

Anti-bacterial and Anti-mold Efficiency of Silver Nanoparticles Present in Melamine-laminated Particleboard Surfaces

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Laminated surfaces of wooden composites are created from decorative papers and impregnation thermosetting resins, mainly melamine-formaldehyde (MF). This type of surface treatment is not always resistant to microorganisms, especially when polluted with organic substances. Bioactive additives are often needed to improve the microbial resistance. In this study, silver nanoparticles (Ag-NPs), in amounts of 0.15×10^{-3} wt.%, 0.5×10^{-3} wt.%, 1.5×10^{-3} wt.%, 5×10^{-3} wt.%, 15×10^{-3} wt.%, and 50×10^{-3} wt.% were added to MF resin. The presence of Ag-NPs in the laminated surfaces of the particleboards improved their anti-bacterial and anti-mold resistances. Growth of gram-positive bacterium *Staphylococcus aureus* on the sterilized surfaces decreased by approximately 53.7% at most, while the growth of gram-negative bacterium *Escherichia coli* decreased by up to 100%. The anti-mold resistance of the polluted laminated surfaces containing Ag-NPs increased against *Penicillium brevicompactum* by up to 62.5%, but there was almost no improvement against *Aspergillus niger*. The Ag-NPs did not affect the resistance of the laminated surfaces towards aggressive chemicals and only minimally towards dry heat at 180 °C. This inorganic biocide decreased the abrasion resistance of the laminated surfaces by up to 12.6%.

Keywords: Ag nanoparticles; Antimicrobial surface; Bacteria; Melamine laminate; Molds

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INTRODUCTION

Surface lamination of wood-based materials consists of pressing one layer or multiple layers of thin papers initially impregnated with thermosetting resins. The interconnection between the laminate layer and underlying material is ensured by melting the resin and subsequently curing it (Reinprecht and Perlác 1983). Mostly, décor papers impregnated with amino thermosetting resins are used for the lamination of the surface of wood-based composites, *e.g.* for particleboards and fiberboards (Roberts and Evans 2005). Nowadays, lamination is done in short-cycle hot presses, within which impregnated décor papers are pressed onto wood-based composites for a few seconds at high temperatures and pressures (Kandelbauer and Teischinger 2010; Nosál and Reinprecht 2017). Laminated surfaces of wood-based materials are commercially produced in many designs and various colors, or with an imitation of wood, rocks, wall tiles, *etc.* (Kohlmayr *et al.* 2014). Although laminated surfaces of wood-based composites secure a certain level of resistance against bacteria and microscopic fungi, these surfaces are not sufficiently resistant against microorganisms when first polluted by organic substances (Vidholdová *et al.* 2015; Nosál and Reinprecht 2017). Antimicrobial properties of laminated surfaces can be achieved

through several methods (Nosál' and Reinprecht 2017). A lot of research has dealt with the possibility of creating antimicrobial surfaces in wood-based composites, including studies about their physical and chemical modification (Magina *et al.* 2016; Saleem and Bokhari 2017). Antimicrobial laminates can also be created by treatment of impregnation resin with organic or inorganic biocides (Hanrahan *et al.* 2006; Kim and Kim 2006).

Inorganic biocides, especially in the form of nanoparticles, added into the surface layers of laminated materials can remarkably improve antimicrobial properties. Biocides added into amino impregnation resins should be chemically and physically inert to the resin to avoid a decrease in the final quality of the laminated surfaces. A biocidal treatment of amino resins could theoretically be used several metal nanoparticles (*e.g.* Ag, Co, Cu) and metal-oxide nanoparticles (*e.g.* CuO, TiO₂, ZnO), which have a good inhibition effect against bacteria (Kamal *et al.* 2016, Khan *et al.* 2016, Ali *et al.* 2017) and fungi (Reinprecht *et al.* 2015, Nosál' and Reinprecht 2018). In previous experiments, good results with ZnO nanoparticles have been achieved at their application into melamine-laminated surfaces of particleboards (Nosál' and Reinprecht 2017) as well as into a whole matter of particleboards (Reinprecht *et al.* 2018).

