J PREV MED HYG 2008; 49: 26-33

Original article

Silver zeolite antimicrobial activity in aluminium heating, ventilation and air conditioning system ducts

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Key words

HVAC ducts • Silver zeolite • Antimicrobial activity

Summary

Introduction. *Air pollution in confined environments is a serious health problem, in that most people spend long periods indoors (in homes, offices, classrooms etc.). Some people (children, the elderly, heart disease patients, asthmatic or allergic subjects) are at greater risk because of their conditions of frailty. The growing use of air-conditioning systems in many public and private buildings aggravates this health risk, especially when these systems are not correctly installed or regularly serviced. The aim of our study was to verify the capacity of Ag + ions to stop the growth of bacteria and moulds inside the ducts of Heating, Ventilation and Air Conditioning system ducts (HVAC) systems when these ducts were lined with active Ag + ions zeolite-coated panels.*
Material and methods. *A Y-shaped HVAC model with two branches was used; one branch was made of traditional galvanized iron, as was the whole system, while the other was lined with active Ag + zeolite-coated polyurethane panels. During the test, samples of dust present inside both ducts were collected and seeded in liquid and solid media to detect bacteria and moulds. The presence of bacteria was also sought in the air emerging from the outlets of both ducts.*
Results. *Tests made on samples of particulate collected from the two different ducts revealed a lower total bacterial load in the samples collected from the Ag + zeolite-coated duct than in the samples from the traditional Zn galvanized duct. In addition, the values of bacterial load found in the air emerging from the Ag + ions zeolite-lined duct were 5 times lower than those found in the air from the traditional galvanized iron duct.*
Conclusions. *The utilization of Ag + zeolite-coated panels in air-conditioning systems could improve the quality of the emerging air in comparison with traditional installations in galvanized iron. This innovation could prove particularly advantageous in the event of accidents during the installation of air-conditioning systems or of contaminated aerosols coming from outside.*

