Anti-bacterial and Anti-mold Efficiency of ZnO Nanoparticles Present in Melamine-laminated Surfaces of Particleboards

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Lamination is one of the most widely used techniques for the surface treatment of wood-based composites such as particleboards, fiberboards, *etc.* It is usually carried out using décor papers impregnated with amino thermosetting resins, mostly melamine-formaldehyde, urea-formaldehyde, or their mixture. Conventional laminates with non-bioactive surfaces are not able to reduce or stop microbial growth when contaminated with organic substances. In this work, zinc oxide (ZnO) nanoparticles were applied into their surface structure to improve their anti-bacterial and anti-mold properties. Melamine-formaldehyde (MF) resin, for the white décor paper impregnation, was modified with ZnO in amounts of 0.1 wt.%, 0.3 wt.%, 0.6 wt.%, and 1 wt.% and pressed onto particleboards. The presence of ZnO in the melamine-laminated surfaces somewhat improved their resistance to the Gram-positive bacteria *Staphylococcus aureus* (by 20.7% or 9.5%). However, the improvement was considerable (~65% or 46.8%) against the Gram-negative bacteria *Escherichia coli.* The presence of ZnO in MF resins increased the anti-mold resistance of the intentionally contaminated laminated surfaces against the microscopic fungi *Aspergillus niger* and *Penicillium brevicompactum* at most by approximately 50%. ZnO nanoparticles had none or only a small negative effect on the resistance of the laminated surfaces towards aggressive chemicals and dry heat 180 °C, and their abrasion resistance decreased at most by approximately 17%.

Keywords: Bacteria; Melamine laminate; Molds; ZnO nanoparticles

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INTRODUCTION

Surface Lamination of Wood-based Composites

The surface treatment of wood-based composites (particleboards, fiberboards, *etc.*) by lamination is mostly carried out using décor papers impregnated with amino thermosetting resins. The paper´s core is usually impregnated with melamine-formaldehyde (MF) or melamine-urea-formaldehyde (MUF) resins, and then its top surface with MF resin (Reinprecht 1982; Roberts and Evans 2005; Kandelbauer *et al.* 2010). Subsequently, the papers are dried to a moisture content of 6% to 9% and pressed onto the wood-based composite. In the past, the pressing process was performed in multi-platen hot presses. In recent years, the laminated surfaces are produced in short-cycle hot presses in which the impregnated papers are pressed onto wood-based composites at temperatures of approximately 180 °C, pressures from 4 MPa to 8 MPa, and press charging time of approximately 9 s, with a pressureless exposure time less than 1.5 s (Kandelbauer and

Teischinger 2009; Kandelbauer *et al.* 2010). Thus, this newer technology can be performed at speeds up to 200 press cycles per hour. Overall, short-cycle hot pressing was achieved by the development of technological equipment and also by using new types of reactive MF resins (Pizzi 1994; Kamoun *et al.* 2003; Hse *et al.* 2008; Biron 2014).

Amino resins for the impregnation of décor papers must fulfil a number of requirements (Kandelbauer *et al.* 2010). The most relevant in terms of the technological process and the final quality of laminated surfaces are the characteristics of MF and MUF resins: (1) sufficiently low viscosity required for their penetration into the porous paper structure; (2) optimal solid content, usually 52% to 56 %; and (3) their high resistance after curing in papers, *i.e.* in created laminates, against water, aggressive chemicals, abrasion, heat, UV radiation, biodegradation, and others – with intention that the laminates will be sufficiently hydrophobic, as well as resistant to weathering and mechanical wear. Laminates are commercially produced in many designs, *e.g.* gloss, semi matt, matt, in various colors, or as an imitation of wood, rocks, wall tiles, and others (Kohlmayr *et al.* 2014). Recently, specific requests for the laminated surfaces have been achieved, *e.g.* laminated wood-based composites, which are applied in hospitals, restaurants, kitchens, and other public places. Such laminated products should have an increased microbial resistance against bacteria and microscopic fungi.

