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Evaluation of Antimicrobial Effect of Zinc Pyrithione against Airborne Fungi and Bacteria Growth Collected onto New and Loaded HVAC Fibrous Filters

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Abstract: Microbial growth onto HVAC filters was observed in real conditions with possible degradation of the indoor air quality. The filtration performance of marketed antimicrobial filters containing zinc pyrithione was tested under laboratory conditions and compared to that of similar filters with the same classification, F7 (EN779:2002). The filtration performance of the two tested filters during loading with PM10 particles was quantified in an experimental setup with filter pressure drop measurement and particle counting upstream and downstream of the filters. The microbial growth on the new and loaded filters, both contaminated with a microbial airborne consortium composed of two bacteria (Gram-positive and -negative) and fungi, was quantified by colony-forming units after conditioning the filters for a few days under controlled temperature (25 °C) and humidity (50% or 90% relative humidity). The results reveal that there was no degradation of the filtration performance of the filters treated with the antimicrobial agent. The efficiency of the antimicrobial treatment, i.e., the ability to inhibit the growth of microorganisms during the incubation period, was significant with the new filters regarding the fungal growth, but the results demonstrate that the antimicrobial treatment became inefficient with the loaded filters.

Keywords: HVAC fibrous filter; filtration performance; microbial aerosol; airborne bacteria and fungi; antimicrobial treatment; zinc pyrithione

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1. Introduction

Indoor air pollution refers to a variety of chemical, biological and physical agents that pose a health threat or cause discomfort. Airborne particles or aerosols are responsible for about 5–34% of all indoor pollution, and their abundance depends on indoor moisture and temperature conditions [1,2]. Bioaerosols are essentially composed of allergens, bacteria, molds, fungi, spores, endotoxins, mycotoxins and all types of particles produced by living organisms with highly variable and complex characteristics. Bioaerosols and, more specifically, microbial aerosols are of major concern in office and commercial buildings because of the close relationship with health diseases (episodes of asthma, rhinitis, conjunctivitis, etc.) [3].

The number of people exposed to bioaerosols in indoor environments can be reduced by appropriate air renewal or air cleaning processes such as filtration, ultraviolet germicidal irradiation, photocatalytic oxidation [4] or plasmacluster ions [5]. For example, the effectiveness of negative ions was investigated on the survival of *Serratia marcescens* and *Staphylococcus epidermidis* in an air duct flow [6]. Despite the existence of advanced treatment technologies for bioaerosol inactivation, the most common technique implemented in air handling units of heating ventilation and air-conditioning (HVAC) systems remains filtration with fibrous filters, allowing the collection of bioaerosols, although leading to a possible microbial contamination of the filters with microbial growth.

Microbial growth onto HVAC filters was observed in the literature under laboratory conditions [7–10], and also in real conditions [11,12], with possible degradation of the

microbial indoor air quality. To address this issue, innovative air filters with photocatalytic properties [13] or containing antimicrobial treatments are designed to be implemented in HVAC systems, or in portable room air cleaners or automobiles. The antimicrobial agents are various, e.g., nanoparticles of copper or silver, or carbon nanotubes [14]. Many natural antimicrobial products have been discovered; e.g., *Sophora flavescens* nanoparticles revealed an inactivation efficiency against *S. epidermidis* between 35 and 72% according to the level of humidity [15]. The activity of *Melaleuca alternifolia* oil (tea tree oil) against a range of microorganisms including *Staphyloccocus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* was shown [16]. The antimicrobial activities of 44 pure natural compounds and two derivatives were determined with only 23 compounds effective in inhibiting the growth of the tested organisms (*S. aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*) [17].

The antimicrobial efficiency of the coating or treatment varies according to the antimicrobial agent and, in particular, may depend on whether the filters are new or loaded. The main issue with antimicrobial air filters is that the coating may lead to changes in the filtration performance [18,19], particularly by increasing the airflow resistivity of the filter.

Zinc pyrithione (ZPT) with the chemical formula $C_{10}H_8N_2O_2S_2Zn$, also known as Zinc Omadine or Zinc 2-pyridinethiol-1-oxide, is used to prevent microbial degradation and deterioration of manufacturing starting materials such as plastics, polymers and latexes, and in a wide range of finished articles made from these starting materials. This chemical acts to prevent the growth of bacteria, fungi, mildew and algae that can cause various types of deterioration such as discoloration, staining and odors. ZPT was found to have a safety profile and/or antimicrobial efficacy that exceeded iodine, chlorhexidine gluconate and triclosan [20]. It acts as an ionophore, interacting nonspecifically with the plasma membrane and shuttling copper into the cell. There is a precedent for pyrithione-mediated ionophore activity across intracellular membranes, as it was previously reported that pyrithione affected zinc transport across vacuole vesicles in vitro [21]. The results of most studies on fungi showed that ZPT inhibits fungal growth through damaging iron–sulfur clusters of proteins essential for fungal metabolism and also by depolarizing the cell membranes and preventing membrane transport.

The aim of this study was to investigate, under laboratory conditions, the antimicrobial efficiency of a marketed filter containing zinc pyrithione which is known for its effect against microorganisms. The filtration performance of the filter, i.e., the particle collection efficiency and the pressure drop, and the antimicrobial effectiveness regarding a bacteria–fungi consortium, i.e., the ability to inhibit the growth of microorganisms during the incubation period, were evaluated and compared to the performance of a regular filter with the same classification. The effect of the level of humidity during filter conditioning as well as the presence of filter loading with organic particles was investigated.