Introduction

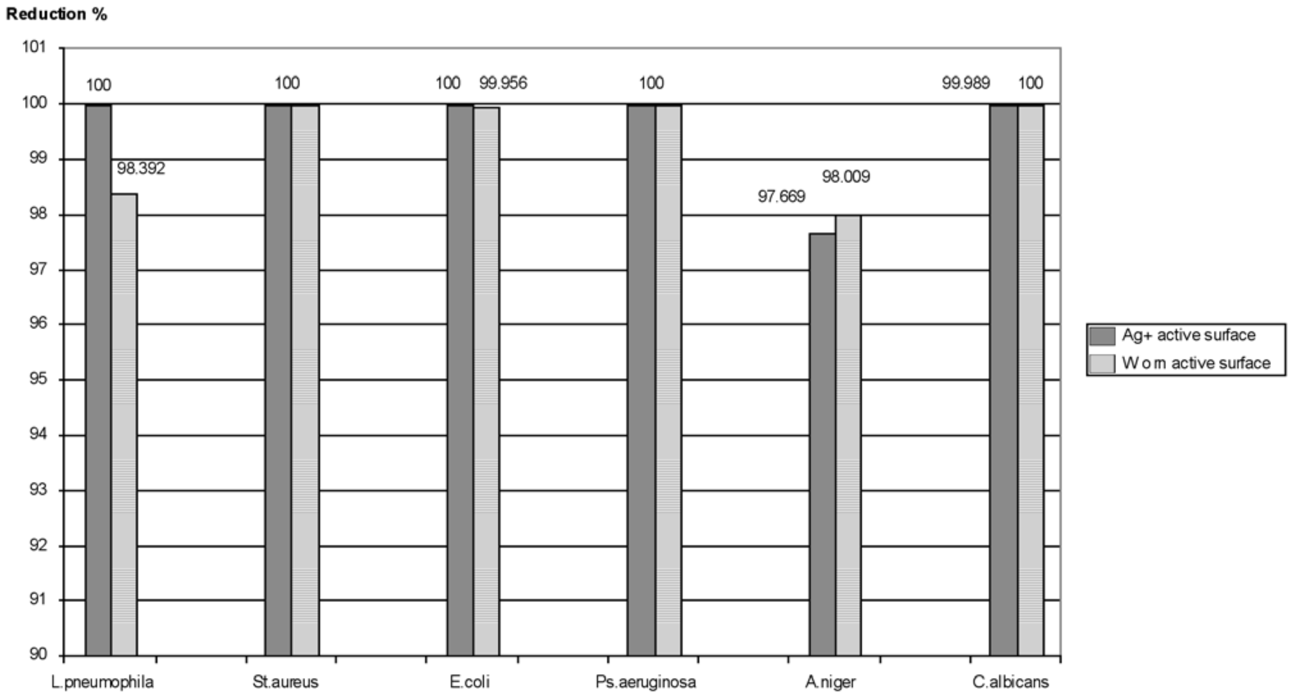
For many years, silver and other heavy metals have been used as antimicrobial agents to coat bio-materials in medicine, dentistry and cosmetics, and a variety of surfaces and materials in the food and construction industries [1-7]. Furthermore, silver can be incorporated into many substances which can be used as coatings able to act as surface antimicrobial agents. Indeed, over time, these Ag + coated substances tend to release the ionic form of silver, which acts against a wide range of micro-organisms (including *L. pneumophila, L. monocytogenes, Ps. aeruginosa, E. coli*, *St. aureus,* moulds and yeasts), inhibiting their enzymatic activity, interfering with electron transport to the cytoplasm and firmly binding the bacterial cell DNA [7-9]. Many Authors have reported that silver sulfadiazine is a first-rate antimicrobial in treating burns [10, 11]. Moreover, as the continuous release of Ag + ions protects catheterized patients against bacteria and moulds, this quality of silver is becoming increasingly appreciated in clinical practice [4, 5].
One of the substances that displays these properties is zeolite. Zeolite is an open-structure ceramic Al-silicate capable of releasing silver ions in a controlled manner through interchange with Na and Ca ions in external wet backgrounds.
Zeolite epoxy coverings have already been experimented on stainless steel surfaces, with a view to reducing the presence of bacteria [12, 13]. Moreover, zeolite-coated surfaces have proved to be more resistant to corrosion and scratching and easier to clean [14]. Active Ag + zeolite has already been registered as an antimicrobial agent for use in the food industry by the Food and Drug Administration (FDA) (FLN000047), Environmental Protection Agency (EPA) (R.71227-1) and National Science Foundation (NSF) (standard 51 n. NSF/3A).
Since the presence of some micro-organisms (such as *L. pneumophila*) inside air ducts could be dangerous to human health [15, 16], we decided to estimate the possibility of protecting the inner surfaces of Heating, Ventilation and Air Conditioning system ducts (HVAC) with a covering of this antimicrobial substance.
The goals of the study were:
- to verify the antimicrobial *in vitro* activity of Ag + vitreous zeolite incorporated as an active layer on the aluminium surface of some polyurethane panels;
- to verify the capability of zeolite to reduce the number of bacteria present in dust samples collected from inside coated HVAC ducts in comparison with

Tab. I. Antimicrobial effect of Ag + zeolite coatings on contaminated tiles (% reduction and germicidal effect on intact or worn surfaces – test according to ASTM E 2180-1).

	L. pneumophila			*St. aureus*			*E. coli*		
	Coated surfaces		Unprotected	Coated surfaces		Unprotected	Coated surfaces		Unprotected
Tiles:	Active	Worn	Controls	Active	Worn	Controls	Active	Worn	Controls
CFU/tile	1	1	30	1	1	164	1	1	1027
CFU/tile	1	8	96	1	1	101	1	9	1040
CFU/tile	1	13	25	1	1	96	1	2	804
Geom. Mean	0	0.6723	1.61911	0	0	2.06715	0	0.418424	2.977953
Anti Log10			41.82			116.72			950.502
% Reduction*	100	98.392		100	100		100	99.95598	
G.E**	1.70186	0.8365		2.0804	2.0804		2.9809	2.37885	
	Ps. aeruginosa			*Aspergillus niger*			*Candida albicans*		
	Coated surfaces		Unprotected	Coated surfaces		Unprotected	Coated surfaces		Unprotected
Tiles:	Active	Worn	Controls	Active	Worn	Controls	Active	Worn	Controls
CFU/tile	1	1	538	26	9	27	1	1	2300
CFU/tile	1	1	552	12	15	35	4	1	1510
CFU/tile	1	1	536	1	1	48	1	1	1950
Geom. Mean	0	0	2.73962	0.83139	0.7101	1.55224	0.2007	0	3.276913
Anti Log10			541.935			35.66			41.82
% Reduction*	100	100		97.6686	98.009		99.989	100	
G.E**	2.73399	2.734		0.45033	0.6435		2.9828	3.283301	