Laminated surface of wood-based composites with antimicrobial properties

Antimicrobial properties of laminated surfaces of wood-based composites can be achieved by several methods that include a number of physical and chemical modifications of the impregnating resins, décor papers, or final laminates, in order: (1) to reduce adhesion of microorganisms into surfaces; (2) to prevent attaching of organic nature impurities into surfaces; and (3) to actively eliminate bacteria and microscopic fungi present in the surfaces.

Kandelbauer and Widsten (2008) suggested some strategies that are possible to achieve the antimicrobial properties of surface treatments (Fig. 1).

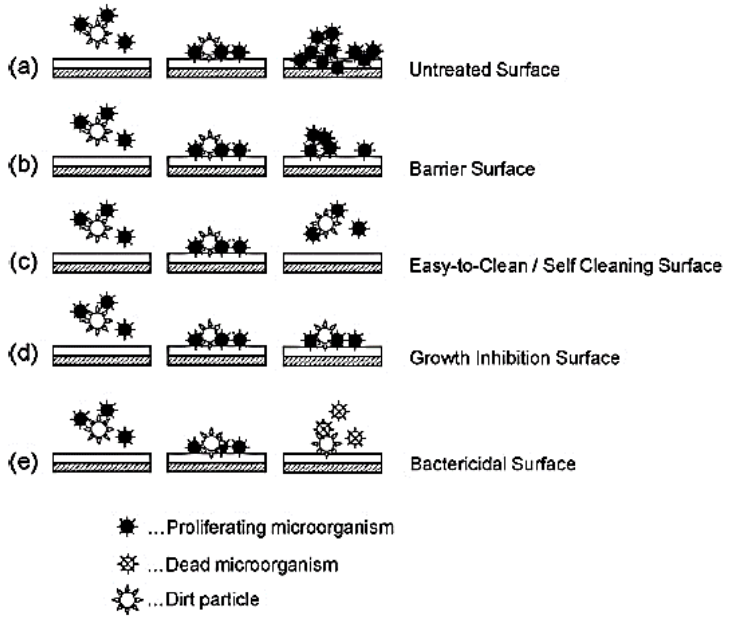


Fig. 1. Basic strategies to achieve antimicrobial properties of surface treatments (Kandelbauer and Widsten 2008)

Surfaces of commercial wood-based composites treated with lamination technologies, *i.e.* using papers impregnated with amino resins, secure a certain level of their resistance against bacteria and microscopic fungi (Vidholdová *et al.* 2015). Such a surface treatment creates a so-called "barrier surface," which implies that there is a barrier between the wooden elements in the composite, which are usually easily attacked by micro-organisms, and the microbe cells (Fig. 1b).

However, dirt particles or other impurities (especially of organic origin) can be attached to the surfaces of laminated wood-based composites. Such particles may serve as a substrate for microbial growth. It is possible to prevent contamination of laminated surfaces by creation of so-called "self-cleaning surfaces" (Fig. 1c). Dirt and other impurities cannot easily attach themselves to the laminated surface due to its high hydrophobicity and low porosity, and therefore they can be easily removed. Badila *et al.* (2014) increased the self-cleaning-ability of the laminated surfaces through the addition of some substances– silicon dioxide (SiO_2) and polydimethylsiloxane (PDMS = $(C_2H_6OSi)_n$) oxidized by pyridinium chlorochromate (PCC = $[C_5H_5NH][CrO_3Cl]$) – into impregnating MF resin, and the resultant laminated surface had significantly increased hydrophobicity and oleophobicity. Gao *et al.* (2015) treated wood-based composites by spraying their surfaces, using titanium dioxide (TiO_2) modified by floroalkylsilane, with the aim to improve their surface cleaning ability. Such surfaces were found to be highly hydrophobic, as well as resistant against solution of hydrochloric acid (HCl) and UV radiation.

However, in production and by using this surface type, secondary defects can be created, such as impaired resin coating homogeneity or increased susceptibility for microscopic scratches, which negatively affect its final cleaning ability. Generally, surfaces of wood-based composites laminated with papers impregnated with MF or MUF resins – but without bio-active additives – in the above view, are still partially susceptible to be attacked by microorganisms.

