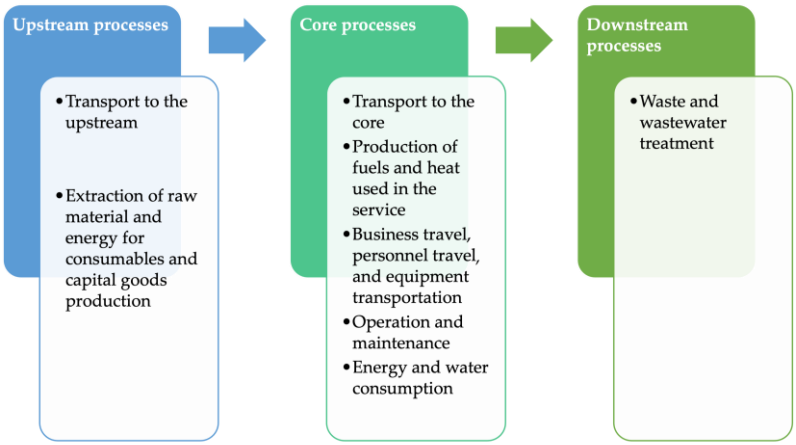

Figure 1. Diagram illustrating the different phases of the analysis process.

The impact assessment methodology is put into practice by computing the impact category known as Global Warming Potential (GWP). This calculation is based on a model established by the Intergovernmental Panel on Climate Change (IPCC). This model assesses how certain gases in the atmosphere, namely, CO_2, CH_4, N_2O, SF_6, HFCs, and PFCs, contribute to the greenhouse effect's escalation. It accomplishes this by establishing a correlation between the quantity of these gases emitted and a specific indicator, "kg CO_2 equivalent", utilizing distinctive characterization factors for each gas.

These characterization factors consider the gas's effectiveness in influencing radiative forcing and its average residence time in the atmosphere. This enables us to relate the GWP of each substance to the GWP of CO_2, which is set at 1 for reference. The evaluation considers a timeframe of one hundred years, and the characterization factors employed align with the data provided in the 6th IPCC Report released in 2021.

To arrive at the total impact score, the contributions of each substance are summed after being converted into kg of CO_2-equivalent units.

2.6. Statistical Analysis

Statistical assessment was carried out using one-way ANOVA, followed by Dunnett's multiple comparisons test, with the use of GraphPad Prism version 9.0.0 designed for MacOS, developed by GraphPad Software in San Diego, CA, USA.

3. Results and Discussion

3.1. Microbiological Assessment

The experimental approach denoted as the "GREEN" protocol, which involves the utilization of entirely natural and biodegradable substances combined with disposable microfiber cloths and mops in sanitization procedures, has exhibited notable significance. This experimental methodology has demonstrated, to the extent of the conducted sampling,

that its performance aligns with that of the traditional system. The sampled areas were consistently found to be adequately cleaned. All spaces exhibited a noteworthy reduction in microbial contamination, with the "GREEN" protocol achieving equivalent or even superior results compared to the traditional technique.

It is important to acknowledge that variations in microbial content across diverse environments can be attributed to differences in usage patterns and the nature of the environment itself. Hence, the key data for evaluation pertains to the extent of microbial reduction (expressed as a percentage) between the non-treatment (NT) and post-treatment (traditional, TT, or GREEN, TG) stages.

The percentage reduction (comparing TT and TG to NT) in the sampled microorganisms via swabs and the overall counts determined via RODAC plates on TSA (tryptic soy agar), MSA (Mannitol salt agar), MCA (MacConkey agar), and SDA (Sabouraud dextrose agar) media are subsequently presented in Figures 2–8.