*% Reduction = (a - b) x100/a;
a = AntiLog of Geometric Mean of CFU recovered from control samples after the incubation period; b = Geometric Mean of CFU recovered from treated samples after the incubation period.
** G.E. = Germicidal effect = Log Nc- Log Nd; Nc = Arithmetic Mean of CFU recovered from control samples after the incubation period; Nd = Arithmetic Mean of CFU recovered from treated samples after the incubation period

Fig. 1. Antimicrobial effect of Zeolite on contamined protected tiles.



samples from non-coated traditional galvanized iron-zinc ducts;

- to verify the bacterial indoor air quality (IAQ) of the air emerging from each type of duct.

Materials and methods

We tested aluminium polyurethane tiles (2.5 x 2.5 cm) (ALP Active S.r.l., Calcinate – BG, Italy) treated with a layer of active Ag + zeolite (AgIon Technologies, Inc.) against six different micro-organisms: (*L. pneumophila* American Type Culture Collection (ATCC) 33152, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15422, *E. coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC6275). All tests were carried out in accordance with the American Society for Testing and Materials (ASTM) E2180-01 Directive and every sample was analyzed in triplicate *vs.* each micro-organism. Each micro-organism was therefore tested against three zeolite-treated samples, three non-treated samples (negative controls) and three zeolite-treated samples previously subjected to hard brushing and to high temperature treatment (150°C/72 h) in order to simulate severe environmental working conditions.

The tiles were contaminated by depositing on them 1 ml of an Agar slurry containing a different micro-organism each time. All the tiles were then incubated at 37°C ± 1 °C (RH ≥ 75%)/24 h and then plunged into a neutralizing broth (Tryptone Soy Broth (TSB) + LPHI Tween-80).

For moulds and yeasts, the temperature and incubation period used were 25°C ± 1°C/72h; the growth substrates were Sabouraud Dextrose Broth/Sabouraud Dextrose Agar (DSB/DSA).

As regards the second and third goals, we used a Y-shaped air-conditioning installation with two branches: one branch made of galvanized iron, as was the whole installation, the other branch made of polyurethane panels lined on both sides with aluminium foil (ALP S.r.l., Calcinate – BG, Italy). The foil on the inner side of this duct was coated with Ag + zeolite in order to release Ag ions that would act against any micro-organisms which might come into contact with it and also inhibit their proliferation over time.

This air conditioning installation worked for 5 months at a temperature of 25°C and at a relative humidity of 45%-90%, as guaranteed by the heating and moisturizing system installed.

The particulate present in both ducts was detected every 15 days, 30 days and 5 months by means of a 1,500 W Cartridge Vacuum Cleaner (PBI-International-MI), the head of which was drawn over a surface of 3,000 cm^2 for each duct in two different points. The dust load for each surface was obtained by weighing the cartridge before and after aspiration of the dust.

Each dust sample present on the filters was dissolved 1/10 w/v in TSB; from this first suspension, we then made successive 2-fold dilutions in TSB. Aliquots of 1 ml of each dilution were then seeded by inclusion in TSA and DSA plates in duplicate and incubated at 37°C ± 1°C/24 h and at 25°C ± 1°C/72 h, respectively. At the end of the incubation period, the bacterial and

fungal counts were registered; only values between 30 and 300 CFU were accepted. The data were tested individually for reproducibility (K < 1.96) and Dilution Linearity was verified (ISO TR 13483). All the counts were expressed in CFU/g; each sample recovered from the 3,000 cm^2 surfaces was analysed considering Bacterial and Fungal Count per g of dust.
The results regarding the two different types of ducts (galvanised iron and Ag + zeolite-coated panels) were then statistically elaborated (Student's t test).
Concerning the IAQ study of the air emerging from the ducts in working conditions, we aspirated volumes of 200, 400, 600 and 800 litres of air by means of an Surface Air Sampler (SAS) (PBI Int.-MI) on TSA Rodac Plates. All the plates were then incubated at 37°C ± 1°C/24 h.
The test was carried out in the presence and in the absence of the outlet grille-filter.
At the end of the incubation period, all the counts were transformed into CFU/m^3 of air and, from the arithmetic mean obtained, we calculated the total Bacterial load and the Staphylococcus load present in one cubic meter of air aspirated.