Laminates unsusceptible to biological attacks can be created by modifying the impregnation amino resins with biocidal additives. Biocidal additives present in amino resins are able to reduce the ratio of microbial growth or completely inhibit the microbial growth on laminated surfaces (Fig. 1d). Some of them are able to actively kill microbial cells (Fig. 1e). Hanrahan *et al.* (2006) used triclosan (2,4,4´-trichloro-2´-hydroxydiphenyl ether) as a bio-active additive into resins for the preparation of the antimicrobial laminated surfaces. Triclosan has strong antimicrobial properties; it does not chemically react with amino resins, but in contrast, it has a negative impact on the human health and environment, and therefore it is necessary to regulate its use in commercial products.

Inorganic biocides are most often applied in the form of nanoparticles. More and more studies are dealing with the addition of nanoparticles to surfaces both interior and exterior to improve their antimicrobial properties (Ali *et al.* 2014). Suitable additives for amino impregnation resins could be mainly zinc oxide (ZnO) and silver (Ag) nanoparticles, especially for their good antimicrobial activity, as well as chemical and physical inertness to various materials. Additionally, at low concentrations they are harmless to human health.

Zinc Oxide Nanoparticles for Anti-microbial Surfaces

Because of zinc oxide nanoparticles' unique characteristics, such as high chemical stability, high electrochemical coupling coefficient, wide radiation absorbance spectra, high photostability, and biological activity, they are a multifunctional substance with a wide range of use (Kolodziejczak-Radzimska and Jesionowski 2014). Nanoparticles of ZnO can by synthesized by various methods depending on their particle size and shape

requirements, purpose of use, and price. They are usually synthesized from various precursors – *e.g.* from ZnCl, $Zn(NO_3)_2.6H_2O$, $Zn(CH_3COO)_2$ – in the form of nanorods (Li *et al.* 2016; Nandi and Major 2016; Yu *et al.* 2017), nanospheres (Onwudiwe *et al.* 2015; Schöttle and Feldman 2016), nanotubes (Samadipakchin *et al.* 2017), nanorings (Liu and Cai 2008), nanowires (Marimuthu *et al.* 2016; Roso *et al.* 2016), or nanospirals (Gao *et al.* 2008). The type of ZnO nanostructure depends on the used chemical synthesis or physical preparation method, *i.e.* classical chemical method (Bhunia *et al.* 2016; Shimpi *et al.* 2016), chemical bath deposition (Qu *et al.* 2017), mechano-chemical method (Rajesh *et al.* 2012), sol-gel method (Gilliot and Hadjadj 2016; Hasnidawani *et al.* 2016), solvothermal method (Feng *et al.* 2016), microwave decomposition (Sirelkhatim *et al.* 2015), or hydrothermal and microwave-hydrothermal method (Politi *et al.* 2015). Each ZnO nanostructure has its specific physicochemical properties (Wang 2004).

Zinc oxide exhibits good antimicrobial activity, especially when it is in the form of nanoparticles (Rao *et al.* 2014; Shi *et al.* 2014). Nanoparticles of ZnO can interact with the surfaces of a living cell, or rather after penetration to the living cell with its core (Seil and Webster 2012). Several authors confirmed the antimicrobial activity of ZnO nanoparticles against the Gram-positive and Gram-negative bacteria, *e.g.* to *Bacillus subtilis*, *Streptococcus pneumoniae, Pseudomonas aeruginosa*, or *Escherichia coli* (Tam *et al.* 2008; Raghupathi *et al.* 2011; Wang *et al.* 2012; Martins *et al.* 2013; Paul and Ban 2014). The ZnO nanoparticles are also active against wood destroying fungi and molds. For example, Reinprecht *et al.* (2015, 2016) found that nanoparticles of ZnO present in maple and lime-tree woods suppressed decay activity of the white-rot fungus *Trametes versicolor* and partly also of the brown-rot fungus *Coniophora puteana*.

