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Analysis of Civil Environments Cleaning Services—Microbiological and LCA Analysis after Traditional and Sustainable Procedures

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Abstract: The development of sustainable processes has an important role to play in balancing social productivity requirements, protecting the environment and natural resources. Up to date, efforts to combat microbial contamination have focused on the use of chemical-based sanitation procedures, which may have various limitations, as testified by the persistence of contamination itself, by the growing antimicrobial resistance of microbes and by the chemical related pollution. The purpose of this paper is to present a comparative analysis of the use of conventional and sustainable cleaning products and processes in civilian environments. The sampling campaign is conducted in a sports hall in Turin, Italy. Each sample comes from a specific environment, surface, furniture and sanitary and is taken using RODAC contact plates and swabs with a neutralizing agent in order to standardize the result of the microbiological evaluation. Sampling occurred before and after traditional and sustainable cleaning procedures. The sustainable experimental design using ecological products in the cleaning procedures of the analyzed areas proved to be a relevant technique. From CAM (minimal environmental criteria) requirements, the sustainable protocol must give equal or better results than the traditional protocol from both microbiological and environmental (LCA) point of view. It can be concluded that the ecological experimental system meets this criterion and has demonstrated better performance both in antimicrobial activity and in environmental impact than the conventional system: all findings are in an acceptable state of sanitation, with no evidence of pathogenic micro-organisms specified in the guideline.

Keywords: Life Cycle Assessment; antimicrobial; green sustainable products; environmental monitoring

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1. Introduction

The public has become increasingly sensitive to environmental issues in recent years. Hence, developing sustainable processes has an important role to play in balancing demands for social productivity, environmental protection and natural resources [1]. In sustainable development studies, Life Cycle Assessment (LCA) is known as a significant tool for the verification of appropriate sustainability along the evaluation of the environmental impacts of the design of a certain product or process [2,3]. The European Commission regards the LCA as the best framework currently available to evaluate the potential effects of products on the environment [4–6].

Hygiene plays a very important role in ensuring that adequate levels of safety are maintained in healthcare facilities, the food industry and any other working environment. Cleaning once a day is usually enough to remove microbial that may be on surfaces. This helps maintain a healthy facility, which further reduces any risk of spreading infection. Microbiologic sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is an expensive and time-consuming process that is complicated by many variables in

protocol, analysis, and interpretation. Therefore, monitoring of airborne microbial and surface contamination is of paramount importance in all situations where poor hygiene can lead to contamination and the possible spread of infection or disease. Such control is important and necessary to verify the effectiveness of cleaning procedures that are usually applied in environments such as hospitals, canteens, and food industries. Studies have been carried out over time on the dynamics of contamination and the effectiveness of disinfectants and sterilisation and adequate cleaning, antiseptic and disinfection procedures have been developed, considering the type of material to be treated and its use. These environments are normally also monitored thanks to guidelines and protocols specifically drawn up. There is a current lack in a standardized procedure for civil work environments not involving food processing or health facilities, which need to be implemented in the near future, also considering the impact of COVID-19 on civil workplaces [7–11].

The objective of the project is to develop a comparative LCA study to highlight the environmental added value of the protocol «PFE Green» in accordance with the new CAM (Minimum Environmental Criteria) for cleaning services compared to a traditional cleaning protocol in a Sport Hall of the city of Turin. CAMs represent the national reference point for green public purchases that can be used by procurement stations, to enable the Green Public Procurement Action Plan to maximize environmental and economic benefits.

The CAM of the cleaning service, published in the Italian Official Journal on 19 February 2021 (in force since 19 June 2021), identifies among the rewarding requirements the ability of the bidder to demonstrate the environmental and qualitative benefits of its protocol offered during the tender, compared to traditional protocol. In particular, the comparative LCA analysis study promoted by PFE S.p.A. (hereinafter abbreviated as PFE), according to the new CAM (D.M. 29 January 2021), meets the need to demonstrate the ability to reduce environmental impacts compared to traditional cleaning and sanitizing techniques, by presenting a comparative Life Cycle Assessment study in compliance with UNI EN ISO 14040–14044 technical standards.

Cleaning services' companies able to demonstrate this way the benefits of a "Green" protocol compared can obtain competitive gains on both the public and private markets. Moreover, this kind of study also demonstrates that it is possible to design a green cleaning service capable of concretely reducing the environmental impact. Therefore, it becomes a model for the cleaning sector so that systems, products and working methods with high environmental efficiency can be researched, tested and put on the market.

2. Materials and Methods

2.1. Cleaning Plan

The case study object of this analysis is represented by the cleaning service provided by PFE at the Palazzetto dello Sport Gianni Asti in viale Bistolfi, 10—Turin.

The contracting station is the City of Turin and the contract refers to the tender "For the cleaning, environmental hygiene and sanitization service at the sports facilities under the responsibility of the municipality of Turin" open procedure N.56/2021.

The reference yard is therefore an area of civil cleaning, consisting of grandstands, race field, changing rooms, services, offices, corridors, technical rooms, and warehouses. It has been chosen for this analysis because it is representative of the type of surfaces, the degree of dirt, the frequencies and the methodologies used in civil sites cleaning.

The cleaning protocol responds to the service specifications defined by the City of Turin for "ordinary and periodic activities, related to cleaning services, programmable and executable with certain periodicities and frequencies".

In accordance with the international reference standards for LCA assessment, the identification of the aspects characterizing the PFE cleaning service, and their schematization was the first step in the development of the analytical and calculation system.

Specifically, the sampled areas represent all the rooms of the structure for a total of 38,150 square meters (sqm).

2.2. Sampling Plan and Microbiological Evaluation

The sampling plan was carried out based on clinical evidence and standard protocols, collecting each sample in triplicate. Sampling involves collecting environmental samples from a variety of areas of use: changing rooms (where floor and hand-touchable surfaces have been sampled), bathrooms (where floor and hand-touchable surfaces, i.e., toilet, sink, shower, etc. have been sampled), playground and grandstands, infirmary room and the guardroom. Sampling occurred before and after cleaning procedures [12,13].

Swabs and Plates

RODAC (Replicate Organism Detection and Counting) contact plates (Liofilchem, Roseto degli Abruzzi, TE, Italy) were used and swabs with a neutralizing agent (Dey Engley) (Liofilchem) were also employed to standardize the results. The overall condition, cleanliness and moisture of the surfaces were initially visually assessed. Microbiological assessment in the form of aerobic colony counts (ACC) was based upon growth, after incubation at 37 °C for 48 h, on tryptic soy agar (TSA) RODAC plates coated with plate count agar with neutralizer. MacConkey Agar (MCA) with neutralizer were used for enterobacterial counts, mannitol-salt agar (MSA) for staphylococcal counts, sabouraud dextrose agar for yeasts and molds (SDA), Clostridium difficile agar (ClDA) for Clostridium count. RODAC plates were inoculated directly by pressing on to flat surfaces with the aid of a contact plate weight applicator for 30 s (VWR International, part of Avantor, Milano, Italy) or, for irregularly shaped surfaces, the entire hand contact area was swabbed using a sterile pre-moistened cotton wool swab, which was then used to inoculate agar plates. Swabs were sampled using a sterile 10 cm × 10 cm template to sample an area of 100 cm^2.