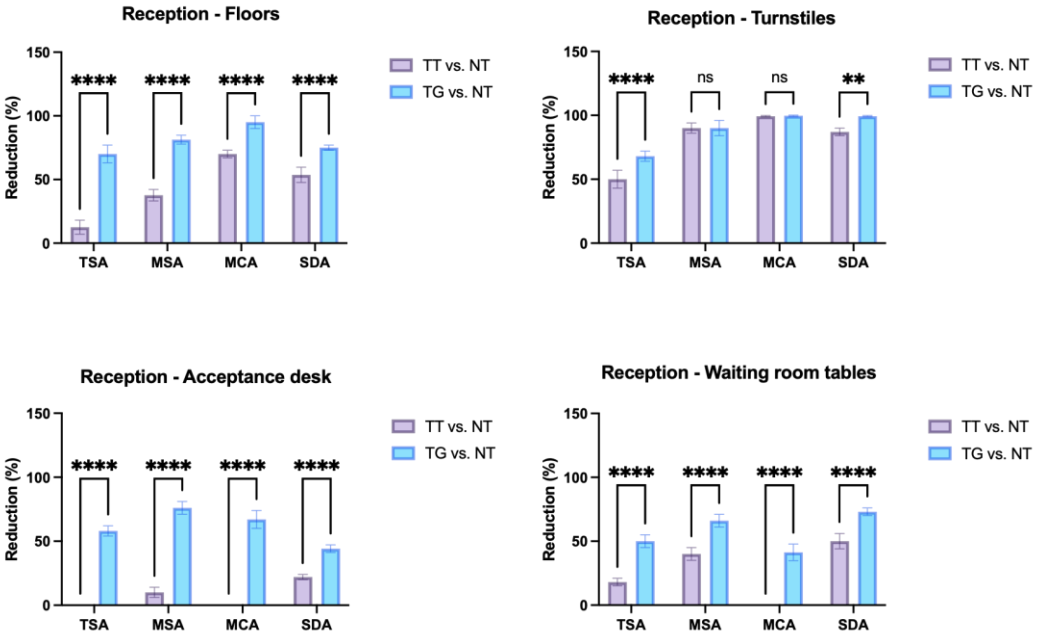

Figure 2. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of the reception area, including the floor area, acceptance desk, turnstiles, and waiting room tables. The data represent the average of two separate experiments conducted in triplicate (mean ± standard deviation), ** p-values < 0.01, **** p-values < 0.001, ns: not significant.

The existing body of the literature concerning the dissemination of infectious agents causing illnesses among patients and healthcare workers highlights the prominent role played by surfaces in propagating microorganisms within the healthcare sector. Cases of infections stemming from methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been recorded, not only among healthcare professionals but also among individuals closely associated with animals, such as breeders, farmers, and veterinarians [25,26]. Due to the absence of universally accepted standards for assessing workplace exposure to biological agents and commonly applied reference values, it is reasonable to consider adopting the criteria proposed by Górny (2004) (>2×10^2–5×10^2 CFU/m^3) when interpreting the findings, including the total counts of molds and yeasts in enclosed spaces [27]. According to these criteria, the status of microbiological contamination in the examined workspaces, as well as in-office stations and restrooms, appears to be quite satisfactory. Under normal conditions, the primary source of fungal bioaerosols in the air within enclosed areas such as offices is aerosols that migrate from the external environment. This phenomenon becomes particularly significant during the summer and autumn months when the concentrations of fungal

spores in the atmosphere rise. Since this study was conducted during the spring/summer period, the level of fungal contamination did not reach critical values.