EXPERIMENTAL

Materials

Commercial particleboards (PBs) with a thickness of 16 mm were produced in Kronospan Bučina DDD, Co. Ltd., Zvolen, Slovakia. White décor papers (DP) – UNI white 0500 with areal weight 90 $g.m^{-2}$ – used for the impregnation process were produced in Malta Décor Sp. Z o. o., Poznan, Poland. The melamine-formaldehyde (MF) resin was produced in Kronospan Bučina DDD, Co. Ltd., Zvolen, Slovakia (viscosity 61.5 mPa.s). The biocide, dispersion of zinc oxide (ZnO) nanoparticles, was produced in Sigma-Aldrich Co. Ltd., Munich, Germany (< 100 nm dynamic light scanning (DLS) particle size; < 35 nm average particle size (APS); 50 wt.% in water).

MF resin modification with ZnO nanoparticles

Melamine-formaldehyde (MF) resin was modified with ZnO nanoparticles in amounts of 0 wt.%, 0.1 wt.%, 0.3 wt.%, 0.6 wt.%, and 1 wt.%. Modified MF resins had been, before using for the impregnation of papers, catalyzed with hardener DeuroCure KS (Deurowood, Hard, Austria), at which the condensing time of all resins was 330 ± 15 s at 100 °C.

Impregnation of papers with MF resins

White décor papers with dimensions of 300 mm by 280 mm were impregnated on a laboratory application device with basic and modified MF resins to achieve their uniform saturation and retention of 120 $g \cdot m^{-2} \pm 10$ $g \cdot m^{-2}$. A uniform dispersion of ZnO nanoparticles

in MF resins used for impregnation process was achieved by mechanical stirring (Makita 6600, Makita Corporation, Anjō, Japan) at 250 rev.min^{-1} for 3 minutes. Impregnated papers were then pre-cured in a laboratory kiln Eccocell 55 – Comfort (MMM Medcenter Einrichtung GmbH, Munich, Germany) for 3 min at 160 °C ± 3 °C.

Preparation of laminated PBs

Pre-cured impregnated papers were pressed onto PBs (300 mm by 280 mm) in a laboratory press Table Press TP 600 (Fontijne Presses & Services BV, Barendrecht, Netherlands) with a pressure of 0.3 MPa, at a temperature of 175 °C, for 4 min. Thicknesses of the laminated layers on the surfaces of PBs varied from 0.2010 mm (at addition of 0.1 wt.% ZnO) to 0.2076 mm (without addition of ZnO), and were not significantly influenced by the amount of ZnO. From the laminated PBs were prepared samples with dimensions of 50 mm by 50 mm for the bacterial and mold tests. Samples with dimensions of 100 mm by 100 mm were used for the abrasion test and samples with dimensions of 250 mm by 250 mm for resistance tests against 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 collection of microorganisms at the Department of Clinical Microbiology of Hospital Zvolen, Zvolen, Slovakia) were used for the bacterial test.

Samples of laminated PBs with cleaned (using mixture of 96% ethanol and 2-propanol in a weight ratio of 8.8:1.2) and sterilized (2-times by UV-light for 20 min) surfaces were placed into sterilized Petri dishes and inoculated with 0.1 mL of bacterial suspensions. Two densities of bacterial suspensions were applied, *i.e.* 0.5* and 1.0*, in the physiological solutions according to the McFarland scale (1.5×10^8 CFU/mL and 3.0×10^8 CFU/mL). Incubation of bacteria on the inoculated laminated surfaces was performed at 37 °C for 48 h. Afterwards, bacteria were stripped from the laminated surfaces using a sterile swap and taken up 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 laminated PBs in the diagnostic soil was assessed on basis of the bacterial activity (BA) valued from 0 to higher numbers in CFU/mL.

Mold test

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

The samples of laminated PBs were divided into two groups: (1) cleaned with 96% ethanol and sterilized (2-times by UV-light for 20 min); and (2) sterilized and then intentionally contaminated. Intentional contamination of the top surfaces of samples was made with 15 wt.% mixed solution of three organic substances (proteins, sugars, and lipids in weight ratios of 8:1.3:0.7). The solution was applied by sprayer – two sprays of 0.5 mL – on the whole surface of samples from distance of 10 cm. Mold tests started with placing of samples into 100-mm Petri dishes filled with a 3 mm to 4 mm thick layer of the Czapek-Dox agar (one sample a dish), and inoculation of their top surfaces with water spore

suspensions of *A. niger* or *P. brevicompactum*. Incubation of molds on the laminated surfaces lasted 4 weeks at 27 °C temperature and 90% relative humidity of air.