2. Materials and Methods

2.1. Fibrous Filters Tested

Two multi-layer polypropylene (PP) fibrous filters marketed by Lydall, both of them containing electrostatic charges and classified as F7 according to the European standard EN 779:2002, were studied: a PP filter containing antimicrobial treatment with zinc pyrithione (ZPT) named the PP/ZPT filter, and a regular PP filter without antimicrobial treatment. Few antimicrobial HVAC filters are currently commercially available, perhaps because of the potential toxicity or negative effect of some antimicrobial agents, such as triclosan, which is suspected of increasing antibiotic resistance. The antimicrobial filter with ZPT selected for this study is one of the few commercially available filters.

F7 class filters, in particular, the 2 tested filters, are widely used in the air handling unit of HVAC systems of commercial or office buildings in the first or second treatment stage. The multi-layer PP filters provide high filtration efficiency and dust holding while minimizing airflow resistance, but the fibers lose their charge due to particle loading and air humidity, reducing the efficiency of the filters.

The two filters were each composed of five layers: a spunbond layer (SP1), a meltblown layer (MB1) containing the zinc pyrithione in the case of the PP/ZPT filter, a meltblown layer (MB2), a meltblown layer (MB3) containing pink pigments (reference color for F7 class efficiency), a spunbond layer (SP2). The two SP layers ensure the mechanical resistance of the filters, and there is an increasing gradient of efficiency between the MB1 and MB3 layers. The MB1 layer of the PP/ZPT filter is obtained by fusion of regular PP granules and granules containing ZPT, extrusion and then spinning.

The structural properties of the two filters were quantified (PP/ZPT filter vs. PP filter): the thickness (Z) was 1.41 ± 0.08 mm vs. 1.46 ± 0.12 mm (averages and standard deviations for N = 15 quantified by micrometers); the porosity was 91.1 ± 0.3% vs. 89.0 ± 0.8% (averages and ranges for N = 2 quantified by mercury intrusion with mercury porosimeter Autopore IV 9500); the fiber median diameter of the most effective filtering layer (i.e., MB3) was 5.1; 2.9 µm vs. 4.5; 2.6 µm (median diameters; standard deviations for N = 100 fibers analyzed by picture analysis with ImageJ software from scanning electron microscopic observations with EOL JSM-5800LV); the basic weight was 136 g/m^2 vs. 139 g/m^2 (obtained from the manufacturer). The airflow resistance (K) of the new PP/ZPT and PP filters was quantified from filter pressure drop (ΔP, Pa) measurements vs. filtration velocity (ϑ) in a ventilated duct with airflow control:

$$\Delta P = K \times \mu \times \vartheta \tag{1}$$

where μ is the air dynamic viscosity considering the conditions of temperature and humidity during the tests. The two types of filters were tested in a flat geometry in this study. The airflow resistance was 3.9 ± 0.1 × 10^7 m^{-1} vs. 3.5 ± 0.1 × 10^7 m^{-1} (averages and ranges for N = 2) for the PP/ZPT filter vs. PP filter. The results indicate a higher airflow resistance of the PP/ZPT filter compared to the PP filter. In particular, the PP/ZPT filter displayed a higher pressure drop than the PP filter by around 10% at the nominal filtration velocity of 0.12 m/s (recommended by the manufacturer), i.e., 84 Pa vs. 77 Pa. The airflow permeability of the filters (B, m^2) was calculated from K considering Darcy's law for laminar airflow:

$$B = \frac{Z}{K} \tag{2}$$

The airflow permeability of the filters was 3.6 ± 0.2 × 10^{-11} m^2 and 4.1 ± 0.4 × 10^{-11} m^2 for the PP/ZPT filter and the PP filter, respectively. Note that the range in the airflow permeability data is higher, due to the high experimental error in the thickness measurement.

The 2 tested filters display equivalent porous structural properties, but they are not similar.

The quantity of zinc in the filters was quantified at the laboratory by an atomic absorption spectrometer (AAnalyst 200, PerkinElmer) after calcination of filter samples at 550 °C for 4 h and acid attack with HCl (3N). The quantity of zinc measured was 0.15 ± 0.02 ppm for the PP/ZPT filter and 0.19 ± 0.02 ppm for the MB1 layer of the PP/ZPT filter, while there was no quantifiable value for the PP filters (i.e., below the quantification limit of 0.09 ppm). Thus, considering the basic weight of the PP/ZPT filter, the mass of zinc per surface of filtration was around 20 µg/m^2.

2.2. Water Retention Capacity

The microbial growth onto fibrous filters is influenced by the air humidity [8], but even more by the water retention capacity of the filters [8,10]. Water is sorbed by the fibers of the filter and/or by the particles collected by the filter according to the hydrophilic characteristics of the fibers and the particles.

The water retention capacity of the tested filters was quantified by a Karl Fisher device (870 KF Titrino plus, 803 Ti Stand, 860 KF Thermoprop, Metrohm) following the methodology described by [10], after conditioning in an airtight container (see Section 2.3.4) for two levels of humidity exposure: 25 °C and 90% RH (i.e., 21 g of water per m^3 of air), and 25 °C and 50% RH (i.e., 11 g of water per m^3 of air). The water retention capacity was

expressed in mg of water per surface of filtration. The average values were compared by using the statistical *t*-test.

2.3. Study of the Microbial Behavior on the Tested Filters

The antimicrobial effect of ZPT was tested with new filters, i.e., filters with just microbial contamination representative of the antimicrobial effect in the first operating days, and with loaded filters, i.e., loaded filters with organic and microbial particles. The tests were performed with the PP/ZPT filter and the PP filter with 3 filter samples to ensure the reproducibility of the results.