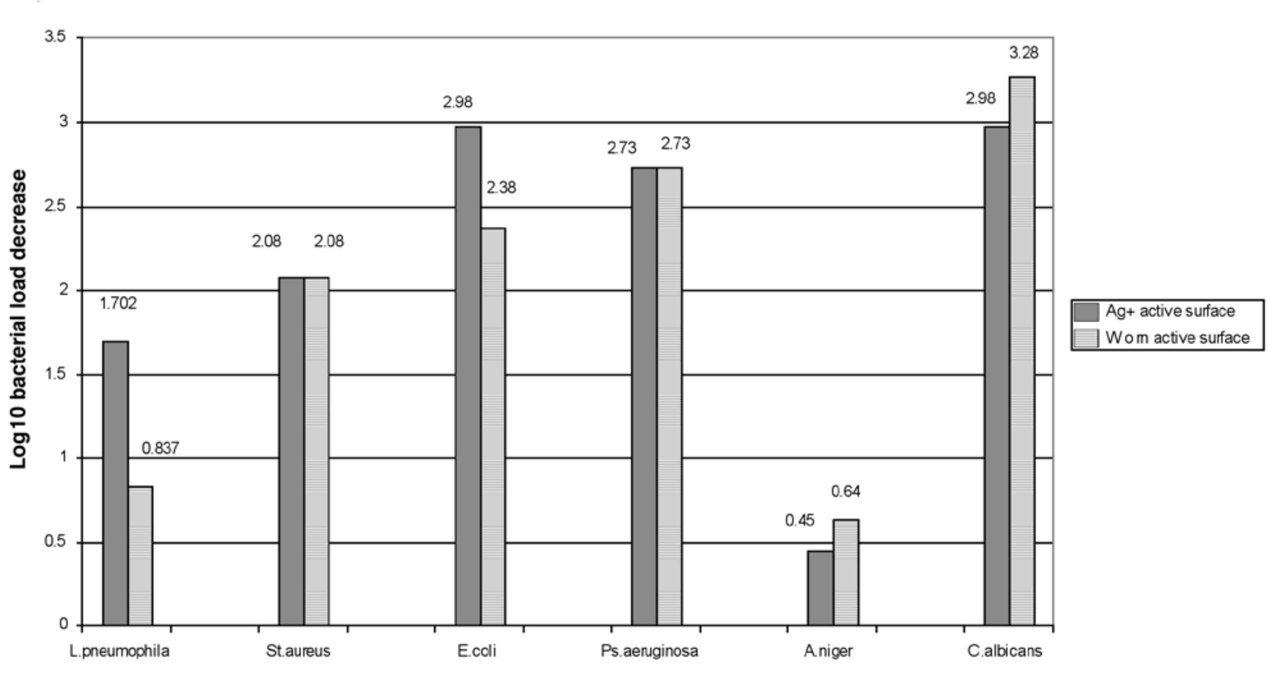
Results

In the first test, aluminium polyurethane tiles protected and unprotected with active Ag + zeolite were used; on Ag + zeolite-coated tiles, the percentage reduction in all micro-organisms tested was about 97.67%-100% (regardless of subjection to hard brushing) (Tab. I) (Fig. 1). The corresponding Germicidal Effects (GE) are reported in Table I and Figure 2.
In the second test, samples of particulate collected from the different ducts and the total bacterial load were analyzed; the mean reduction was 25.77% in the particulate collected from the Ag + zeolite duct. Furthermore, comparison of the bacterial loads of the two ducts (galvanized iron and Ag + zeolite-coated panels) revealed a statistically significant difference (Tab. II). This reduction was registered at both sampling sites (1 m and 4 m from the common duct) without significant differences between the two sites (Figs. 3 and 4).
The particulate collected from the Ag + zeolite duct consistently contained fewer moulds than that from the galvanized iron duct, with a mean difference of 23.14% (Tab. II); however, the values were too scattered for their difference to be considered significant.
In the third test, the total bacterial load was detected in the air emerging from the two ducts, which worked for 5 months constantly. The reduction in bacterial load was 74.85% in the presence of the outlet grille-filter and 80.19% without it (Fig. 5). Similar reductions were seen in the Staphylococcus load (67.44% and 69.84%).