The anti-mold resistance of laminated surfaces was evaluated visually after 7, 14, 21, and 28 days. The determination of growth activity of the molds (GAM) used the following scale from 0 to 4 in accordance with the Standard STN 49 0604 (1980): 0 = no mold; 1 = mold up to 10%; 2 = mold up to 25%; 3 = mold up to 50%; 4 = mold more than 50% on the top surface.

Standard quality tests

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

RESULTS AND DISCUSSION

Bacterial Test

The results of the bacterial test are shown in Table 1.

Table 1. Bacterial Activity on Laminated Surfaces at Application of ZnO Nanoparticles into MF Resins

ZnO in MF (wt.%)	Bacterial Activity (10^8 CFU/mL)			
	Staphylococcus aureus		*Escherichia coli*	
	0.5*	1.0*	0.5*	1.0*
0	0.29	0.63	0.20	0.47
0.1	0.25	0.54	0.18	0.49
0.3	0.26	0.59	0.09	0.23
0.6	0.26	0.53	0.10	0.26
1	0.23	0.57	0.07	0.25

* Density of bacterial suspension according to McFarland Standard; N = 6: Number of tested samples in one series

Because of the presence of ZnO nanoparticles in MF resins, the laminated surfaces of PBs had a negligibly higher resistance against the activity of the Gram-positive bacteria *S. aureus*, *i.e.* its activity decreased either approximately by 20.7% from 0.29 CFU/mL to 0.23×10^8 CFU/mL (for 0.5* McFarland), or approximately by 9.5% from 0.63 CFU/mL to 0.57×10^8 CFU/mL (for 1.0* McFarland). However, zinc oxide evidently reduced the activity of the Gram-negative bacteria *E. coli* more on the surfaces of laminated PBs – approximately by 65% (from 0.2 CFU/mL to 0.07×10^8 CFU/mL for 0.5* McFarland) or 46.8% (from 0.47 CFU/mL to 0.25×10^8 CFU/mL for 1.0* McFarland).

Mold Test

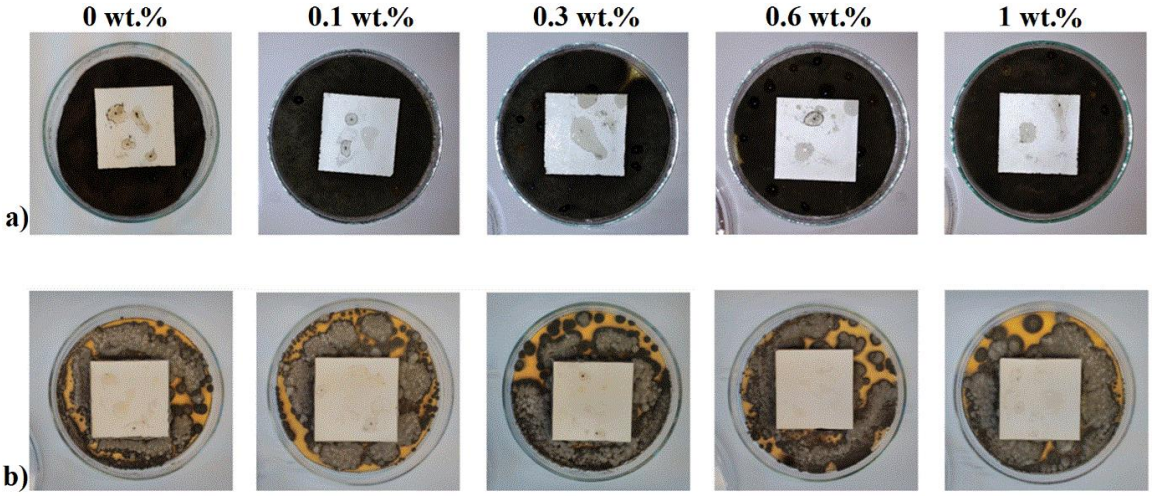
The results of the mold test on the sterilized surfaces are shown in Table 2 and on the contaminated surfaces in Table 3.