2.3.1. Preparation of the Microbial Consortium

The microbial contamination of the filters was ensured with a bacteria–fungi consortium, consisting of *Staphylococcus epidermidis* cells (CIP 53 124), *Serratia marcescens* cells (DSM30121) and *Penicillium chrysogenum* spores (wild strain).

S. epidermidis is a bacterial species found on the skin, and thus it is frequently present in indoor air from office buildings in particular. *S. marcescens* is a motile, short rod-shaped, Gram-negative, facultative anaerobe bacterium, classified as an opportunistic pathogen, and thus its presence in air could cause some pneumonia and urinary tract infections. It is capable of producing a pigment called prodigiosin, which ranges in color from dark red to pale pink, depending on the age of the colonies [22]. *P. chrysogenum* is a species from the type *Penicillium* often found in indoor air [23]. Many protease allergens from different species of *Penicillium*, including *P. chrysogenum*, are implicated in the occurrence of symptoms found in occupants of contaminated buildings [24].

The culture medium used for the microbial growth was a liquid nutrient broth (Biokar diagnostics) composed of tryptone (10 g/L), meat extract (5 g/L) and sodium chloride (5 g/L) for *S. epidermidis* and *S. marcescens*, while soy-based Rose Bengal Chloramphenicol agar (Biokar diagnostics) was used for *P. chrysogenum* spore production. After their growth, *S. epidermidis* and *S. marcescens* cells were collected in an isotonic solution (NaCl 9 g/L), while *P. chrysogenum* spores were collected in peptone water on the surface of the agar plate. All the harvested strains were washed three times with isotonic solution and by centrifugation for 10 min at 2000× *g*.

The microbial consortium was prepared on the same day as filters were contaminated. The 2 bacterial species were harvested at the beginning of the stationary growth phase.

2.3.2. Filter Contamination with the Airborne Microbial Consortium

The microbial contamination of the tested filters with the airborne bacteria and fungi was performed in a vertical filtration column (Figure 1). The microbial suspension was aerosolized with a pneumatic nebulizer, AGK 2000 (Palas). The experimental setup was composed of a straight stainless-steel cylindrical pipe of 45 mm in diameter and 1.5 m in length. The tested filter was located in the middle of the column in a removable filter holder. The airflow through the tested filter was ensured by the nebulizer connected at the top. The microbial contamination was performed at the nominal filtration velocity of the filters, i.e., 0.12 m/s, with the filters tested in a flat geometry. The microbial suspension composed of the 3 species simultaneously was aerosolized upstream of the filters for 10 min at a flow rate of 6 L/min.

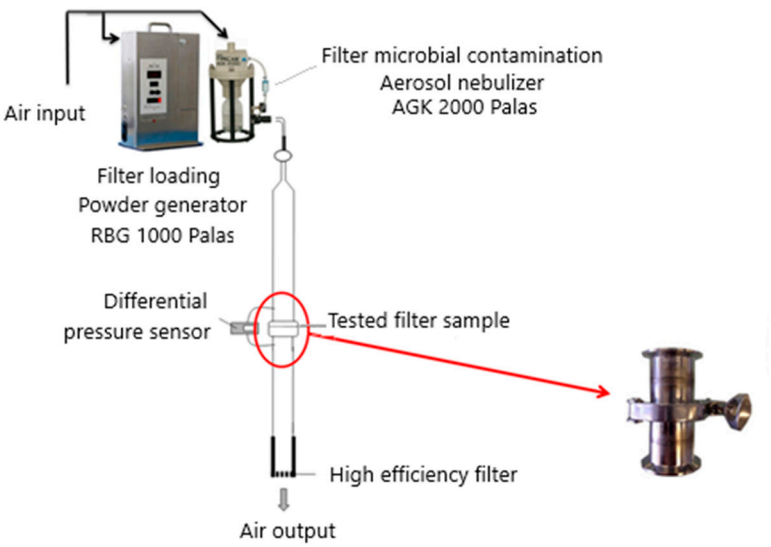

Figure 1. Schematic diagram of the experimental system for the filter contamination with the airborne microbial consortium and for the filter loading with micronized rice particles.

2.3.3. Filter Loading with Organic Particles

The loaded filters were obtained under laboratory conditions by clogging new filters with organic particles of micronized rice with a size distribution representative of PM10 particles. The micronized rice was selected to provide nutrients for microbial growth. The clogging was carried out in the same vertical filtration column as previously described for microbial contamination using a rotating brush particle generator (RBG 1000, Palas) operating at a rate of 5 m^3/h for 2 min. The average generated particle concentration was around 1.5 g/h. The micronized rice particles naturally contain the fungus *P. chrysogenum*, with a concentration of 2250 ± 850 CFU/g of micronized rice (colony-forming units) [9]. The particle size distribution of the micronized rice particles was characterized with the optical particle counter WELAS 2100 (Palas) with a mass and a number median diameter of 7.4 µm and 0.6 µm.

After particle loading, the filters were contaminated with the microbial consortium in the filtration device as explained previously for the new filters. Note that the quantity of *P. chrysogenum* coming from the micronized rice during the 2 min of loading was estimated to a maximal value of 10^2 CFU, i.e., assuming negligible loss of cultivability with generation and a negligible deposit in the setup. This quantity was negligible compared to the CFU coming from the microbial suspension nebulized upstream of the filter (10^{12} CFU).