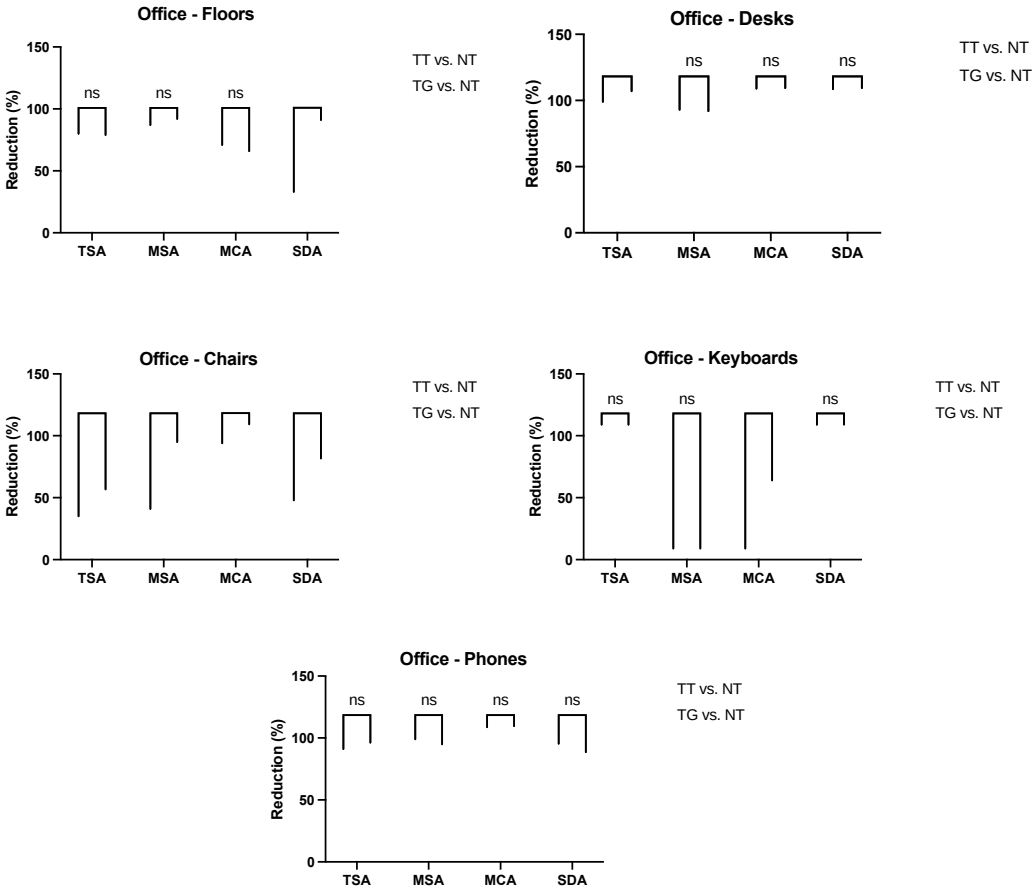

Figure 3. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of office-related elements, such as the floor area, desktop, chairs, keyboard, and phones. The data are the average values from two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages: ns: not significant.

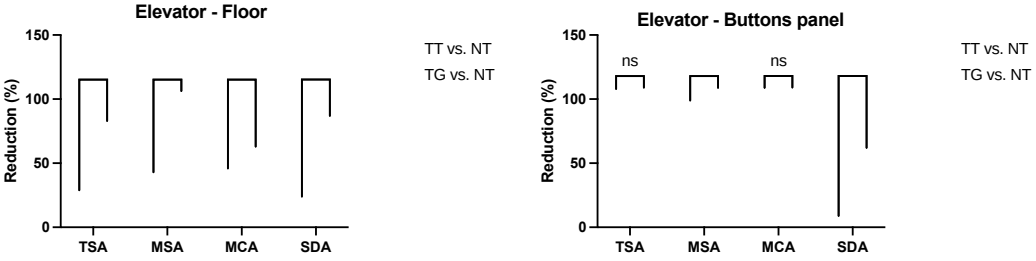

Figure 4. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of elevator elements, such as the floor area and button panel. The data are the average values from two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages: ns: not significant.

The research identified non-fermenting Gram-negative bacilli, more precisely, *Acinetobacter* spp., and filamentous fungi (listed as harmful biological agents). Additionally, a broad spectrum of coagulase-negative staphylococci (CoNS) was observed. The bacterial and fungal contamination in all areas varied widely, ranging from 0 to 3.0×10^2 CFU/25 cm^2. This range is consistent with the levels of microbiological contamination observed on office

surfaces by other researchers [26,27]. However, it is worth noting that higher concentrations of microorganisms were observed on the entrance and restroom floors, especially when a traditional cleaning system was used. This situation may indicate the necessity of implementing proper disinfection, decontamination, and floor cleaning procedures. Nigam and Cutter previously showcased the efficacy of standard practices involving the cleaning and disinfection of both the insides of ambulances and the equipment utilized by medical services [28,29]. Numerous other studies in the same vein have documented comparable findings regarding the extent of microbiological contamination on internal surfaces [25,26,30–34].