Silver nanoparticles (Ag-NPs) are well-known biocide additives, potentially applied also into melamine-formaldehyde (MF) resins for laminated surfaces. The numerous favorable properties of Ag-NPs have led them to be used in many industrial sectors. Silver nanoparticles are often used as an antimicrobial/disinfectant agent in biomedicine (Panáček *et al.* 2006; Lim *et al.* 2015; Salar *et al.* 2015), in air and water purification, cosmetics, the textile industry, packaging materials, and home appliances, such as washing machines, refrigerators, and others (Eckhardt *et al.* 2013; Carbone *et al.* 2016). Commercial products contain various amounts of Ag-NPs from an almost undetectable level up to 700 mg/kg (Bauer *et al.* 2016). The antimicrobial efficiency of Ag-NPs strongly depends on the particle size because particles with smaller dimensions can more easily penetrate microbial cells. Likewise, smaller particles have a larger active surface, and therefore there is greater contact between the nanoparticle and microbial cells (Lok *et al.* 2006). Generally, the minimum inhibitory concentration of nanoparticles against microbes decreases with a decreasing particle size (Martínez-Castañón *et al.* 2008).

The aim of this study was a complex analysis of selected bactericidal, fungicidal, and user properties of melamine-laminated surfaces of particleboards (PBs) containing Ag-NPs added to the MF resin at various amounts.

EXPERIMENTAL

Materials

Commercial PBs (Kronospan Bučina DDD, Co. Ltd., Zvolen, Slovakia) with a thickness of 16 mm were used as an underlay. White décor papers (UNI white 0500) (Malta Décor Sp. Z o. o., Poznan, Poland) with an area weight of 90 g/m² were used for impregnation with basic and modified MF resins. The basic MF resin was produced by Kronospan Bučina DDD, Co. Ltd. at a melamine to formaldehyde molar ratio of 1 to 1.72 and was workable in 2 d with a preserved dynamic viscosity of 61.5 mPa·s and dry mass of 58%. The Ag-NPs (US Research Nanomaterials, Inc., Houston, TX, USA) had the following characteristics: average particle size of less than 15 nm and 5 wt% in water.

MF resin modification with Ag-NPs

The MF resin was modified with silver nanoparticles (Ag-NPs) present in amounts of 0.15×10^{-3} wt%, 0.5×10^{-3} wt%, 1.5×10^{-3} wt%, 5×10^{-3} wt%, 15×10^{-3} wt%, and 50×10^{-3} wt%. The modified MF resins, before using for impregnation of papers, had been catalyzed with the hardener DeuroCure KS (Deurowood, Hard, Austria). The condensing time for all of the resins was $330 \text{ s} \pm 15 \text{ s}$ at $100 \text{ }^\circ\text{C}$.

Impregnation of the papers with MF resins

White décor papers with the dimensions $300 \text{ mm} \times 280 \text{ mm}$ were impregnated on a laboratory mechanical applicator with the basic and modified MF resins to achieve uniform saturation and a retention of $120 \text{ g/m}^2 \pm 10 \text{ g/m}^2$. A uniform dispersion of Ag-NPs in the MF resins used for the impregnation process was achieved by mechanical stirring (Makita 6600, Makita Corporation, Anjō, Japan) at 250 rpm for 3 min. The impregnated papers were then pre-cured in a laboratory kiln (Eccocell 55 – Comfort, MMM Medcenter Einrichtung GmbH, Munich, Germany) for 3 min at $160 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$.

Preparation of the laminated PBs

The pre-cured impregnated papers were pressed onto PBs ($300 \text{ mm} \times 280 \text{ mm}$) with a laboratory press (Table Press TP 600, Fontijne Presses & Services BV, Barendrecht, Netherlands) at a pressure of 0.3 MPa and temperature of $175 \text{ }^\circ\text{C}$ for 4 min. The thicknesses of the laminated layers on the PB surfaces ranged from 0.2022 mm to 0.2069 mm and were not remarkably influenced by the Ag dispersion amount. From the laminated PBs, samples were prepared with the dimensions $50 \text{ mm} \times 50 \text{ mm}$ for the bacterial and mold tests. Samples with the dimensions $100 \text{ mm} \times 100 \text{ mm}$ were used for the abrasion test, and samples with the dimensions $250 \text{ mm} \times 250 \text{ mm}$ were used for the resistance tests against aggressive chemicals and dry heat.

Methods

Bacterial test

Staphylococcus aureus ATCC-25923 as a representative of gram-positive bacteria and *Escherichia coli* ATCC-25922 as a representative of gram-negative bacteria (from the microorganism collection at the Department of Clinical Microbiology of Hospital in Zvolen, Slovakia) were used for the bacterial test.