2.3.4. Filter Incubation and Quantification of Microbial Concentration Extracted from the Filters

Once contaminated by the microbial consortium, the new or loaded filters, still located in their filter holder, were incubated in an airtight container with controlled temperature and humidity for 8–10 days at 25 °C and 50% RH or 90% RH (Figure 2). The two humidity conditions were selected because 50% RH is the setpoint in HVAC systems, and a previous study [8] showed that filter conditioning at 90% RH ensured significant microbial growth onto the filters. During the conditioning period, the filters were no longer subjected to airflow. Ventilation stops in HVAC systems are quite common during nights, weekends or vacations. The time period of 8–10 days corresponds to a deteriorated situation representative of a ventilation stop during a 1-week vacation period, and it was selected to ensure significant microbial growth quantifiable with the culture-based method.

After conditioning, the microbial concentration in the filters was quantified by colony-forming unit (CFU) counting. Microorganisms were extracted from the conditioned filters

according to the following methodology [8]: filter stirring for 1 h in 50 mL of extraction solution ($MgSO_4$ (0.01 mol/L), Tween-20 (0.25%)), and then ultrasound for 1 min. The application of this protocol allowed recovery between 80 and 85% of the microorganisms present on the filter media.

Extracted solutions were then diluted and spread on 3 different plates according to the microbial species:

- *P. chrysogenum* was cultivated on Rose Bengal Chloramphenicol agar [25], and the plates were incubated at 25 °C for 5 days;
- *S. epidermidis* was cultivated on Chapman agar culture medium, and the plates were incubated at 37 °C for 24 h;
- *Serratia marcescens* was cultivated on nutrient agar culture medium, and the plates were incubated at 30 °C for 48 h.

Finally, bacteria and fungi colonies were counted, and concentrations were expressed in colony-forming units (CFU) per filter surface. The limit of quantification (LQ) was set to 10 CFU/cm^2.

The possible presence of Zn in the extracted solution, adversely affecting the plating (CFU) results, was evaluated by comparing the microbial concentrations extracted from new and loaded PP and PP/ZPT filters right after contamination, i.e., at t = 0.

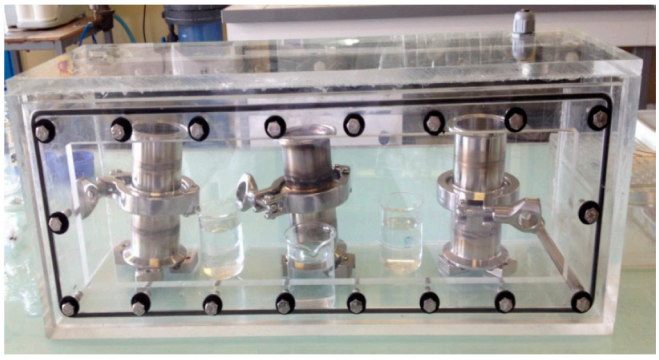

Figure 2. Picture of the airtight container for filter conditioning at controlled temperature and humidity conditions.

2.4. Setup for the Study of the Filtration Performance Regarding PM10

The filtration performance of the 2 tested filters was compared regarding PM10 particle collection. The PP/ZPT and the PP filters were loaded with the micronized rice particles at the nominal filtration velocity of 0.12 m/s to evaluate the evolution of their pressure drop and filtration efficiency.

The experimental setup was a straight horizontal filtration device of 5 m length and a section of 9.6 × 9.6 cm. The airflow was ensured by a centrifugal fan located downstream of the filter. The airflow was maintained constant during the test. Two straight lengths of flow stabilization upstream and downstream of the tested filter allowed the generation and measurement of particles using sampling probes. The pressure drop of the tested filter was performed with a differential pressure sensor. Particle counting was ensured by an aerodynamic counter (APS 3321, TSI) from isokinetic sampling upstream and downstream of the tested filter at 5 L/min.

The spectral filtration efficiency at time t was quantified from 3 successive measurements upstream/downstream/upstream of the tested filter according to

$$E_i = \frac{\overline{C}_{up,i} - C_{down,i}}{\overline{C}_{up,i}} \tag{3}$$

where E_i is the filtration efficiency of the tested filter at time t of the clogging for particles with diameter d_{pi}; $C_{down,i}$ is the number concentration of particles with diameter d_{pi} mea-

sured by the particle counter located downstream of the tested filter; $\overline{C}_{up,i}$ is the average number concentration of particles with diameter d_{pi} measured by the particle counter located upstream of the tested filter, calculated from the 2 measurements before and after the downstream measurement.

The total filtration efficiency at time t was quantified according to

$$E = \frac{\sum_{i=1}^{N} \left(\overline{C}_{up,i} - C_{down,i}\right)}{\sum_{i=1}^{N} \overline{C}_{up,i}} \quad (4)$$

The changes in the filter pressure drop and total filtration efficiency during the clogging were expressed as a function of the mass per unit area of collected particles (g/m^2) calculated from the mass of particles weighed at the end of the clogging and the particle concentrations measured upstream and downstream of the tested filter.

3. Results and Discussion

3.1. Water Retention Capacity of the Tested Filters

The water retention capacities of the new and loaded PP/ZPT filters are presented in Figure 3 after conditioning at 50% or 90% RH. The first observation is that the PP/ZPT filters, new or loaded, demonstrate quantifiable water retention capacities. Specifically, the results demonstrate that (i) the level of humidity during conditioning (i.e., 50% vs. 90% RH) influences the water retention capacity of the tested PP/ZPT filters: the water retention capacity of the new and loaded PP/ZPT filters is higher with RH conditioning of 90% than 50% with an increase factor of 38% and 27% and a confidence level of 97.2% and 99.7%, respectively; (ii) the water retention capacity is significantly higher with the loaded PP/ZPT filter than the new PP/ZPT filter by around 18 times for conditioning at 50% or 90% RH (with a confidence level of >99.9%).