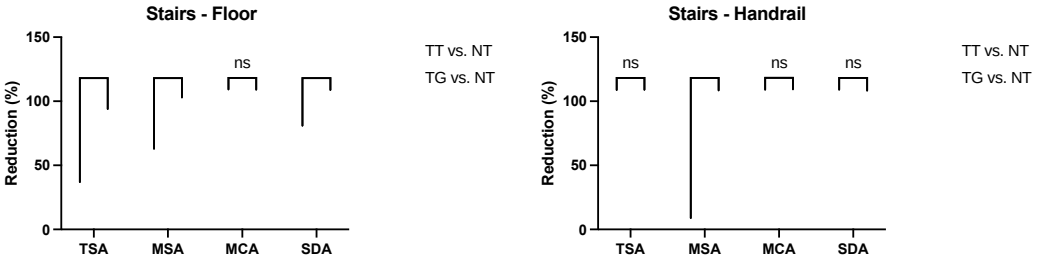

Figure 5. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of stair elements, such as the floor area and the handrails. The data are the average of two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages; ns: not significant.

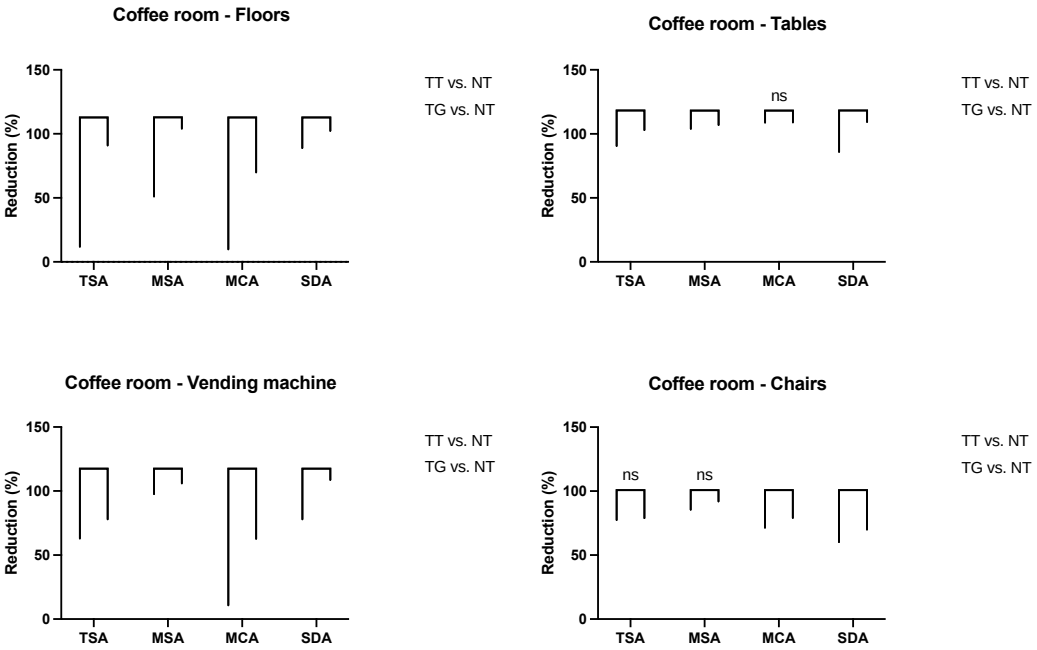

Figure 6. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of the coffee room, including the floor area, vending machine, and tables. The data are the average of two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages: ns: not significant.