RODAC plates were then incubated within 2 h, colonies were counted after 24–48 h. A very slight growth (6–39 colonies) ≅ 10 CFU/25 cm^2 and a scant growth (<6 colonies) ≅ < 10 CFU/25 cm^2, where considered as acceptable for standard INAIL (Istituto Nazionale Assicurazione Infortuni sul Lavoro—National institute for insurance against industrial injuries) protocols, as intended for civil environments. Swabs were also plated in each different isolation agarized media (TSA, SDA, MSA, MCA and ClDA).

A total of 208 samples were collected. In Figure 1, a representative image of the sport yard while sampled by RODAC plates and swabs. Surface samples were collected from floors, tables, chairs, benches, toilets, sink, shower, and bathroom floors. Air samples were collected at the center of each room at 150 cm from the floor. The samples were transported to the laboratory in refrigerated insulated bags (0–4 °C). The temperature was monitored via data logger. The plates were incubated at 36 ± 1 °C for 48 h and SDA plates at 25 °C for 72–120 h. The colonies were then counted, isolated and identified [14–18].

Figure 1. Collection of swabs and plates from the PalaRuffini sport yard.

2.3. Microbial Isolation and Identification

Swabs samples were vortexed to aid the release of microbes into the diluent, followed by transfer and spreading on 90 mm petri dishes each containing 20 mL of the different agar (TSA, MCA, MSA, SDA, ClDA) for growth of microbes. The petri dishes were incubated at 36 °C (25 °C for SDA plates), where bacterial colony growth was observed every day up for 5 days. Colonies with unique phenotype (morphology, shape and color) were picked for further experiments and stored with 50% (v/v) glycerol at −80 °C. To identify bacterial isolated colonies, API (Analytical Profile Index) systems (Biomerieux Italia, Grassina, FI, Italy) have been used according to manufacturer's instructions. The analytical profile index, or API, is a bacterial classification based on biochemical tests, allowing rapid identification. This system is developed for quick identification of clinically relevant bacteria based on wells containing dehydrated substrates to detect enzymatic activity by the inoculated microorganism.

2.4. Cleaning Procedure

The trial was carried out subsequently with the two selected protocols: use of the "traditional" system (TT) for four weeks, followed by the "GREEN" experimental system (TG), for a total period of 8 weeks. In this way it was possible to compare the results of the different cleaning methods in areas with the same intended use, type of use and characteristics of contamination. Non-clean/non-treated surface sampling was carried out as our control (NT).

Protocols used are listed in Table 1. Subsequently, the cleaning protocols were designed thanks to the collaboration of some leading specialized green cleaning manufacturers: È Così Srl, Filmop International Srl and Fimap SpA.

Table 1. Cleaning operations and environments.

Cleaning Operation	Environment	Frequency
Wet dusting with detergent	Furniture in all indoor areas	Daily (7/7)
Wet dusting with detergent	Area 3—Toilets/changing rooms	Daily (7/7)
Periodic descaling toilets and sanitary	Sanitary in all internal areas	Bi-monthly (2/30)
Wet dusting of electronic equipment with suitable detergent	Cleaning of electronic, mechanical and computer equipment in Area 1—Offices and Area 4—Technical areas	Daily (7/7)
Disinfection of changing rooms	Inside and outside lockers in changing rooms in Area 3—Changing rooms	Daily (7/7)
Disinfection furniture accessories of bathrooms and showers	Area 3—Toilets	Daily (7/7)
Manual floor washing	All indoor areas (central arena excluded)	Bi-weekly (2/7)
Manual floor washing with detergent-disinfectant	Changing rooms	Bi-weekly (2/7)
Mechanized floor washing (with scrubbing machine)	Corridors in all internal areas	Bi-weekly (2/7)
Dry sweeping and punctual stain removal	Central Arena in Area 9—Sport spaces/stands/pools	Bi-weekly (2/7)
Washing of entrance doors and windows	Area 2—Connective spaces	Monthly (1/30)

Briefly, the treatment differed for:

- Use of scrub cloths in TT treated at higher temperatures (60 °C) than TG (40 °C).
- The use of eco-labelled products for the cleaning of floors and surfaces in TT, with lower dilution of use (2–100%) compared to the products of the experimental protocol TG (dosage 0.8–10%).
- Use of eco-labelled textiles for the cleaning of floors and surfaces in the experimental protocol TG. Specifically, the cloths used for the cleaning of floors passed the test

conducted by an independent laboratory following the ISO:23231 as for the release of microplastics in the water of washing and rinsing [19].

- The experimental protocol "GREEN" involved more durable textiles for the cleaning of sanitaria, taps and furniture. Such textiles can be used up to 500 washing cycles at 95 °C [20,21].

PFE Green and Traditional Protocols

The TG and TT system were carried out as follows:

2.5. Standard/GREEN Protocol Active Ingredients

2.5.1. Peracetic Acid

Combines the disinfectant and germicidal actions of hydrogen peroxide with an enhanced liposolubility, and the resistance towards the decomposition mediated by peroxidases and catalases. It remains effective even in presence of organic material and its degradation products (acetic acid, oxygen, hydroxide peroxide and water) are not dangerous and are easily disposed. It is considered that the biocidal mechanism is linked to the oxidant action on lipidic membranes, DNA and other essential components to the life of a cell. Its sporicidal effect can be explained by its peptidic degrading action. Focusing on hydrogen peroxide, it is considered a local irritant, however, the effects depend on the concentration and are characterized by irritations of the mucous membranes of mouth, esophagus, stomach and intestinal tract as well as the lung. Concentrated hydrogen peroxide ($\geq$50%) causes severe skin burns and eye damage. Concentrations of 35 $\leq$ 50% cause skin irritation and may cause respiratory irritation. Up to concentrations of 5%, no irritation is expected. As for the environmental toxicity, based on available literature, concentrated hydrogen peroxide is toxic to aquatic organisms, but the product is readily biodegradable in H_2O and O_2 and it does not have any bioaccumulation potential [22–24].

2.5.2. Sodium Hypochlorite

If stabilized in aqueous solution it generates hypochlorous acid (HOCl), which acts as an active chlorine reservoir characterized by a strong oxidant potential towards bacterial cells. Sodium hypochlorite-based disinfectants have a wide range of action. Therefore, they are extremely effective towards bacteria, fungi, spores and viruses.

2.5.3. Phenylphenol

Organic compound well known for its antimicrobial properties. If combined with surfactants is effective against gram+ and gram- bacteria, fungi, and lipophilic viruses. Phenylphenol reacts with a variety of macromolecules and microbial structures via its reactive hydroxyl groups. It attacks integral membrane proteins turning them in a colloid state. In some cases more specific mechanisms of action have been described in the inhibition of fatty acid synthesis (by inhibiting the activity of enoyl reductases).