Discussion

Scientific studies indicate that there are many sources of pollution inside buildings and that pollutants may remain in recycled air; furthermore, some indoor pollution comes from outdoor air.

Fig. 2. Antimicrobial effect of Ag^+Zeolite on contamined protected tiles.



Tab. II. Comparison between mean values.

Total bacterial counts (Mean values in Colony-Forming Units per gram (CFU/g) of dust collected)								
	Galvanized Iron Duct		Ag + Zeolite-Coated Duct					
Date of Test	mean	± S.D.	mean	± S.D.	Diff.%	t -Value	D.F.	P value
30.01.07	90311	7012	71566	8517	-20.76	3.40	6	0.0145
19.03.07	44825	6663	33446	6170	-25.39	3.42	14	0.0042
05.06.07	40233	6249	25169	5374	-37.44	5.78	18	0.0001
All values	58456	20066	43394	18284	-25.77	2.54	42	0.0147

Total mould counts (Mean values in Colony-Forming Units per gram (CFU/g) of dust collected)								
	Galvanized Iron Duct		Ag + Zeolite-Coated Duct					
Date of Test	mean	± S.D.	mean	± S.D.	Diff.%	t -Value	D.F.	P value
30.01.07	24028	17334	20699	13057	-13.85	0.31	6	NS
19.03.07	4557	1571	3397	1627	-25.46	1.03	6	NS
05.06.07	8148	4190	2010	485	-75.33	2.06	2	NS
All values	13064	13925	10041	11924	-23.141	0.52	18	NS

Total bacterial count in air emerging from the ducts (Mean Values in CFU/m^3)					
Date of Test	Zeolite Panel Duct	Iron Duct	t-Value	D.F.	P value
5/6/07	49.25 ± 4	216.25 ± 78	4.28	6	0.0051

Staphylococcal load in air emerging from the ducts (Mean Values in CFU/m^3)					
Date of Test	Zeolite Panel Duct	Iron Duct	t-Value	D.F.	P value
5/6/07	8 ± 3	27 ± 6	5.27	6	0.0017

Indoor exposure to the main pollutants could be ten times higher than outdoor exposure [17]. Air pollution in confined environments is a serious health problem, in that most people spend long periods indoors (in homes, offices, classrooms etc.). Some people (children, the elderly, heart disease patients, asthmatic or allergic subjects) are at high risk of developing respiratory disease because of their conditions of frailty. The growing use of air-conditioning systems in many public and private buildings aggravates this health risk, especially when these systems are not correctly installed or regularly serviced.

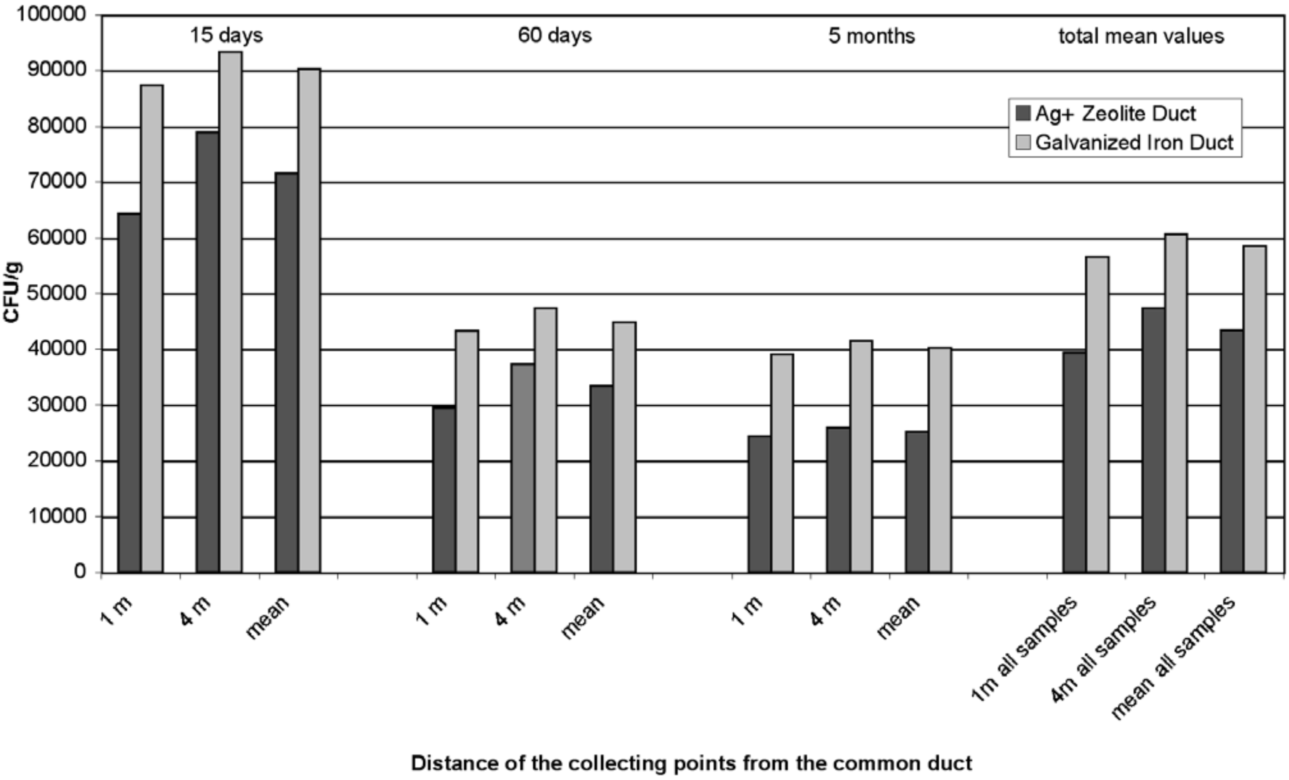
The fine dust entering air-conditioning systems from outside, the consequent presence of particulate inside air-conditioning ducts and its constant accumulation promote the growth of mites, moulds and bacteria. These agents can be found in indoor environments if suitable terminal filters are not used.