The cleaned and sterilized top surfaces of the laminated surfaces of PBs were resistant to growth of molds at all used concentrations of ZnO nanoparticles in MF resins

(Table 2, Fig. 2). However, laminated surfaces without ZnO had a slightly lower resistance to molds after 21 and 28 days of incubation (GAM = 1) (Figs. 2a, 2b). Similar results that regard a high resistance of cleaned laminates to molds were achieved by Vidholdová *et al.* (2015) for commercially produced laminated PBs without the presence of fungicides. In contrast, they found that various commercial raw particleboards were quickly and intensively attacked by molds. Generally, it can be concluded that the laminated surfaces create a so-called "barrier surface" on the wooden-composites against activity of molds, *i.e.* surfaces without digestible organic compounds do not have suitable conditions for growth of microscopic fungi.

Table 2. Mold Activity on Cleaned Laminated Surfaces

ZnO in MF (wt.%)	Growth Activity of Molds – On Cleaned Surfaces (0 to 4)							
	A. niger				*P. brevicompactum*			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
0	0	0	1	1	0	0	1	1
0.1	0	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0	0
0.6	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
* n = 6: Number of tested samples in one series								



The marks on the surfaces were caused by mold spores from inoculation. Mold growth was observed only on the surfaces of unmodified laminates (0 wt.%).

Fig. 2. Cleaned laminated PBs inoculated with a) *A. niger* and b) *P. brevicompactum* – top surfaces after 28 days of incubation in molds

In contrast to the cleaned laminated surfaces, the intentionally contaminated laminates were not completely resistant to growth of molds (Table 3, Fig. 3). After 21 days, on the contaminated laminated surfaces with ZnO nanoparticles a partly inhibited growth of *A. niger* and *P. brevicompactum* (GAM = 2) was observed in comparison to surfaces without ZnO (GAM = 4).

Table 3. Mold Activity on Contaminated Laminated Surfaces

ZnO in MF (wt.%)	Growth Activity of Molds – On Contaminated Surfaces (0 to 4)							
	A. niger				*P. brevicompactum*			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
0	1	3	4	4	1	3	4	4
0.1	1	2	2	3	1	2	2	3
0.3	1	2	2	2	1	2	2	2
0.6	1	2	2	2	1	2	2	2
1	1	2	2	2	1	2	2	2
* n = 6: Number of tested samples in one series								

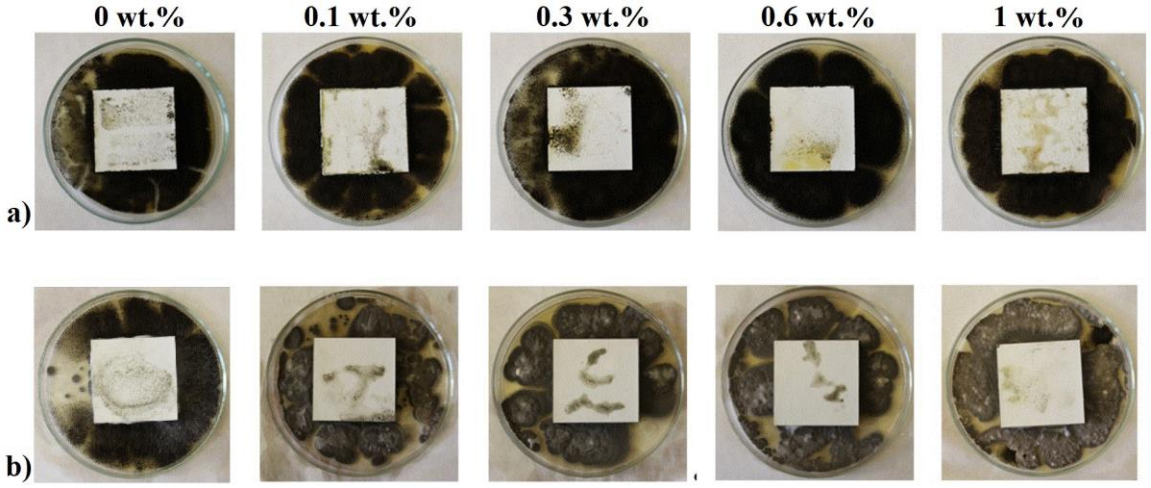


Fig. 3. Contaminated laminated PBs inoculated with a) *A. niger* and b) *P. brevicompactum* – top surfaces after 28 days of incubation in molds