2.6. LCA Analysis

LCA is a methodology standardized by ISO 14040:2006 [25] and ISO 14044:2006 [26]. Furthermore, ISO 14067:2018 standard was also taken as a reference [27] in order to quantify the Carbon Footprint of the cleaning service, i.e., its greenhouse gases emissions.

ISO 14067:2018 standard establishes that reference shall be made to relevant Product Category Rules (PCR), if existing. In this case, PCR 2011:03 v3.0.1 "Professional cleaning services for buildings" were considered for further specific indications for the UN CPC 853 product [27,28]. PCR establishes the main analysis requirements, in terms of functional unit, system boundaries—that is processes included in the analysis—type and quality of data to be used and applicable cut-off criteria.

The functional unit used is that defined by the existing PCR, namely 1 square meter of representative average surface kept clean for 1 year. In defining the representative

surface, the different types of environments considered in the sample area were taken into consideration.

The system boundaries adopted are of "cradle-to-grave" type. The processes included in the analysis, divided into the three phases (as seen in Figure 2), "upstream", "core" and "downstream", are as follows:

- The "upstream" phase included:
  - extraction and processing of raw materials;
  - transport of raw materials and semi-finished products to suppliers;
  - the production of consumer goods, namely chemicals (detergents) and textiles (fringes);
  - production of cleaning trolley;
  - the production of machinery (washer and dryer, washing machine).
- The "core" phase included:
  - the supply chain of consumer goods from producers to the yard;
  - the implementation of the service through the use of chemicals and textiles;
  - production of transport fuels;
  - the production of electricity used in the construction site for the implementation of the service;
  - water consumption for dilution of chemicals and by the washing machine.

Transport of personnel and maintenance workers was excluded from the study. Transport of durable goods (shelf-life longer than 3 years) has been excluded according to point 4.3.1.2 of the PCR because it does not take place continuously during the service life.

- The "downstream" phase included:
  - the transport and treatment of solid and wastewater generated by the processes of the "core" phase.

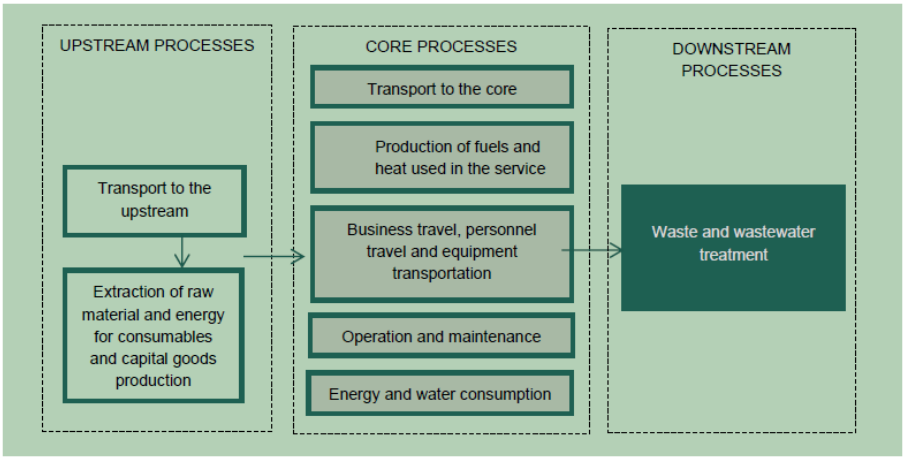

Figure 2. A diagram illustrating the different processes included in the analysis.

The main assumptions adopted for the study refer to the principles set out in ISO:14026 for comparative analysis. Specifically, it is assumed that:

- The two systems compared have the same functional unit, they are equivalent in terms of spatial characteristics and interventions;
- Being a comparative analysis study, similar processes have not been considered, for example the transport of personnel at the site, which does not vary between the two protocols;
- The areas investigated are the same;
- The criteria for inclusion of inputs and outputs are identical;
- Data quality requirements are the same;

- Life cycle inventory units are identical;
- The calculation procedures are similar;
- Allocation rules are equivalent;
- The selected impact categories and characterization factors are identical (ISO:14067, GWP100);
- The types of intervention and the frequency of operations are identical;
- The surfaces are comparable by type of floor, functions of use of the property and overall dimensions of the surface itself;
- The degree of use of the sampled areas and dirt level are equivalent;
- As regards electricity consumption, due to the lack of specific data of the yard-specific supply contract, the residual national energy mix has been taken as a precautionary measure;
- The total area of the building is 38,150 sqm.

The Impact Assessment methodology is implemented through the calculation of the Global Warming Potential (GWP) impact category, based on the model created by the Intergovernmental Panel on Climate Change (IPCC). This model evaluates the contribution to the increase in the greenhouse effect of some gases present in the atmosphere (namely CO_2, CH_4, N_2O, SF_6, HFCs, PFCs), correlating the quantity emitted to the category indicator "kg CO_2 equivalent", using the specific characterization factor. This factor varies according to the substance efficacy in influencing the radiative forcing and its average residence time in the atmosphere, allowing to relate the GWP of each substance to the GWP of CO_2, set equal to 1. The time threshold considered is one hundred years and the characterization factors refer to the 5th IPCC Report of 2014. The total impact score is obtained by adding together the contributions of each substance once translated into kg CO_2-equivalent.

The impact assessment phase is limited to a single impact category because the quality of available data for quantifying other impact categories is judged insufficient by the authors. Although limited to a single impact category, the analysis takes into consideration the one currently considered of greatest relevance. Moreover, the use of Ecolabel-branded ecological detergents in the adopted protocols guarantees the exclusion of chemical substances potentially harmful to aquatic ecosystems and human health. As a matter of fact, wastewater generated by the cleaning service is assimilated to urban sewage.

Among the processes considered by the reference PCR for the professional cleaning service of buildings, there is no other industrial processes with a particularly hazardous.

2.7. Statistical Analysis

Data were analyzed using Graphpad Prism (Graphpad software). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test with GraphPad Prism version 9.0.0 for MacOS (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

3.1. Cleaning Effectiveness

The "GREEN" experimental protocol using ecological and super-concentrated detergents products in combination with the use of new-generation cloths and mops in microfiber and high performance scrubbing machine, in the sanitization procedures, was found to be a technique of relevant interest. The experimental system has proved, within the limits of the sampling performed, that the performances are in agreement with the conventional system.

All the sampled spaces were found acceptably cleaned: in fact, all spaces resulted in a significant decrease of microbial contamination, and the TG has always been comparable (if not better) to the TT with every technique used. The data reported are the most representative sampling for each sampled space.

It should be noted that variability in the number of microorganisms found in different environments is subject to variability in use and use of the environment. Therefore, the

data to be evaluated are the microbial abatement (or percentage reduction) between pre-treatment (NT) and post-treatment (TT or TG).

The percentage reduction (TT and TG vs. NT) of sampled microorganisms with swabs and total bacterial RODAC count plates on TSA, MSA, MCA and SDA are subsequently reported from Figures 3–16.