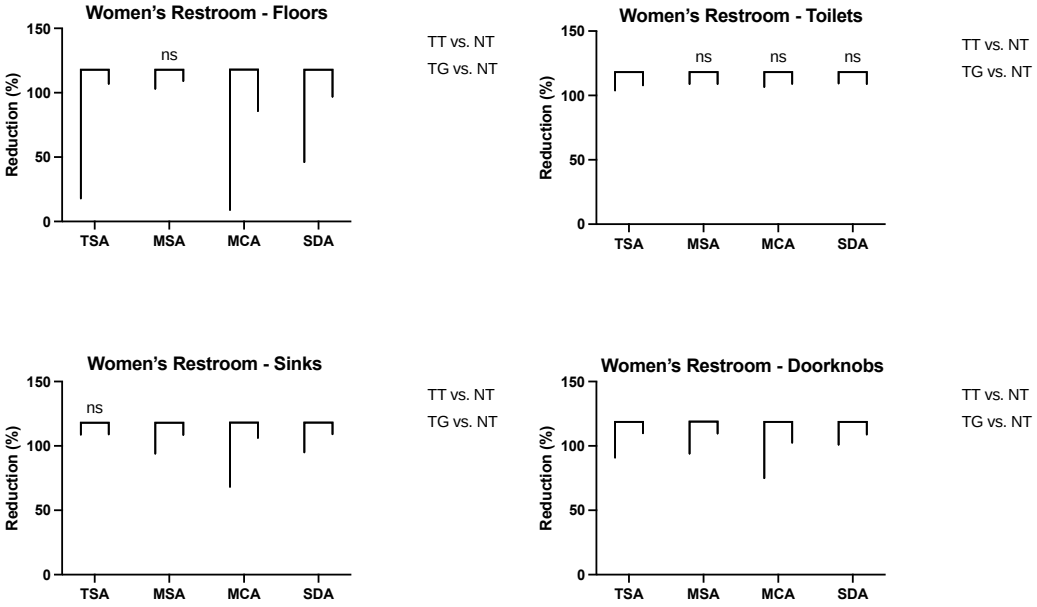

Figure 7. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of the women's restroom, such as the floor area, toilet surfaces, sink surfaces, and doorknobs. The data are the average of two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages: ns: not significant.

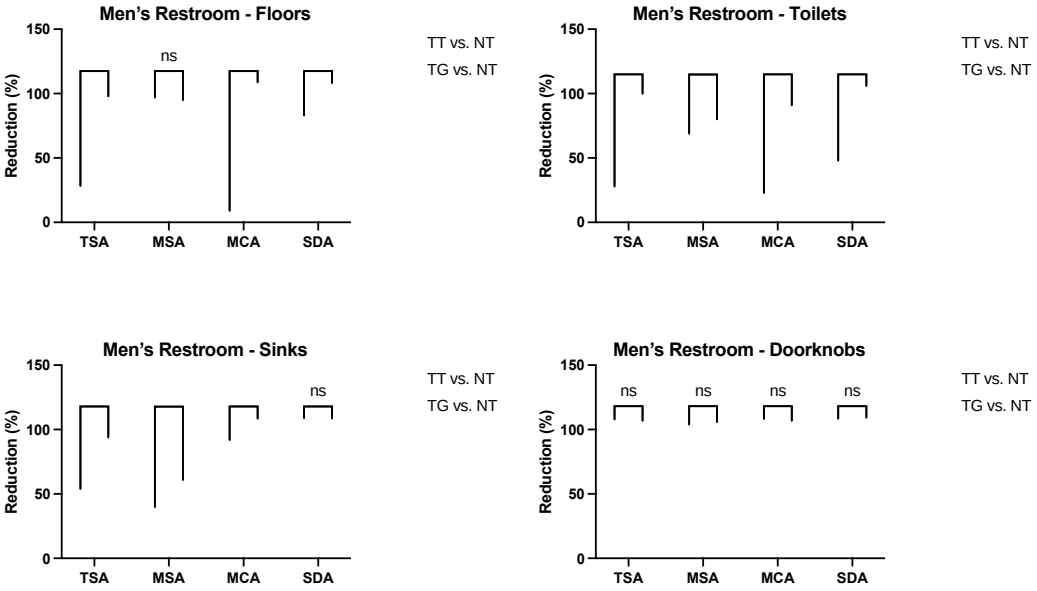

Figure 8. Reduction in colony formation units expressed as a percentage between non-treated (NT) and treated conditions (TT and TG) for various aspects of the men's restroom, such as the floor area, toilet surfaces, sink surfaces, and doorknobs. The data are the average of two separate experiments conducted in triplicate (mean ± standard deviation), and the results are expressed as percentages: ns: not significant.