New PP filters were also tested after conditioning at 25 °C and 90% RH, and the quantity of water sorbed for a given surface of filtration was slightly lower than that of the new PP/ZPT filters for the same conditions with a confidence level of 97%; by considering the same PP material of the fibers, this result may be explained by the porous structural parameters of the two tested filters.

In conclusion, the presence of water on the new or loaded PP/ZPT filters, required for microbial growth, is confirmed by these results. Moreover, the results indicate that the organic loading of filters, simulated by micronized rice particles under the tested conditions, sorbs a large amount of water, attributed to the nature of the particles used.

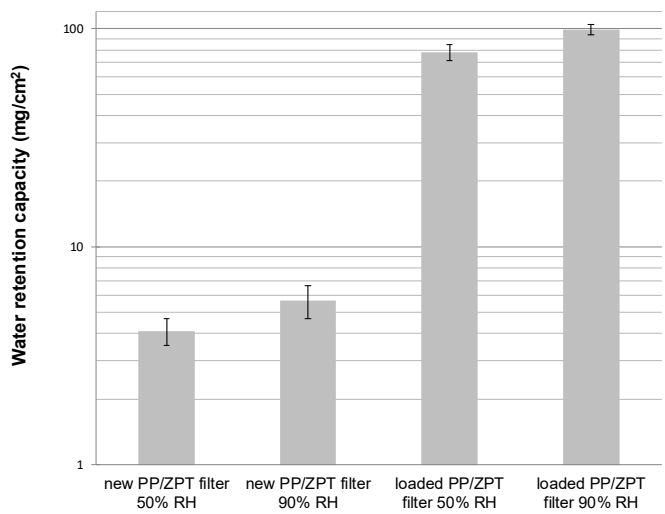

Figure 3. Water retention capacity of new or loaded PP/ZPT filters (averages and standard deviations for N = 4 filter samples).

3.2. Evaluation of the Influence of ZPT on the Filtration Performance Regarding PM10

The evolutions of the filter pressure drop and total filtration efficiency according to the areal mass of particles collected are presented in Figure 4. The results indicate a fairly similar evolution of the increase in the filter pressure drop and total filtration efficiency. The two tested filters lead to similar depositions of the particles during the first in-depth filtration stage and the following stage of cake filtration.

The evolutions of the spectral efficiency of the two tested filters during their clogging are presented in Figure 5. The results indicate that the particle collection efficiency is higher than 96% for the two tested filters whatever the particle diameter and the level of clogging. In particular, for the PP/ZPT filter, for the high level of clogging (i.e., from 36 g/m^2 of areal particle mass collected), we observe a slight decrease in the particle collection efficiency for the submicronic particles, characteristic of electret filters. Basically, according to filtration theory, the filtration efficiency of fibrous filters containing electrostatic charges is enhanced in the beginning of clogging due to the particle collection mechanism by electrostatic forces; the forces disappear with the clogging, leading to a possible decrease in the filtration efficiency if the cake filtration does not compensate it.

To conclude, the two tested filters display a fairly similar filtration performance regarding PM10 particle collection, meaning that the zinc pyrithione has no effect on the filtration performance.

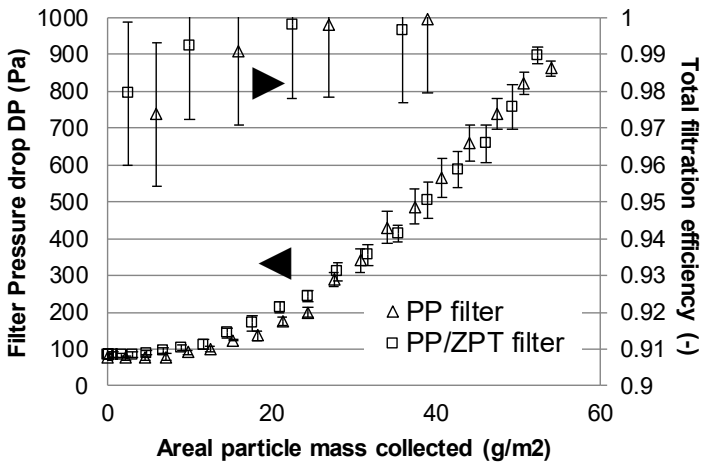

Figure 4. Evolution of pressure drop and total filtration efficiency of the PP and the PP/ZPT filters during the clogging vs. areal particle mass collected (averages and standard deviations for N = 1 filter sample, t = 1 min for pressure drop and 2% of coincidence for filtration efficiency).