Samples of laminated PBs with cleaned (using a mixture of 96% ethanol and 2-propanol at a weight ratio of 8.8:1.2) and sterilized surfaces (twice by ultraviolet light for 20 min) were placed into sterilized Petri dishes and inoculated with 0.1 mL of the bacterial suspensions. Two densities of bacterial suspensions, *i.e.*, 0.5^* (1.5×10^8 CFU/mL) and 1.0^* (3.0×10^8 CFU/mL), were applied in the physiological solutions, according to the McFarland scale. Incubation of bacteria on the inoculated laminated surfaces was performed at $37 \text{ }^\circ\text{C}$ for 48 h. The bacteria were then stripped from the laminated surfaces using a sterile swab and put in a liquid culture medium for 48 h. Finally, the bacteria were pre-inoculated from the liquid medium into the sodium chloride diagnostic soil in the Petri dishes.

The anti-bacterial resistance of the laminated PBs in the diagnostic soil was assessed on the basis of the bacterial activity and valued from 0 to higher numbers in CFU/mL.

Mold test

The microscopic fungi *Aspergillus niger* and *Penicillium brevicompactum* (from the fungi collection at the Mycological laboratory of Technical University in Zvolen, Slovakia) were used in the mold test.

The laminated PB samples were all sterilized, and then one half of the samples were intentionally polluted. Intentional pollution of the top surfaces of the samples was done with a 15 wt% mixed solution of proteins, sugars, and lipids at a weight ratio of 8:1.3:0.7. The solution was applied with a sprayer (two sprays of 0.5 mL) on the whole sample surface from a distance of 10 cm. The mold tests were started by the placement of the samples into 100-mm Petri dishes filled with a 3-mm- to 4-mm-thick layer of Czapek-Dox agar (one sample per dish), and their top surfaces were inoculated with water spore suspensions of *A. niger* or *P. brevicompactum*. Incubation of the molds on the laminated surfaces lasted 4 weeks at a temperature of 27 °C and relative humidity of 90%.

The anti-mold resistance of the laminated surfaces was evaluated visually after 7 d, 14 d, 21 d, and 28 d. The growth activity of the molds (GAM) was determined with the following scale from 0 to 4 using standard STN 49 0604 (1980), where 0 is no mold, 1 is up to 10% mold, 2 is up to 25% mold, 3 is up to 50% mold, and 4 is more than 50% mold on the top surface.

Standard quality tests

First, the laminated PB samples were conditioned for 1 week at a temperature of 23 °C ± 2 °C and a 50% ± 5% relative humidity. Next, the top surfaces were tested for their resistance to cold liquids of aggressive chemicals, dry heat, and abrasion in accordance with EN 12720:2009+A1 (2013), EN 12722:2009+A1 (2013), and EN 15185 (2011), respectively.

RESULTS AND DISCUSSION

Bacterial Test

The results of the bacterial activity on the laminated surfaces of the PBs are shown in Table 1.

Table 1. Bacterial Activity on the Laminated Surfaces after Application of Ag-NPs in the MF Resins

Ag-NPs in MF (wt%)	Bacterial Activity (10 ⁸ CFU/mL)			
	<i>S. aureus</i>		<i>E. coli</i>	
	0.5*	1.0*	0.5*	1.0*
0	0.41	0.73	0.30	0.62
0.15 × 10 ⁻³	0.36	0.74	0.28	0.61
0.5 × 10 ⁻³	0.32	0.65	0.18	0.47
1.5 × 10 ⁻³	0.32	0.65	0.09	0.20
5 × 10 ⁻³	0.28	0.52	0.00	0.04
15 × 10 ⁻³	0.25	0.55	0.00	0.02
50 × 10 ⁻³	0.19	0.42	0.00	0.02
N (number of tested samples in one series) = 6				

The addition of Ag-NPs to the impregnation MF resin caused a moderate reduction in the gram-positive bacterium *S. aureus* activity on the laminated surfaces. The highest reduction of this bacterium, approximately 53.7% from 0.41×10^8 CFU/mL to 0.19×10^8 CFU/mL (for 0.5* McFarland) and approximately 42.5% from 0.73×10^8 CFU/mL to 0.42×10^8 CFU/mL (for 1* McFarland), was observed on the laminated surfaces containing the highest Ag-NP amount, *i.e.*, when the MF resin contained 50×10^{-3} wt% silver. In contrast, the Ag-NPs remarkably reduced (up to 96.8% from 0.62×10^8 CFU/mL to 0.02×10^8 CFU/mL for the 1* McFarland density) or even totally reduced (up to 100% from 0.30×10^8 CFU/mL to 0 CFU/mL for the 0.5* McFarland density) the activity of the gram-negative bacterium *E. coli* for lower amounts of Ag-NPs in the laminated surfaces. An example of this was when the MF resin contained 15×10^{-3} wt% or only 5×10^{-3} wt% silver (Table 1).