In the context of non-medical environments (not exclusively related to healthcare or food processing), a study conducted by Reynolds et al. revealed that around 93% of nearly 200 sampled surfaces from locations such as shops, kindergartens, offices, gyms, restaurants, and children's play equipment were contaminated. Some instances displayed substantial bacterial concentrations, reaching 2×10^6 CFU/10 cm^2. An assessment of 60 environmental surface samples revealed the presence of coliforms (7%) and fecal

bacteria (1.5%) as well [35]. Another investigation by Elsergany et al. (2015) scrutinizing surfaces from four different shopping centers in Sharjah (United Arab Emirates) discovered that 80% of the samples exhibited total bacterial loads, with average values ranging from 500 to 1500 CFU/cm^2 (depending on the surface type), and *Staphylococcus aureus* was detected [36]. Even domestic settings were not spared from microbial scrutiny. The interior surfaces of homes, including beams, floors, and flat edges, commonly harbor *Penicillium chrysogenum*, along with *Penicillium glabrum* and *Penicillium corylophilum* (Bech-Andersen and Elborne, 2003) [37]. In the study conducted by Adams et al. (2013), four buildings within a university precinct were sampled despite no evident fungal pollution issues. The internal surfaces exhibited fungal contamination akin to the exteriors of the buildings. The fungal genera *Cladosporium* and *Cryptococcus* thrived on thresholds, which were not observed externally. Thermotolerant genera like *Exophiala*, *Candida*, and *Fusarium* were present in pipes [38].

Various areas were sampled and designated, with selective Mannitol salt agar used for *Staph. aureus* isolation (MSA), MacConkey agar (MCA) for enterococci, and Sabouraud dextrose agar (SDA) for mold and yeast. The microorganisms, primarily those presented in Table 3, were isolated and identified using biochemical API tests from Biomerieux.

Table 3. Microorganisms isolated in the different environments.

Staphylococcus cohnii	*Buttiauxella agrestis*	*Enterobacter aerogenes*	*Pectobacterium carotovorum*
Staph. hominis	*Candida albicans*	*Enterobacter gergoviae*	*Providencia rustigianii*
Staph. gallinarum	*Candida ciferrii*	*Enterococcus gallinarum*	*Raoultella* spp.
Staph. epidermidis	*Candida dattila*	*Enterococcus hirae*	*Firmicutes* spp.
Staph. auricularis	*Trichosporon* spp.	*Escherichia fergusonii*	*Stenotrophomonas* spp.
Staph. xylosus	*Scolecobasidium humicola*	*Escherichia hermannii*	*Curtobacterium* sp.
Staph. simulans	*Cyphellophora olivacea*	*Escherichia coli*	*Dyadobacter* sp.
Staph. sciuri	*Exophiala oligosperma*		*Penicillium* spp.
Staph. capitis			
Staph. aureus			

3.2. LCA

The results of the comparative LCA showed that the "GREEN" protocol, compared to the "Traditional" protocol, allows the avoidance of the emission of 260 g of CO_2-e per square meter of cleaned surface every year. If parameterized to the entire surface of the cleaning site—the premises of the M.A.R.R. Directional Centre situated in Santarcangelo di Romagna, Italy—the avoided emissions amount to 925 kg of CO_2-e every year.

The most significant reduction in absolute terms is associated with the detergents used in the laundry system. Thanks to the use of eco-labeled and more concentrated detergents, it was possible to avoid emissions of 654.1 kg of CO_2-e per year of service (−77% compared with the use of traditional laundry detergents). It was also possible to reduce the total detergent consumption by 104.3 kg every year (−27% compared with the use of traditional detergents).

The two primary processes that determine the most significant impacts are as follows:

- For the "GREEN" protocol: laundry chemical production (29% of the total GWP100 score), laundry energy consumption (25%), and cleaning trolley production (20%);
- For the "Traditional" protocol: laundry chemical production (54% of the total GWP100 score), laundry energy consumption (23%), and cleaning trolley production (8%).