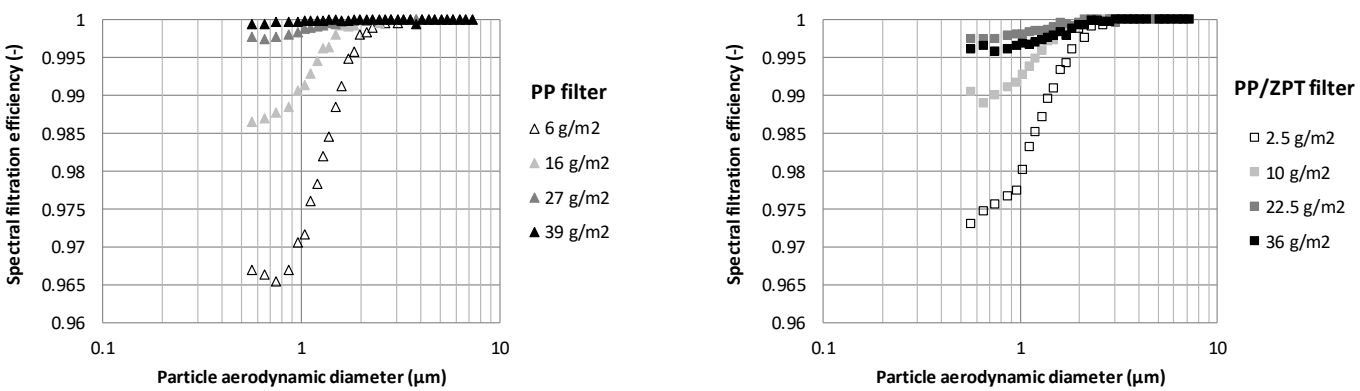

Figure 5. Spectral filtration efficiency of the PP and the PP/ZPT filters according to the level of clogging.

3.3. Influence of ZPT on the Microbial Behaviour on the Tested Filters

3.3.1. Cultivable Microbial Concentrations Nebulized Upstream of the Tested Filters during the Tests

The cultivable microbial concentrations (CFU/mL) in the suspension nebulized upstream of the filters during the several tests are indicated in Table 1; the average values were close to 10^{11} CFU/mL for *S. epidermidis* and *S. marcescens*, and 10^{8} CFU/mL for *P. chrysogenum*. The maximum quantities of species generated upstream of the filters during the 10 min of contamination were calculated assuming a negligible loss of cultivability with generation and a negligible deposit in the setup: around 10^{16} CFU for *S. epidermidis* and *S. marcescens*, and around 10^{12} CFU for *P. chrysogenum*.

Table 1. Cultivable microbial concentrations into the suspension for the several tests (new or loaded filters—RH value of filter conditioning after contamination).

Suspension CFU/mL	***Staphylococcus epidermidis***	***Serratia marcescens***	***Penicillium chrysogenum***
New PP filters—50% RH	n/a	n/a	n/a
New PP filters—90% RH	2.78 × 106	3.03 × 109	7.60 × 105
New PP/ZPT filters—50% RH	1.96 × 109	3.48 × 109	5.60 × 105
New PP/ZPT filters—90% RH	8.50 × 108	1.07 × 109	4.70 × 105
Loaded PP filters—50% RH	1.00 × 1012	2.18 × 1012	5.20 × 108
Loaded PP filters—90% RH	2.34 × 1011	3.20 × 1010	1.00 × 106
Loaded PP/ZPT filters—50% RH	1.46 × 1010	4.00 × 1010	1.40 × 106
Loaded PP/ZPT filters—90% RH	5.38 × 1010	4.84 × 1010	7.50 × 105
Average concentration CFU/mL	1.86 × 1011	3.30 × 1011	7.50 × 107

3.3.2. Evaluation of ZPT Transfer during Microorganism Extraction from the Tested Filters

A possible bias in the methodology was investigated regarding the transfer of ZPT in solution during the step of microorganism extraction from the filters and its potential inhibition in the culture-based method.

The microbial concentrations extracted from the new and loaded PP and PP/ZPT filters right after microbial contamination with the microbial suspension are presented in Figure 6. The results indicate that the cultivable microbial concentrations extracted from the tested filters at t = 0 for each species are comparable regardless of the level of clogging of the filters and the presence of ZPT. The cultivable microbial concentrations quantified are around 10^{7} CFU/cm^{2} for *S. epidermidis* and *S. marcescens*, and 10^{4} CFU/cm^{2} for *P. chrysogenum*. Thus, the extraction methodology of the filters should not significantly transfer ZPT in the solution because no effect on the cultivability of the tested microorganisms was observed between the PP and PP/ZPT filters.

In addition, the cultivable concentration of the fungi onto the filters right after contamination, i.e., t = 0, was determined with new (N = 7) and loaded (N = 8) filters (regardless of the presence of ZPT in the filters). The average cultivable concentrations (±standard deviations) of *P. chrysogenum* were not significantly different ($9.2 \times 10^{3} \pm 2.7 \times 10^{3}$ CFU/cm^{2} and $1.5 \times 10^{4} \pm 1.3 \times 10^{4}$ CFU/cm^{2} for the new and loaded filters, respectively). This result confirms that the quantity of spores of *P. chrysogenum* coming from the micronized rice loading is negligible compared to that collected during the contamination with the microbial suspension.

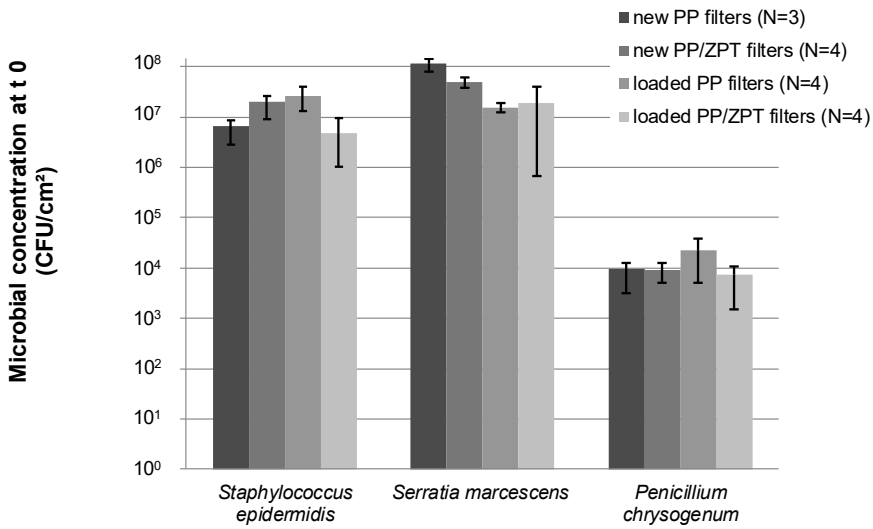

Figure 6. Microbial concentrations extracted from new and loaded PP and PP/ZPT filters right after contamination at t = 0 (averages and ranges).