In most hospital units where the risk of infectious disease is high (operating theatres, surgical units, wards and intensive care units) all duct outlets are equipped with High-Efficiency Particulate Air filters (HEPA) (99.97% efficiency at 0.3 micron sized particles); however, this precaution is not adopted in other hospital environments (hospitalization or casualty wards, reception wards, inner passageways, laboratories, cafeteria, canteen, kitchen etc.).

Air filters are not usually fitted to the outlets of HVAC systems in all public and private buildings. Consequently, people could be exposed to the risk of infectious disease caused by the spread of air containing fine dusts, moulds and bacteria.

The spread of biological agents could be avoided by ensuring that HVAC systems are frequently serviced or by using air duct systems able to reduce the bacterial load. In the test conducted on tiles conforming to the

Fig. 3. Active Zeolite protected polyurethane duct and galvanized iron duct. Bacterial counts in the proximal and distal points of both ducts after 15 days, 60 days and 5 months of working (mean values in CFU/g).



ASTM E 2180-1 Directive, we studied the antimicrobial activity of Ag + zeolite against six different microbial species. The results revealed that the percentage of microbial reduction varied from 97.67% for *A. niger* to 100% for the other micro-organisms. The corresponding Germicidal Effect rose from 0.45 to 2.98 Log10 Units.

Fig. 4. Active Zeolite protected polyurethane duct and galvanized iron duct. Bacterial load reduction% in the proximal and distal points of the active Ag^{+} Zeolite protected duct after 15 days, 60 days and 5 months of working (mean values).