As for the "GREEN" protocol, a greater incidence of water consumption and wastewater treatment was found compared to the "Traditional" protocol. The total water consumption in the "GREEN" protocol was 66 cubic meters, compared with 54 cubic meters

(+22%) in the "Traditional" protocol. This higher impact is partly associated with a higher number of laundry cycles than the "Traditional" protocol (852.3 cycles per year for the first against 833.3 cycles per year for the second protocol) and partly with the greater water consumption for laundry and the dilution of cleaning chemicals. On the other hand, the choice of cleaning and laundry detergents with more sustainable characteristics proved to be a virtuous and winning choice given their significantly lower impact per unit of weight, despite this resulting in greater water consumption in the use phase at the cleaning site.

An assessment of the data quality was conducted, covering checks for completeness and consistency. This assessment revealed that a minimum of 99% of material and energy flows, as well as 99% of environmental impacts, were accounted for. Furthermore, the analysis of proxy data indicated that their influence on the final quantified GWP value was less than 3% for both the "GREEN" and "Traditional" protocols.

The main limitations of the LCA consist of the value choices relating to different aspects, from allocation procedures to the system boundaries to assumptions about specific aspects, such as transport or end-of-life processes. Another important limitation is the availability of the data, whether primary or secondary and their precision and accuracy. Finally, the only impact assessment methodology adopted for the LCA, i.e., climate change, although considered very relevant, is not able to capture all the critical aspects, advantages, and disadvantages inherent to the product system under study.

4. Conclusions

In summary, the literature underscores the recognized and substantiated concern of surface contamination across occupational and non-occupational settings. The evidence from microbiological monitoring substantiates the need for measures to prevent and manage contamination. These measures entail the strategic implementation of environmental microbiological monitoring, the utilization of appropriate disinfectants, and the assessment of the efficacy of cleaning and disinfection processes on surfaces. Nonetheless, based on the collected data, it can be affirmed that both the "Traditional" and "GREEN" protocols have demonstrated satisfactory cleaning effectiveness. This aligns with the findings in the existing literature and falls within the contamination limits outlined in the INAIL guidelines.

In this research, the extent of microbiological contamination within workplaces does not pose an immediate health hazard to employees and visitors. Nevertheless, the presence of potentially pathogenic biological agents in certain areas could serve as a potential source of transmission to workers and cleaning staff. Given the potential exposure to these hazardous biological agents, adopting measures aimed at preventing microbial transmission and interrupting transmission pathways may enhance occupational safety in this work environment.

The degree of microbiological contamination observed in all the areas examined was deemed satisfactory and fell within acceptable limits. Furthermore, this contamination level was consistent with that observed in other office spaces. This could suggest that the existing and proposed cleaning and disinfection procedures are effective in maintaining a safe and sanitary work environment.

Additionally, the examination conducted led to the conclusion that the "GREEN" experimental system exhibits superior performance concerning GHG emissions compared to its "Traditional" counterpart. Its adoption results in the prevention of 260 g of CO_2-e per square meter of cleaned surface annually. Consequently, the yearly implementation of the "GREEN" protocol across the entire cleaning site translates to emission reductions equivalent to 925 kg of CO_2-e.

In the future, the use of textile equipment for floor cleaning with a greater yield in terms of cleaned surface for each use could bring further benefits to the "GREEN" protocol. In this way, the number of washing cycles necessary for textile equipment reconditioning would be reduced, with a consequent reduction in both the water and energy consumption of the washing machine, the use of laundry detergents, and the consumption of the textiles themselves.

Finally, in this work, two different evaluation methodologies were adopted: an environmental metric and a microbiological one, relating to hygienic quality. In the future, other possible metrics could be used, for example, in the socio-economic sphere, to develop this research towards a Life Cycle Sustainability Assessment. The application of such a holistic methodology would be a source of interesting indications in the professional cleaning sector, where the relevance of the workforce is still high.

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

Funding: This research was funded by Formula Servizi—Via Monteverdi 31, 47122 Forlì, Italy.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank all the representatives and the operators of the cleaning service. The authors wanted to thank the Consorzio Interuniversitario per le Biotecnologie (CIB).

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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