Figure 3. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG)—Changing room, floors area. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

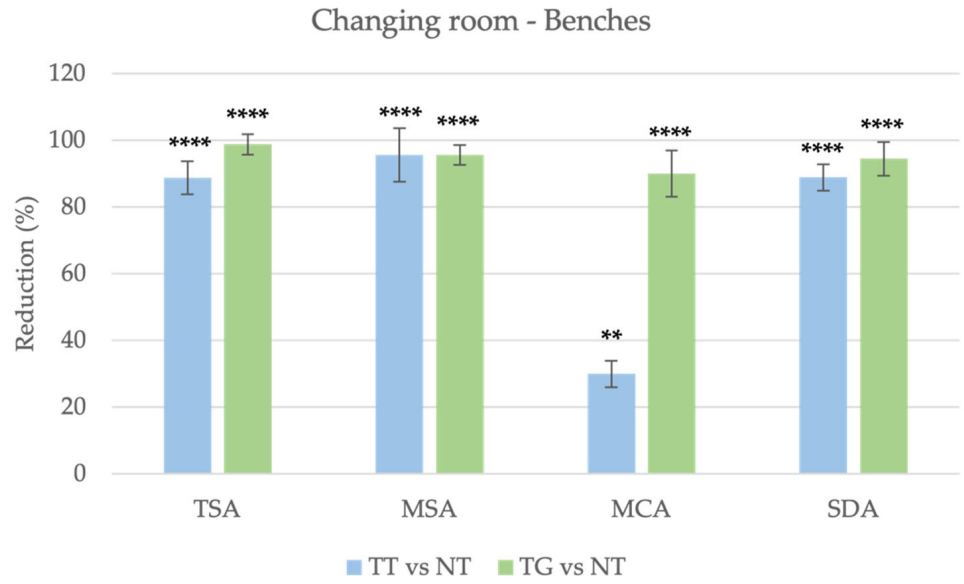

Figure 4. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG)—Changing room, benches top. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

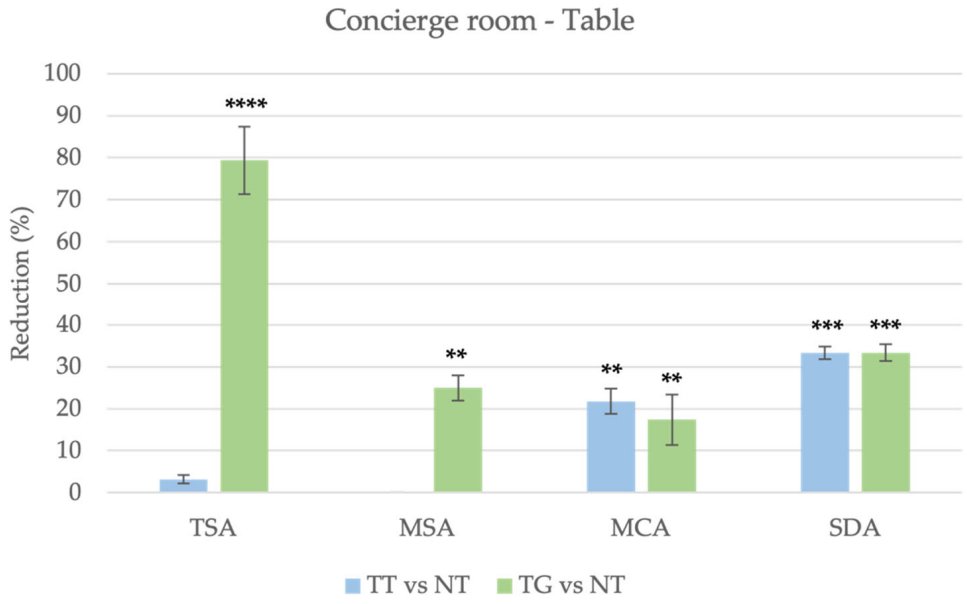

Figure 5. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) concierge room, tabletop. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

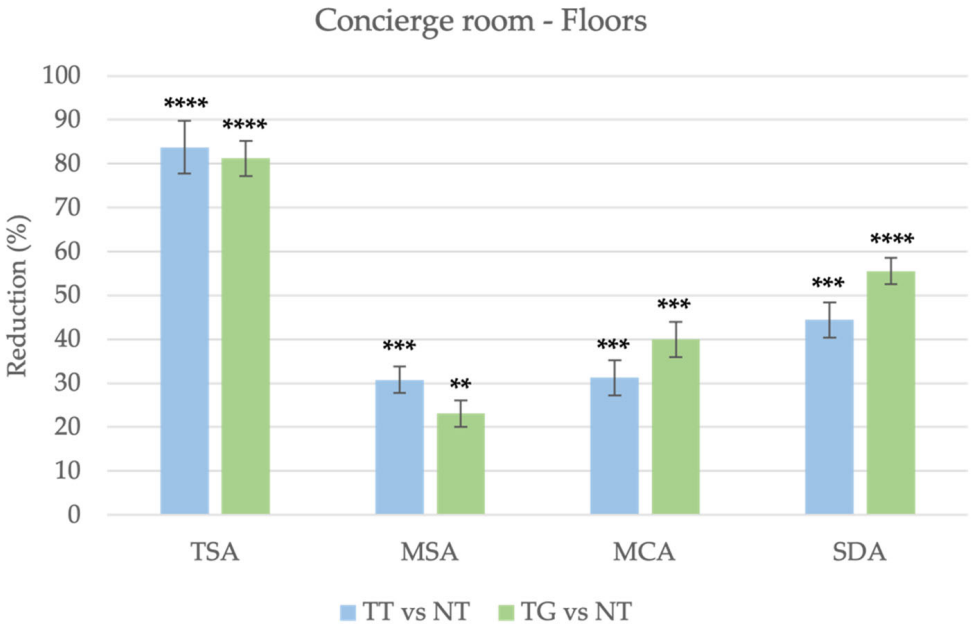

Figure 6. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) concierge room, floor area. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

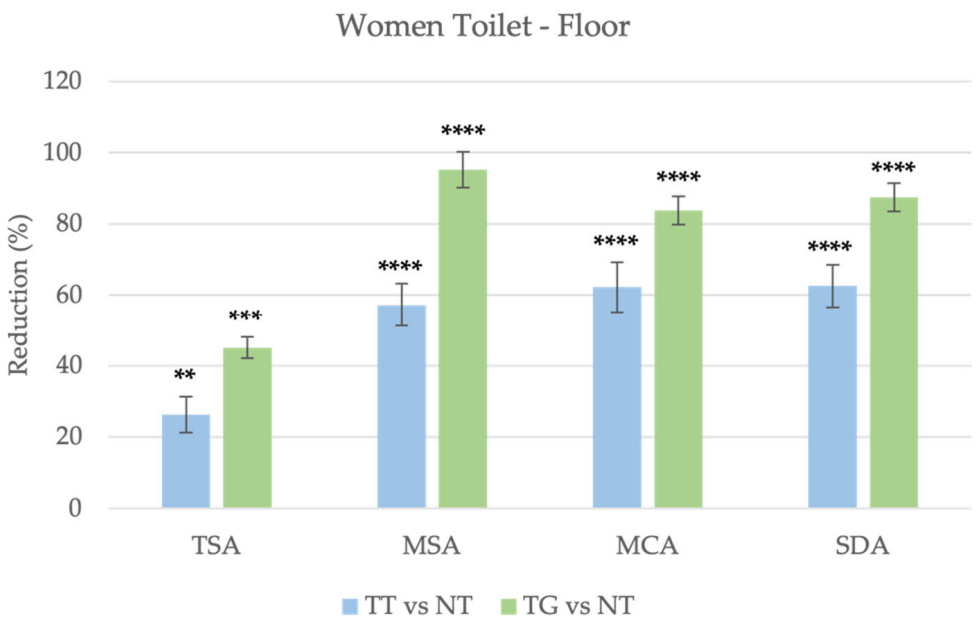

Figure 7. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) women toilet, floor area. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