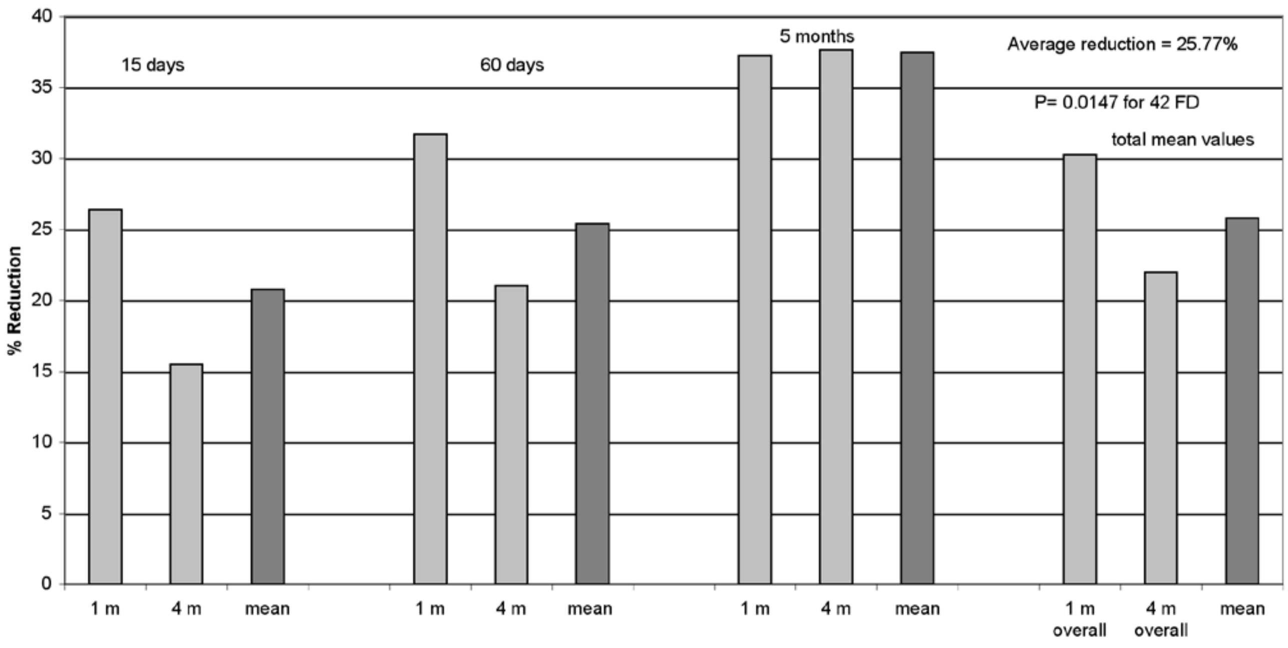
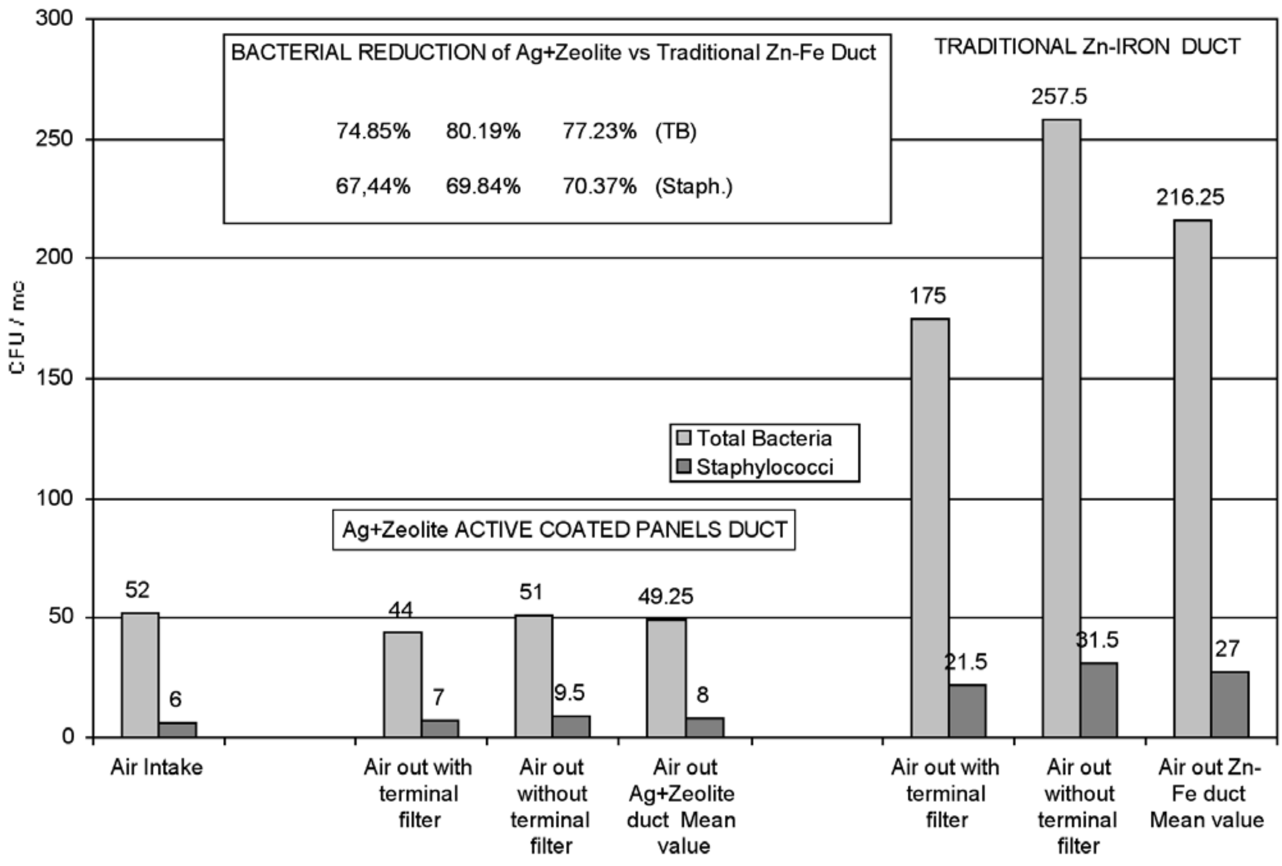