Overall, based on the bacterial and mold tests, it was concluded that ZnO nanoparticles added into MF resin for laminated surfaces of PBs had an evident biocidal effect on their anti-bacterial anti-mold properties. The results achieved in this work were in concordance with the findings of Li *et al.* (2009), Hochmannova and Vytrasova (2010), and Reinprecht *et al.* (2015, 2016), in that these authors indicate a high anti-bacterial and anti-fungal effectiveness of ZnO nanoparticles in coatings or preservatives for wood protection. In contrast, Bellotti *et al.* (2015) observed that ZnO nanoparticles did not have any relevant anti-mold effect in polyurethane coatings for interiors. Li *et al.* (2009) suggested as possible explanation for lowering the anti-mold activity of ZnO nanoparticles that there would be a negligible amount of UV radiation in the interior of the material. Thus, the ZnO would not become activated to a form that would damage the mold cell walls. The knowledge that cleaned laminated surfaces were resistant to molds, but that intentionally contaminated laminated surfaces were not completely resistant to mold attack implies that laminated surfaces have to be maintained and cleaned – in accordance with the work of El-Feky *et al.* (2014).

Standard Quality Tests

The results of the resistance tests of the laminated surfaces of particleboards to cold aggressive liquids, dry heat, and abrasion are shown in Table 4.

Zinc oxide nanoparticles had only negligible negative effects on the standardized quality characteristics of the laminated surfaces of PBs. This can be explained by supposing

that this substance did not change the structural composition of the cured MF resins in the décor impregnated papers and final laminates. A relatively higher decrease occurred only at the abrasion resistance of laminates, maximally about 16.7%, when the MF resin was modified by 1 wt.% of ZnO nanoparticles (Table 4). Such a decrease could be explained by an interaction of small solid ZnO nanoparticles, which are not in the chemical bond with the cured MF resin in laminates, with the abrasive sandpaper.

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

Tested Parameters		ZnO in MF (wt.%)				
		0	**0.1**	**0.3**	**0.6**	**1**
Chemicals	Acetone	5	5	5	5	5
	Alcohol	5	5	5	5	4
	Coffee	4	4	4	4	3
	Ink	5	5	5	5	5
	Ketones	3	3	3	3	3
	Oil	5	5	5	5	5
	Tea	4	4	4	3	3
	Water	5	5	4	4	4
Dry heat (180 °C)		4	4	4	3	3
Relative abrasion resistance (%)		100	100	95.83	91.67	83.33

* 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 light source is mirrored and close to tested surface; 5: no visible changes; n = 5: Number of tested samples in one series for chemicals resistance and dry heat resistance; n = 10: Number of tested samples in one series for abrasion resistance

CONCLUSIONS

1. The addition of zinc oxide nanoparticles into MF resin, used for preparation of laminated surfaces of PBs, had a positive effect on the reduced bacterial activities, less against the Gram-positive bacteria *S. aureus* and more against the Gram-negative bacteria *E. coli*.
2. The cleaned laminated surfaces of PBs well resisted the mold growth even without presence of ZnO nanoparticles. In contrast, the resistance of the intentionally contaminated laminated surfaces of PBs to molds *A. niger* and *P. brevicompactum* was weak, at which due to the addition of zinc oxide nanoparticles it was partly improved.
3. Nanoparticles of ZnO did not have any or had only a small effect on the resistance of melamine-laminates to cold aggressive chemicals and to dry heat.
4. Abrasion resistance of the laminated surface of PBs was partly lowered with an increased amount of ZnO nanoparticles in the laminates.

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