Based on the bacterial test results, it was concluded that Ag-NPs in the melamine-laminated surfaces were able to inhibit bacterial growth. However, the Ag-NPs had different efficiencies for the gram-positive and gram-negative bacteria. The silver only partially suppressed the gram-positive bacterium *S. aureus*, but it was lethal to the gram-negative bacterium *E. coli*. Different efficiencies of Ag-NPs against the two types of bacteria were also observed in previous studies (Yoon *et al.* 2007; Li *et al.* 2011; Suchomel *et al.* 2015). Kim and Kim (2006) reported a high efficiency of Ag-NPs in melamine laminates against *E. coli*, when the number of bacteria on the tested specimen surfaces was reduced by more than 98%. Testing other materials, a significant inhibition of *E. coli* was achieved with CuO (Khan *et al.* 2016) and TiO₂ (Kamal *et al.* 2016) nanoparticles in combination with chitosan.

Mold Test

The results of the GAM on the cleaned and sterilized laminated surfaces of the PBs are shown in Table 2. For the polluted laminated surfaces, the results are shown in Table 3.

The cleaned and sterilized laminated surfaces containing smaller or higher amounts of Ag-NPs were completely resistant to the growth of both *A. niger* and *P. brevicompactum* (Table 2 and Fig. 1). However, a slight growth of *A. niger* was observed on the reference untreated laminated surfaces after 21 d and 28 d of incubation (GAM of individual samples from 1 to 2) (Table 2, and Figs. 1a, 1b, 1c, and 1d).

Table 2. Mold Activity on the Cleaned Laminated Surfaces

Ag-NPs in MF (wt%)	GAM – On Cleaned Surfaces (0 to 4)							
	<i>A. niger</i>				<i>P. brevicompactum</i>			
	7 d	14 d	21 d	28 d	7 d	14 d	21 d	28 d
0	0	0	1	1.5	0	0	0	0
0.15×10^{-3}	0	0	0	1	0	0	0	0
0.5×10^{-3}	0	0	0	0	0	0	0	0
1.5×10^{-3}	0	0	0	0	0	0	0	0
5×10^{-3}	0	0	0	0	0	0	0	0
15×10^{-3}	0	0	0	0	0	0	0	0
50×10^{-3}	0	0	0	0	0	0	0	0
N = 6								

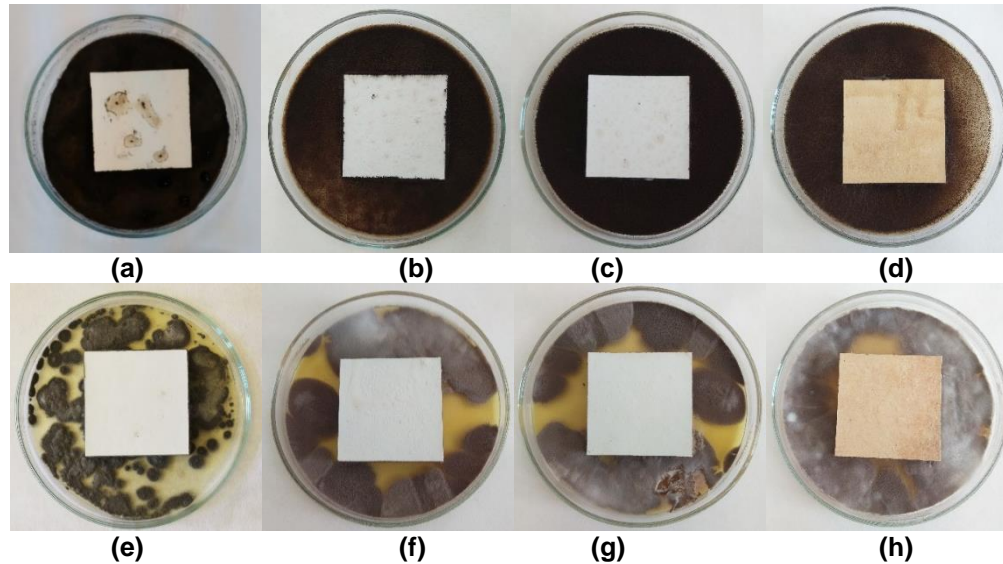


Fig. 1. Top surfaces of the sterilized laminated PBs inoculated with *A. niger* / (a) 0 wt%, (b) 0.15×10^{-3} wt%, (c) 5×10^{-3} wt%, and (d) 50×10^{-3} wt% of Ag-NPs in MF/ or with *P. brevicompactum* / (e) 0 wt% (f) 0.15×10^{-3} wt%, (g) 5×10^{-3} wt%, and (h) 50×10^{-3} wt% of Ag-NPs in MF/ after 28 d of incubation