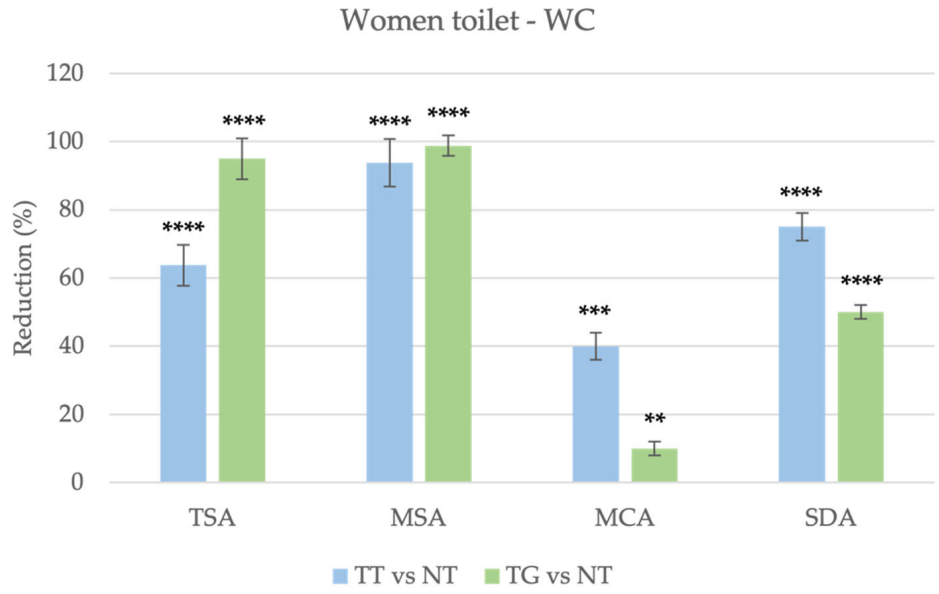

Figure 8. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) women toilet, WC surfaces. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

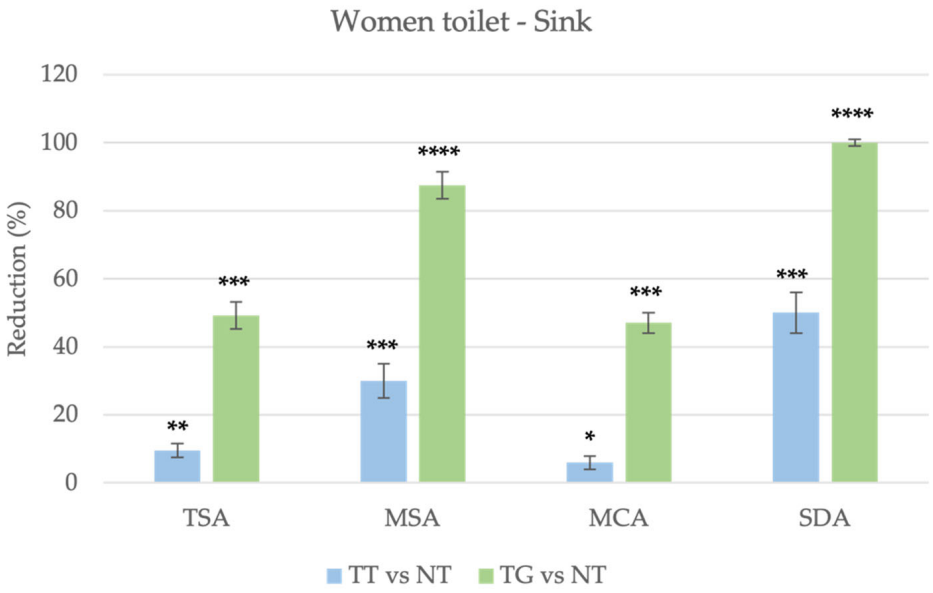

Figure 9. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) women toilet, sink surfaces. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. * $p = 0.01$; ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

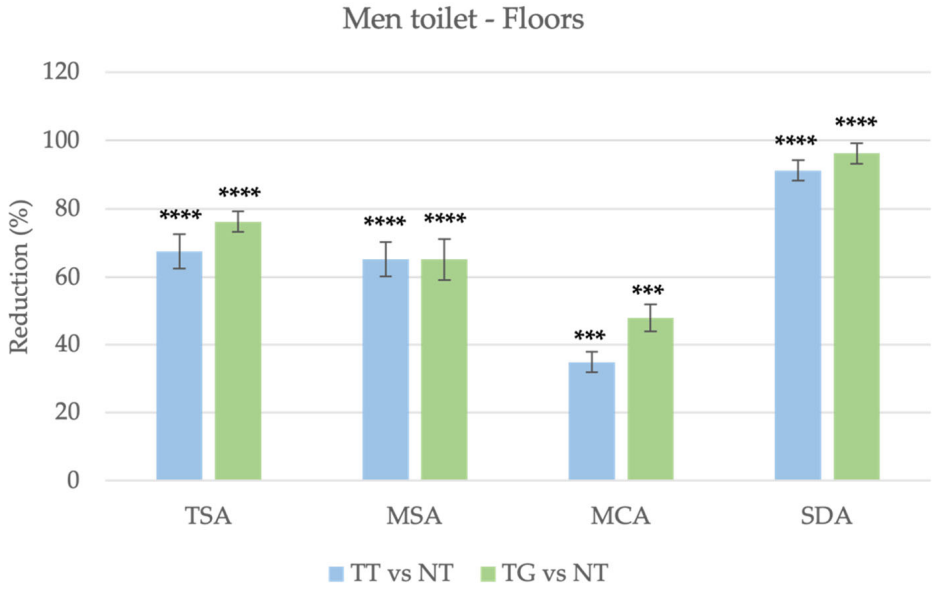

Figure 10. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) men toilet, floor area. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