Fig. 5. Total Bacterial Count (cfu/mc) in the Air at both Air Outlet Ducts of the Plant Model.



Comparison between tiles subjected to simulated hard wear and unworn tiles revealed little difference in the percentage-reduction obtained. This confirms the ability of Ag + zeolite coatings to endure very hard working conditions and to maintain their antimicrobial efficacy for a long time.

A similar antimicrobial effect was subsequently confirmed by the second test, in which a mean 25.77% reduction in bacterial load was yielded by Ag + zeolite-coated ducts *vs*. traditional galvanized iron ducts. In the real conditions concerning Ag + ions showed a significant ability to reduce bacteria. This reduction was detected at both sampling sites: 1 m and 4 m from the air inlet of the common duct into the active Ag + Zeolite-coated duct.

The value of the reduction obtained was different from those yielded by the *in vitro* test on tiles infected with well-defined microbial species in the laboratory in that these species were not necessarily present in the experiment conducted in "real conditions". Indeed, it is easier to verify the antimicrobial activity of a substance on a surface in the total absence of spores than on a surface heavily loaded with spores.

The results of the last test showed a greater antibacterial efficacy of the active Ag + zeolite duct than the traditional non-protective, galvanized iron duct. Likewise, the Ag + zeolite panels also exerted a reductive effect against moulds, though the difference was not statistically significant.

In the third experiment, the dust samples collected from both types of ducts, which worked constantly for 5 months, were tested. The aim of this test was to ascertain whether the active Ag + zeolite-coated panels exerted an effective antimicrobial activity on the air emerging from the outlets. If so, we could quantify the reduction in infectious risk for people who usually spend long periods in confined environments (offices, laboratories, shops etc.) in which HVAC systems are not normally equipped with a terminal filter at the outlet grille.

It is important that the HVAC system model used in the study was equipped with a 60% filter at the outlet grille of both types of duct; this system was chosen in order to prevent bacteria or moulds inside the model from reaching the external environment. The filter was similar to that used normally in traditional HVAC systems downstream of the Air Handling Unit (HAU) and upstream of the ducts from which conditioned air enters the environment.

This survey allowed us to detect bacteria and to assess the quality of air samples from the grille-ended ducts in two different conditions (with or without filter). In both situations, active Ag + zeolite reduced dust-borne bacteria and it is plausible that a similar effect would be exerted on bacteria present in the air emerging from the outlet.

After a working time of 5 months, the total bacterial count of the air emerging from the two different ducts was evaluated. In the presence of the terminal filter, the panels coated with active zeolite achieved a 74.85% reduction in comparison with the galvanized iron panels and an 80.19% reduction in the absence of the filter. Similar reductions were seen with regard to Staphylococci (67.44% and 69.84% respectively).

Samples taken by the same SAS equipment from the air-intake mouth of the HVAC system, at the other side of the building, were used as negative controls. The results obtained showed that, when the terminal filter was installed, the bacterial reduction achieved through the presence of active Ag + zeolite was good, with reductions in Total Bacterial load and Staphylococci of 74.85% and 67.44%, respectively. This reduction effect was more evident when the filter was not installed, a condition in which reductions of 80.19% and 69.84%, respectively, were registered.

These results indicate that using active Ag + Zeolite-coated panels for the construction of public or private HVAC system ducts can reduce the risk of inhaling micro-organisms or dust to 20-30% of the risk encountered when traditional galvanized iron ducts are used.

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■ Received on January 10, 2008. Accepted on February 15, 2008.

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