In contrast, the intentionally polluted “contaminated” laminated surfaces of the PBs were markedly attacked by molds (Table 3 and Fig. 2). The polluted reference laminates without Ag-NPs were not able to inhibit the growth activity of both mold species. The presence of silver in the laminated surfaces only minimally inhibited a strong growth of *A. niger*, but it was able to inhibit *P. brevicompactum* more. Nearly no growth reduction in *A. niger* by Ag-NPs was observed after 21 d and 28 d of the mold test, at which point almost all of the samples were over 50% covered by mycelia of this mold with a GAM of 3 to 4 (Table 3, and Figs. 2a, 2b, 2c, and 2d). However, the presence of Ag-NPs in the laminates caused a more apparent reduction in *P. brevicompactum* (up to 62.5% growth). Considerable growth inhibition of this mold on the polluted laminated surfaces occurred with Ag-NP contents $\geq 1.5 \times 10^{-3}$ wt%, where the mold growth on laminates was only up to 25% with a GAM of 1 to 2 (Table 3, and Figs. 2e, 2f, 2g, and 2h).

Table 3. Mold Activity on the Intentionally Polluted Laminated Surfaces

Ag-NPs in MF (wt.%)	GAM – On Polluted Surfaces (0 to 4)							
	<i>A. niger</i>				<i>P. brevicompactum</i>			
	7 d	14 d	21 d	28 d	7 d	14 d	21 d	28 d
0	2.5	4	4	4	2	3	4	4
0.15×10^{-3}	2.5	4	4	4	2	3	3.5	3.5
0.5×10^{-3}	2.5	4	4	4	1.5	2.5	2.5	2.5
1.5×10^{-3}	2	4	4	4	1	2	2	2
5×10^{-3}	2	3.5	4	4	1	2	2	2
15×10^{-3}	1.5	2.5	3.5	3.5	1	1.5	1.5	1.5
50×10^{-3}	1	2	3.5	3.5	0.5	1	1.5	1.5
N = 6								

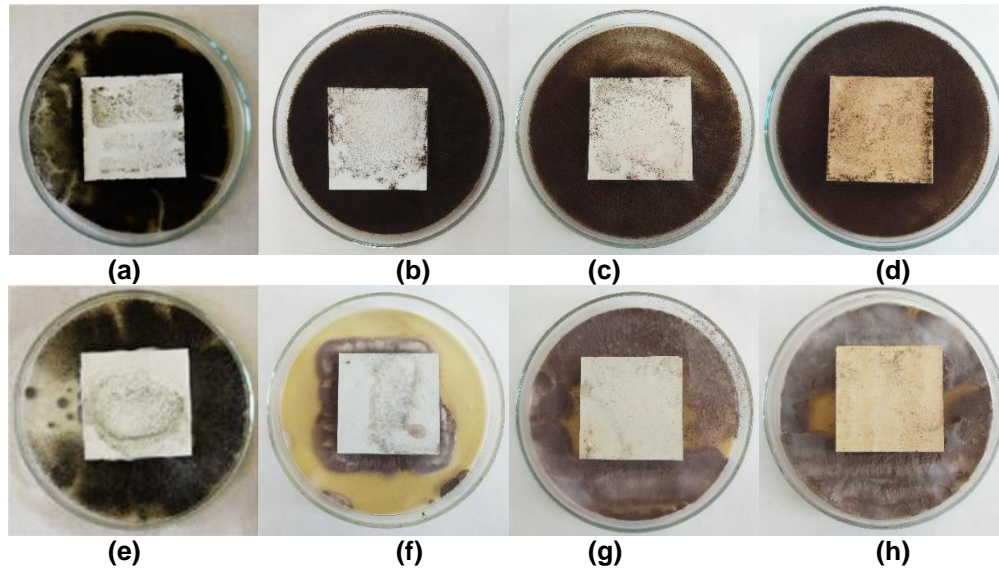


Fig. 2. Top surfaces of the intentionally polluted laminated PBs inoculated with *A. niger* / (a) 0 wt%, (b) 0.15×10^{-3} wt%, (c) 5×10^{-3} wt%, and (d) 50×10^{-3} wt% of Ag-NPs in MF/ or with *P. brevicompactum* / (e) 0 wt% (f) 0.15×10^{-3} wt%, (g) 5×10^{-3} wt%, and (h) 50×10^{-3} wt% of Ag-NPs in MF/ after 28 d of incubation