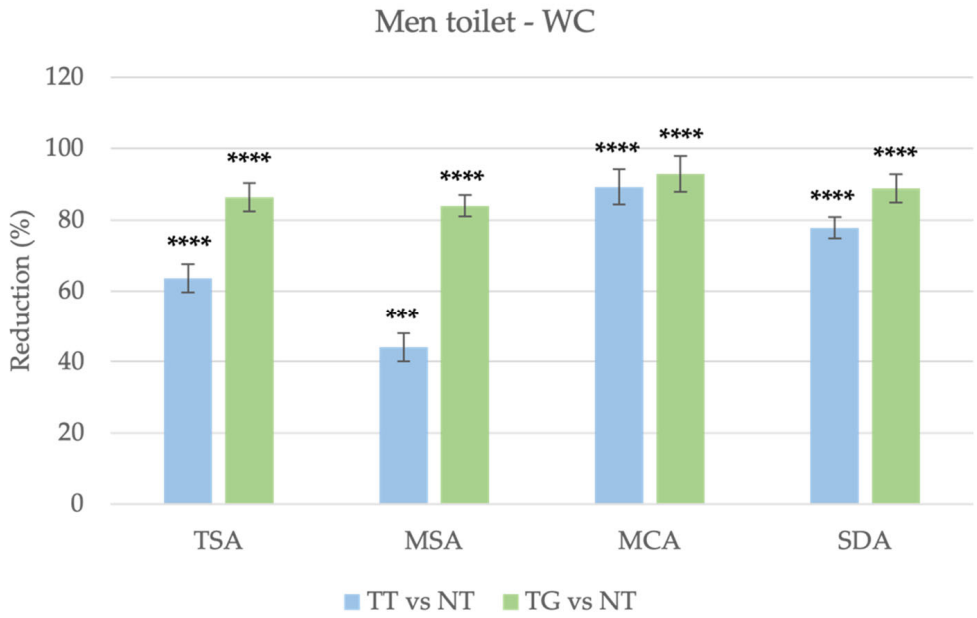

Figure 11. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) men toilet, WC surfaces. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

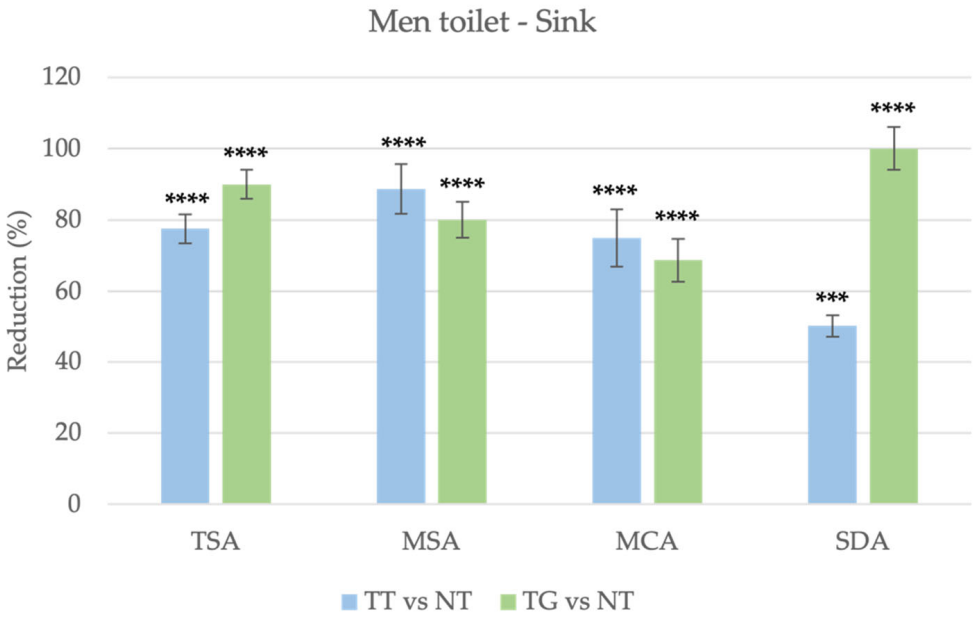

Figure 12. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) men toilet, sink surfaces. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

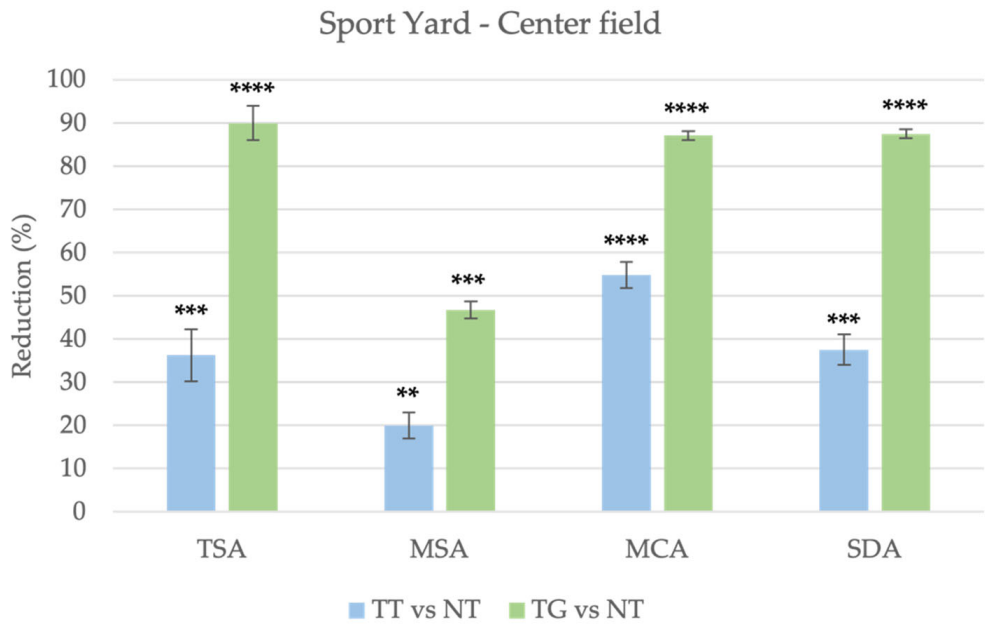

Figure 13. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) yard area of the sport court, center of the field. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. ** $p = 0.001$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