3.3.3. Antimicrobial Effect of ZPT

New Filters

The results of the cultivable microbial concentrations (expressed in CFU/cm^2) extracted from the new PP and PP/ZPT filters right after the microbial contamination of the filters (t = 0) and after 8 days of filter conditioning at 25 °C and 50% or 90% RH (t = 8d 50% RH, or t = 8d 90% RH) are presented in Figure 7 for the three microbial species studied.

After 8 days at high humidity (90% RH), the results demonstrate that for the regular PP filter (without antimicrobial treatment), the fungal spore concentration on the filter increases significantly by around 1 log. On the other hand, the Gram-positive bacteria concentration decreases by 2 log, and the Gram-negative concentration decreases by around 0.5 log (with a confidence level of 91% with the t-test). In the presence of antimicrobial treatment in the filtering media, the results show no growth for *P. chrysogenum* on the new PP/ZPT filter, and the concentration slightly decreases (with a confidence level of 95%). The *S. marcescens* concentration decreases by 1 log, and the concentration of *S. epidermidis* decreases by 4 log (i.e., around 90% and 99.99% of inactivation, respectively).

For conditioning at 50% RH, the bacterial survival is limited, with no living bacteria extracted from the new PP and PP/ZPT filters after 8 days, except for a low concentration of *S. epidermidis* with the PP/ZPT filter, which may be explained by the higher water retention capacity of the PP/ZPT filter; the survival of *P. chrysogenum* at 50% RH is also limited, with a decrease in the fungal concentration by 2 log for the PP and the PP/ZPT filters.

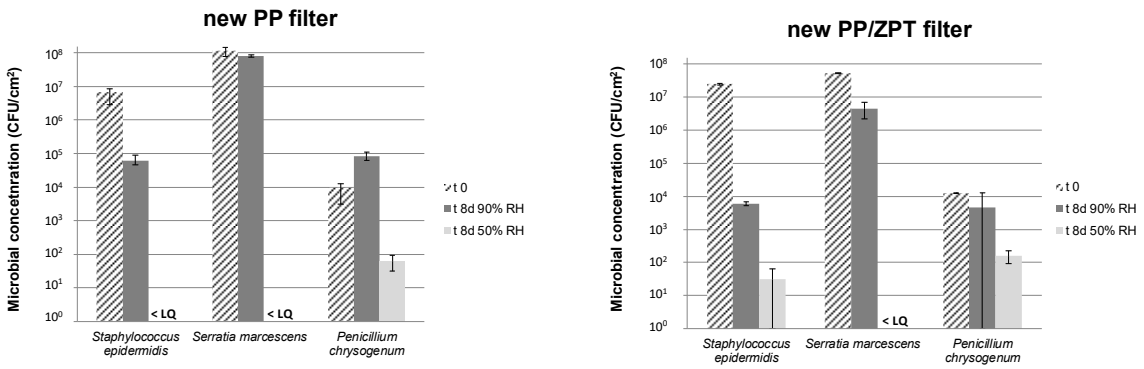

Figure 7. Microbial concentrations extracted from new PP and PP/ZPT filters right after contamination and after 8 days of conditioning at 25 °C and 50% or 90% RH (averages and ranges for N = 3 filters).

Loaded Filters

The same experiments were performed as described previously with the filters loaded with micronized rice particles (PM10) containing the fungus *P. chrysogenum*.

The results presented in Figure 8 demonstrate that the presence of organic particles collected by the filter influences the microbial survival on the tested filters conditioned at 90% RH. The bacterial survival after 10 days is higher for *S. epidermidis* in comparison to the new filters, particularly for the PP/ZPT filter with antimicrobial treatment, with around a 1.5 log reduction (against 3.5 log with the new filters); for *S. marcescens*, the concentration decrease is more significant with the loaded filter, probably influenced by the growth of *P. chrysogenum* on the filters. Indeed, in the presence of organic particles which could be used as a substrate by *P. chrysogenum*, this strain presents significant growth on the PP filter and also the PP/ZPT filter. The microbial growth on the PP filter was observed by SEM (Figure 9).

The low relative humidity (50% RH) during filter conditioning has a strong effect on the bacterial survival, with no living Gram-negative *S. marcescens* extracted from the filters and a sharp decrease in the *S. epidermidis* concentration by 5–6 log for the two tested filters (i.e., 99.999–99.999% of inactivation). The behavior of *P. chrysogenum* with the loaded PP and PP/ZPT filters after incubation at 50% RH is almost the same as that with the new filters, with a sharp decrease in the concentration by 1–2 log.

Verdenelli et al. [19] studied the antimicrobial activity of glass fiber HEPA filters treated with phosphated quaternary amine complexes against microbial strains (10 bacteria and 6 fungi). They concluded that the antimicrobial activity is strongly influenced by the working time of the filters, and they observed a decrease in the antimicrobial activity for several strains with used filters compared to unused filters.