The anti-mold test results were divided into sterilized and intentionally polluted sample groups. The surfaces of the sterilized samples were resistant to growth of both *A. niger* and *P. brevicompactum*. This was explained by an absence of nutrient substances on the samples, and therefore mold developing was an impossibility (Kandelbauer and Widsten 2009). The presence of Ag-NPs in the melamine-laminated surfaces of the intentionally polluted samples only had a mild effect on *A. niger* growth, mainly in the first 2 weeks for the samples containing the highest amounts of Ag-NPs. In contrast, the resistance of the laminated surfaces against *P. brevicompactum* remarkably increased by adding Ag-NPs. These results were in accordance with Zhang *et al.* (2008), who reported a lower efficiency of Ag-NPs against *A. niger* compared with *Penicillium* sp.

Standard Quality Tests

The results of the standard quality tests of the laminated surfaces of the PBs are shown in Table 4.

The Ag-NPs in the laminated surfaces of the PBs had no or negligible negative effects on the standardized quality characteristics. This was explained by the assumption that Ag-NPs did not considerably change the structural composition of the cured MF resins in the final laminates when applied at relatively low concentrations. A remarkable decrease occurred only with the abrasion resistance of the laminated PBs, where a maximum decrease of 12.6% was observed for the laminates prepared with the highest Ag-NP concentration in the MF resin (50×10^{-3} wt%) (Table 4). This decrease was explained by the interaction of the small solid nanoparticles of silver not chemically bonded with the cured MF resin in the laminates and the abrasive sandpaper, and probably by a certain change in the chemical structure of the laminates caused by the presence of Ag-NPs.

It should also be emphasized that the laminates containing the highest amounts of Ag-NPs had a darker color with grey-brown shades (Figs. 1 and 2).

Table 4. Resistance of the Laminated Surfaces to Aggressive Chemicals, Dry Heat, and Abrasion

Tested Parameter		Ag-NPs in MF (wt%)						
		0	0.15×10^{-3}	0.5×10^{-3}	1.5×10^{-3}	5×10^{-3}	15×10^{-3}	50×10^{-3}
Chemicals	Acetone	4	4	4	4	4	4	4
	Ethanol	5	5	5	4	5	5	5
	Coffee	4	3	4	4	4	4	4
	Ink	5	5	5	5	5	5	5
	Ketones	3	3	3	3	3	3	3
	Oil	5	5	5	5	5	5	5
	Tea	4	4	4	4	4	4	4
	Water	5	5	5	5	5	5	5
Dry Heat (180 °C)		4	4	4	4	4	3	3
Relative Abrasion Resistance (%)		100	100	98.77	98.72	97.44	96.16	87.43

1 - noticeable differences, changed structure, or surface treatment fully or partially removed; 2 - noticeable differences, without structural changes; 3 - slight differences visible from several directions; 4 - slight gloss change visible only when the light source is mirrored and close to the tested surface; 5 - no visible changes; N_1 (number of tested samples in one series for the chemical and dry heat resistances) = 5; and N_2 (number of tested samples in one series for the abrasion resistance) = 10

CONCLUSIONS

1. The presence of Ag-NPs in the melamine-laminated surfaces of the PBs reduced the activity of the gram-positive bacterium *S. aureus* and the gram-negative bacterium *E. coli*. However, total effectiveness of the silver was observed only against *E. coli*.
2. The clean and sterilized laminated surfaces were resistant enough to mold growth, even without Ag-NP modification.
3. The presence of Ag-NPs in the polluted laminated surfaces, *i.e.*, after their intentional pollution with organic substances, had only a mild effect on *A. niger* growth, but had a higher effect on *P. brevicompactum* growth.
4. The Ag-NPs had no or negligible negative effects on the resistance of the laminated surfaces to cold aggressive chemicals and dry heat.
5. The abrasion resistance of the laminated surfaces decreased by about 12% in the presence of the highest Ag-NP amount (50×10^{-3} wt% of silver) in MF resin.

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