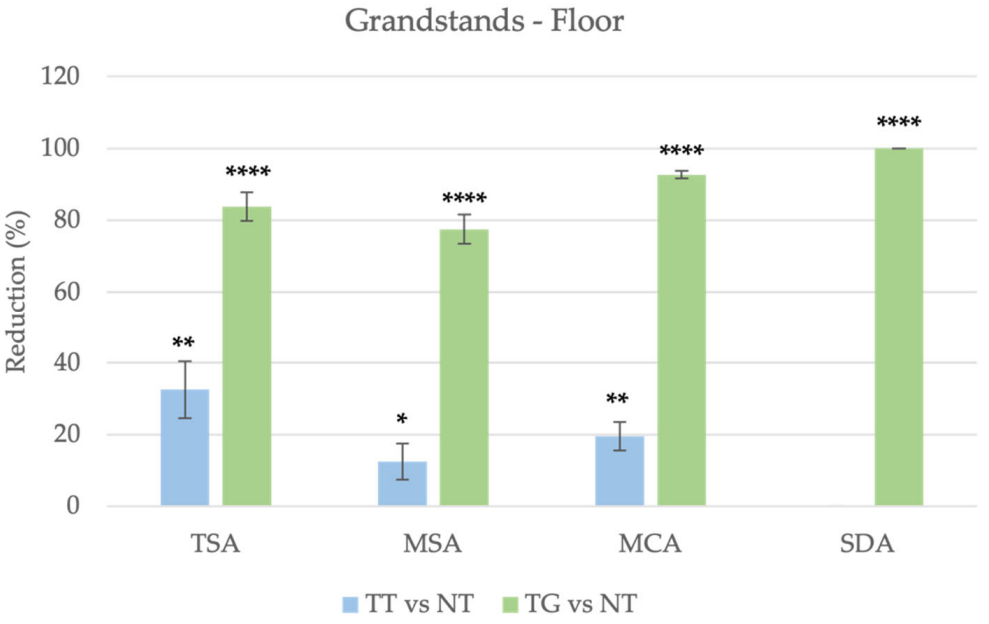

Figure 14. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) grandstand area, floors. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. SDA in TT did not show any reduction. * $p = 0.01$; ** $p = 0.001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

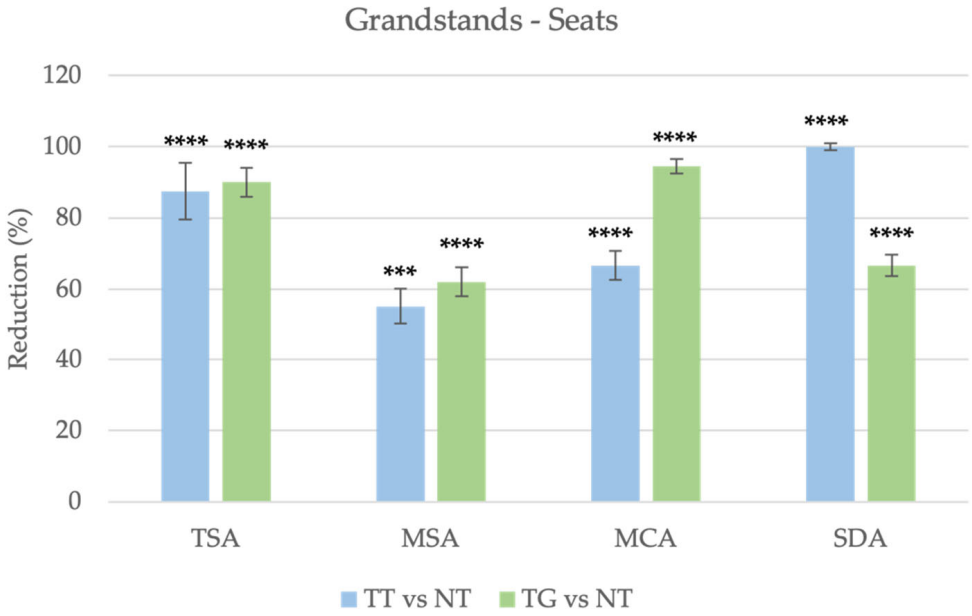

Figure 15. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) grandstand area, seats. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

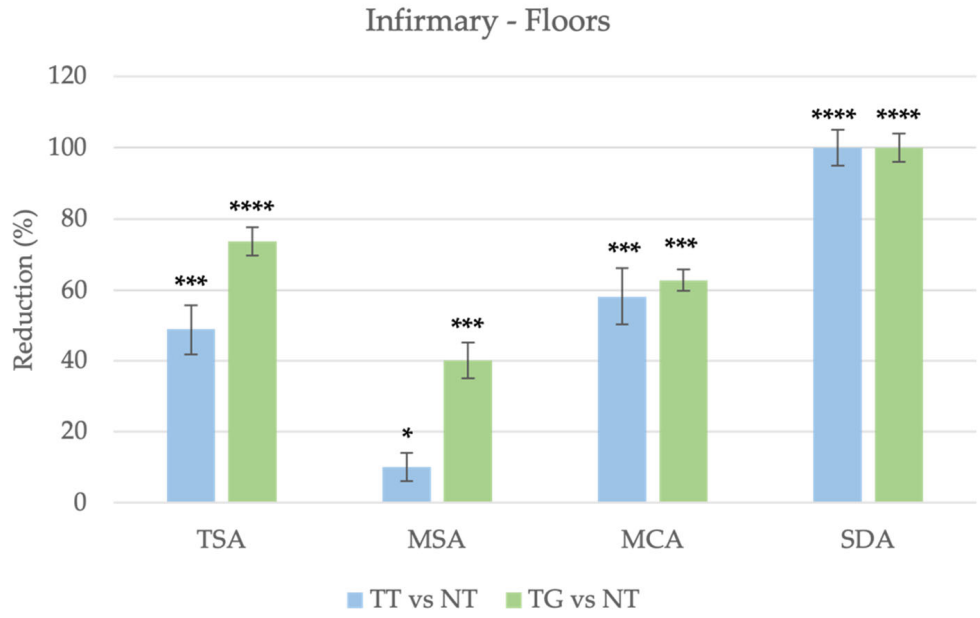

Figure 16. Percentage reduction of Untreated vs. Traditionally Treated (TT) and Green Treated (TG) infirmary area, floors. Data are the mean of 2 independent experiments performed on triplicate (mean ± standard deviation), and values are represented as a percentage. * $p = 0.01$; *** $p = 0.0001$; **** $p < 0.0001$ (ANOVA followed by Dunnett post test).

3.2. Microbial Isolation and Identification

The different spaces were sampled and titled in selective Mannitol Salt Agar soils for the isolation of *Staph. aureus* (MSA), MacConkey Agar for Enterococci Isolation (MCA), Sabouraud Dextrose Agar for Mold and Yeast Isolation (SDA), Clostridium difficile Agar Base for *Clostridium* Isolation (CDBA). The biochemical API Tests (Biomerieux) have been isolated and identified mainly with the following microorganisms, shown in Table 2:

Table 2. Principal microorganisms isolated in the different environments.

Isolated Microorganisms		
Staphylococcus cohnii	*Enterobacter* spp.	*Buttiauxella agrestis*
Staph. hominis	*Enterococcus faecium*	*Candida albicans*
Staph. gallinarum	*Enterococcus gallinarum*	*Candida boidinii*
Staph. epidermidis	*Enterococcus hirae*	*Candida catenulata*
Staph. auricularis	*Escherichia coli*	*Candida ciferrii*
Staph. xylosus	*Pectobacterium carotovorum*	*Trichosporon* spp.
Staph. sciuri	*Providencia rustigianii*	*Saccharomyces cerevisiae*
Staph. capitis	*Raoultella* spp.	