The same filters as in the present study were tested over 7 months in realistic conditions with semi-urban outdoor air [26]. The results confirmed the antimicrobial effectiveness of zinc pyrithione regarding fungi cultivated with DRBC agar, i.e., that the fungal concentration on the regular PP filter was significantly higher than that on the ZPT/PP filter, with no influence of the loading of the filter considering a moderate increase in the filter pressure drop by about 30 Pa. No conclusion was established regarding the bacteria because of the high statistical deviation.

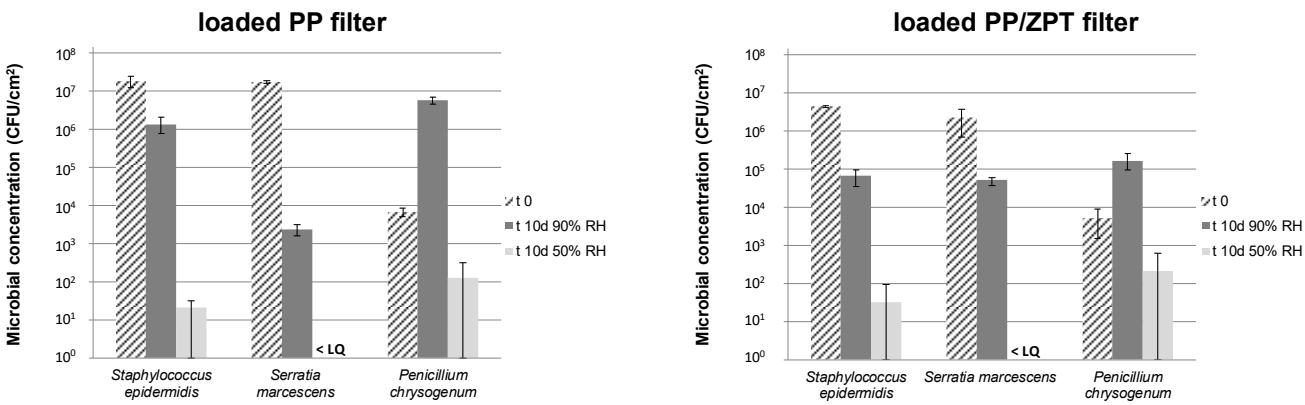

Figure 8. Microbial concentrations extracted from loaded PP and PP/ZPT filters right after contamination and after 10 days of conditioning at 25 °C and 50% or 90% RH (averages and ranges for N = 3 filters).

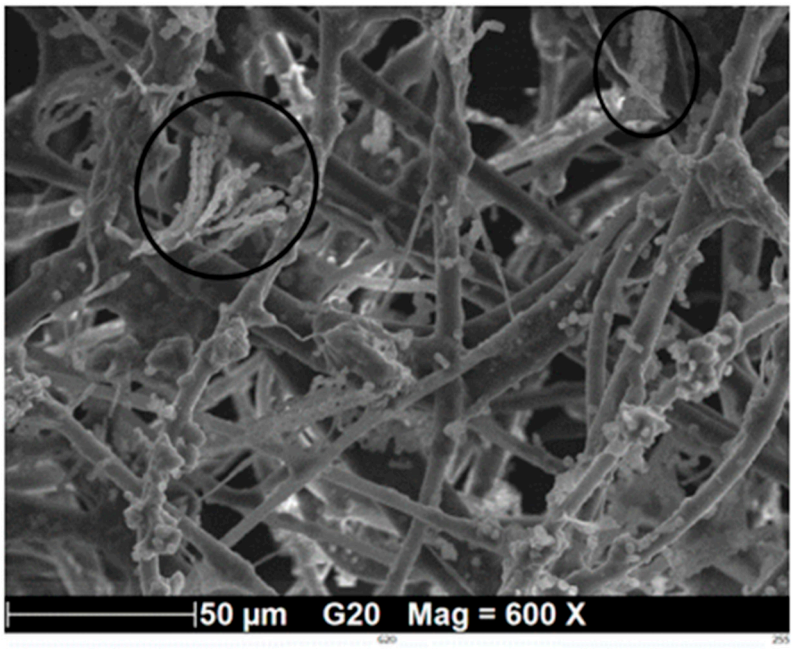

Figure 9. SEM observations of the microbial growth of fungal spores of Penicillium onto PP filter conditioned at 90% RH (upstream side of the filter).

4. Conclusions

Two marketed F7 fibrous filters, one containing zinc pyrithione antimicrobial treatment, were studied. The two filters revealed similar filtration performances during their clogging with PM10 particles, meaning that the antimicrobial treatment did not degrade the filtration performance. The microbial growth on the new and loaded filters contaminated with an airborne consortium of one fungal and two bacterial strains and conditioned at two levels of humidity was studied. At the low humidity value of conditioning (50% RH), with the new or loaded filters, with or without antimicrobial treatment, the microbial population on the filters decreased and possibly did not survive (*S. marcescens*). At the high humidity value of conditioning (90% RH), the bacteria did not grow on the new filters, and only the fungi were able to develop. The effectiveness of the antimicrobial treatment with zinc pyrithione was confirmed for new filters against the fungus *P. chrysogenum*. For the loaded filters, the results indicate that the antimicrobial treatment was not more efficient against the fungus *P. chrysogenum*, the endemic species of the micronized rice particles (PM10) collected by the filters; the two populations of bacteria significantly decreased with or without antimicrobial treatment. To conclude, the data do not support the effectiveness of ZPT in HVAC filters as a single technology implemented for the purpose of protecting occupant health regarding microbial contamination.

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