From literature data on the spread of infectious agents responsible for diseases of patients and health professionals, it is clear that surfaces have a primary role in the spread of microorganisms in the healthcare sector. Infections caused by strains of methicillin resistant *Staphylococcus aureus* (MRSA) have been documented not only among health professionals but also among staff working closely with animals, such as breeders, farmers and veterinarians [29,30]. All of these environmental studies are supported by a strict regulation and rigid safety protocols and microbial contamination threshold.

As for civil environments (not strictly health-care or food-processing associated), a study by Reynolds et al. showed that the total bacterial load in about 200 samples taken from surfaces of shops, kindergartens, offices, gyms, restaurants and children's play equipment, etc. 93% of the samples were contaminated, in some cases with very high bacterial concentrations (up to 2×10^6 CFU/10 cm^2). In 60 samples taken from environmental surfaces, coliforms (7%) and fecal bacteria (1.5%) were also isolated [31]. In another study, Elsergany et al. (2015) found that out of a total of 224 samples taken from surfaces of 4 different shopping centers in Sharjah (United Arab Emirates), 80% of them showed total bacterial loads with average values from 500 to 1500 CFU/cm^2 (depending on the type of surface examined), with the presence of *Staphylococcus aureus* [32]. Bacteria and fungi were also sampled in domestic environments. On the internal surfaces of homes (joists, floors, flat edges) the most commonly found species is represented by *Penicillium chrysogenum*, but also *Penicillium glabrum*, *Penicillium corylophilum* [33]. In the work of Adams et al. (2013), 4 buildings in the university area that did not present obvious problems of fungal pollution were sampled. Internal surfaces showed fungal contamination similar to that the outside of the buildings. Fungal genera *Cladosporium* and *Cryptococcus* were abundant on thresholds and had not been found outside. Thermotolerant genera such as *Exophiala, Candida* and *Fusarium* were found on the pipes [34].

In short, it is clear from the literature that the problem of contamination of surfaces in working and non-working environments, is perceived and confirmed by the results of the microbiological monitoring carried out and how the measures to be implemented for the prevention and control of contamination must necessarily include the programming of environmental microbiological monitoring, the use of suitable disinfectants and the evaluation of the effectiveness of cleaning and disinfection operations carried out on surfaces.

Nevertheless, from the data obtained and reported above, it can be stated that "traditional" and "GREEN" protocols have had a good performance in terms of cleaning effectiveness, in line with literature data and within the limits of contamination reported on INAIL guidelines.

3.3. LCA Analysis

The results of the comparative LCA analysis show how the Green Protocol of cleaning, compared to the Traditional Protocol, allows for the annual avoidance of 7.68 g of CO_2e emissions per square meter of cleaned surface. The environmental added value of the Green Protocol is even more obvious if we consider the scenario of application to the entire pilot site subject of this study—the Palazzetto dello Sport Gianni Asti in Turin. The results

of this comparative analysis show that the cleaning service provided in accordance with the Green Protocol on the entire pilot site avoids the emission of 293 kg of CO_2e every year.

The most significant reduction in absolute terms is associated with the Textile Equipment aspect. In fact, thanks to the use of last generation reconditionable equipment (Filmop Globo Micro-Activa Filmop and Eco Multi-T Filmop) it was possible to avoid emissions of 379.88 kg of CO_2e per year of service at the pilot site (−98.6% compared with the use of traditional protocol textiles).

The first three processes that determine the most significant impacts are:

- for the Green Protocol: cleaning chemicals (36.6%), energy consumption (28.5%) and laundry chemicals (17.3%);
- for the Traditional Protocol: textile equipment (46.2%), cleaning chemicals (24.8%) and energy consumption (12.6%).

As for the Green Protocol, the greater incidence of energy consumption and washing machine products is associated with a number of laundry cycles higher than the Traditional: 222.6 cycles per year for the first one against 123.1 cycles per year for the second protocol. The greatest number of cycles is associated with choices such as pre-impregnation in the machine, for washing the floors, and the dedusting of the floors with reconditionable fringe. On the other hand, these two virtuous choices of the Green Protocol ensure an extremely low consumption in terms of textile equipment (in the Traditional Protocol a disposable equipment is used for dedusting), as well as chemical and water. In addition, you should also consider the washing program that, thanks to laundry products effectiveness even at low temperatures, in the Green Protocol, it has been set to 40 °C instead of 60 °C. An overall reduction in the life cycle that is rewarded by a significant reduction in the system (−35.2%).

A data quality analysis encompassing completeness and consistency checks is performed. The analysis shows that at least 99% of material and energy flows, and 99% of environmental impacts, are included.

The analysis of proxy data shows that their contribution to the quantified final GWP value results in less than 1% for both the Green and the Traditional Protocol.

4. Conclusions

In conclusion, the study conducted for the professional cleaning service carried out by PFE at the Palazzetto dello Sport Gianni Asti in Turin represents a reliable pilot study in terms of correctness and representativeness, and whose contents can be used to both inform stakeholders of the environmental benefits of their Green Protocol, and with a view to a continuous improvement of their environmental performance.

This study is one of the few regarding cleaning effectiveness in civil environments. The analysis helped to realize the urgent need for standardized protocols, procedures and contamination limits, in order to furtherly uniformize methodologies and to better recognize the situations in which a cleaning preventive measure must be taken into consideration.

From the data obtained and reported above it can be stated that “traditional” and “GREEN” protocols both have had a good performance in terms of sanitizing effectiveness, and that “GREEN” protocol has had a better performance in terms of GHG (Greenhouse Gases) emissions than the “traditional” one. From CAM requirements, the green protocol must give equal or better results than the traditional protocol from both microbiological and environmental point of view. Following the analysis carried out, it can be concluded that the “GREEN” experimental system meets this criterion and has shown better performance than the “traditional” system regarding the antimicrobial activity and always within the threshold (all findings are in an acceptable state of sanitation). Overall, it is noteworthy that the antimicrobial decontamination was accompanied by an avoiding of 7.68 g of CO_2e every year for each square meter of cleaned surface, while, at the same time, limiting the use of concentrated chemicals. This means that every year the application of “GREEN” protocol to the entire yard would allow a quantity of avoided emissions equal to 293 kg of CO_2e. Future experiments will involve the evaluation of the cleaning processes in other

public and private spaces, such as food-processing industries and workplaces, such as offices and laboratories.

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Abbreviations

ACC	Aerobic Colony Count
API	Analytical Profile Index
CAM	Minimal Environmental Criteria
CDBA	Clostridium difficile Agar Base
CFU	Colony Forming Unit
ClDA	Clostridium difficile Agar
GHG	Greenhouse Gases
GWP	Global Warming Potential
INAIL	Istituto Nazionale Assicurazione Infortuni sul Lavoro
IPCC	Intergovernmental Panel on Climate Change
ISO	International Standard Organization
LCA	Life Cycle Assessment
MCA	MacConkey Agar
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
NT	Non-treated
PCR	Product Category Rules
RODAC	Replicate Organism Detection And Counting
SDA	Sabouraud Dextrose Agar
TG	Green Treatmnet
TSA	Tryptic Soy Agar
TT	Traditional Treatment

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