

Effect of Different Nanoparticle Treatments on the Decay Resistance of Wood

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The efficacy of five different nanoparticles (zinc-oxide, zinc-borate, silver, copper, and copper-borate) were evaluated after application at different concentrations for protection against *Coniophora puteana* and *Coriolus versicolor*. Tests were performed with two different wood species (beech and pine sapwood). Furthermore, tests were done to investigate the resistance of the nanoparticles to leaching. Overall, the results of the efficiency of the investigated nanoparticles against the brown and white rot fungi were diverse. One of the investigated fungi showed a tolerance to the nanoparticles in some cases (zinc-oxide, silver nanocubes, and copper). The most effective nanoparticle treatments were those containing borate. However, only the zinc-oxide, copper, and silver nanoparticles showed a high resistance to leaching. Unfortunately, zinc-borate and copper-borate showed a low resistance to leaching, as the treated samples showed a similar decay compared with that of the control. Thus, only the zinc-oxide at the highest investigated concentration (5% m/m) provided effective protection after leaching for both of the investigated fungi. Also, the copper nanoparticles showed potential as an effective treatment at higher concentrations.

Keywords: Wood preservation; Nanoparticles; *Coniophora puteana*; *Coriolus versicolor*; Leaching

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INTRODUCTION

The utilization of wood contributes to sustainable development. The technical properties of most European wood species are worse than those of some competing materials that have an unsustainable origin (tropical wood species, plastics, *etc.*). Using nanomaterials to create a new generation of novel cost-effective products is a key issue now in several industries (plastics, chemistry, *etc.*), and is an issue identified by the forest products industry as well. However, the use of nanoparticles to improve major technical properties of wood has currently not been investigated thoroughly. In contrast, in the cases of polymers, textiles, and paper, there are a lot of promising results available related to the improvement of mechanical, hydrophobic, combustion, and other properties (Wang *et al.* 2006; Textor and Mahltig 2010; Chen and Yan 2012; Kumar and Maiti 2016; de Luna and Filippone 2016; Hosseini 2017; Karwowska 2017; Mozumder *et al.* 2017; Zabihi *et al.* 2018).

Currently, there is only some data available on the improvement of wood material properties with the use of nanoparticles, but most of the results are positive. According to the available results, the targeted improvement of different material properties is possible with the use of nanoparticles (nanotubes, nanowires, nanosized metal oxides, nanoclays, *etc.*).

When using different nanoparticles, it is possible to reduce the moisture uptake and improve the ultraviolet protection, mechanical properties, and durability (Mahltig *et al.* 2008; Rassam *et al.* 2012). Using nanoparticles, the fire resistance can be improved as well (Mahr *et al.* 2012). According to the earlier study done in this field by the authors, zinc-oxide nanoparticles have a limited protection effect against *Poria placenta*, but they performed well against *Serpula lacrymans*. However, the zinc-borate nanoparticles showed strong protection against both of those fungi (Lykidis *et al.* 2016).

The combination of different nano-metals (silver nanoparticles in combination with copper- or zinc-oxide) provides strong protection against termites as well (Green and Arango 2007). Pure nano-copper displays wood protective properties against *Gloeophyllum trabeum* and *Trametes versicolor* during mini-agar slant and wood block tests (Weitz *et al.* 2011). Field stake tests and ground proximity tests have been performed at different sites (Hilo, Hawaii; Gainesville, Florida; Dorman and Saucier sites in Mississippi; Australia; New Zealand). Micronized copper particles have performed well in field trials. In some cases, they performed better than their amine-soluble counterparts that served as controls (McIntyre and Freeman 2011). The biological resistance of building materials treated with nano-zinc, nano-copper, and nano-silver against mould (*Aspergillus brasiliensis* and *Penicillium funiculosum*) has been tested by Huang *et al.* (2015). It was found that nano-zinc and nano-copper are the most effective in the case of wooden floorings, followed by nano-silver. Different preparations of nano-zinc-oxide, nano-zinc-borate, nano-copper, nano-titanium, nano-cerium, nano-silver, and nano-boron have been investigated in earlier studies and found to be effective (Kartal *et al.* 2009; Shirakawa *et al.* 2013; Mantanis *et al.* 2014; Terzi *et al.* 2016).

To improve wood properties, immersion and impregnation are the most adequate processes. In the case of some nanoparticles, it is not yet clear whether they are applicable and effective for improving wood properties. The mode of nanoparticle preparation may also influence the efficiency because of different particle sizes and formulations. For that reason, it is necessary to analyze the relationship between these nanoparticles and wood. Nanoparticles have to be in a liquid medium, either dispersed or suspended, for the impregnation of wood to be possible; therefore, the base materials may also be an influencing factor.

Assuming that zinc, copper, silver, and boron nanoparticles show original properties, they may be a tool in developing new wood protection systems. Preparations of nano-metals may possess unique characteristics that are very different from the characteristics of the elemental metal (Mody *et al.* 2010). The objective of this study was to evaluate the preparation of different metal nanoparticles to prevent decay by the brown rot fungus *Coniophora puteana* and the white rot fungus *Coriolus versicolor*. The goal of the planned research was to investigate different nanoparticles and their effect on the durability of wood. Nanoparticle treatments were prepared by using nanoparticles directly from their preparation solutions, without precipitating or re-dispersing them. An important goal of this study was to investigate the leaching resistance of the nanoparticles from the wood material. Instead of surface treatments, a full cross-section treatment was performed, which could make the service life of wooden products longer. An important objective was the improvement of the durability of European wood species.

EXPERIMENTAL

Preparation of Nanoparticles

Five different nanoparticles were tested in this study, namely zinc-oxide, zinc-borate, silver (Ag), copper (Cu), and copper-borate. The suspensions/colloids were used for the treatment of the samples directly, without precipitating or re-dispersing the obtained nanoparticles.

The colloidal zinc-oxide nanoparticles were prepared by hydrolyzing zinc acetate dihydrate (Lach-Ner S.R.O., Prague, Czech Republic) in a basic KOH-methanol (Lach-Ner S.R.O.) solution (Sun *et al.* 2007). With this method, zinc-oxide nanoparticles with an average size of 3 nm to 5 nm were prepared. The concentration of the basic zinc-oxide suspension was 5% (m/m).

The zinc-borate nanoparticle suspension was produced by using a water-based mixture with 1.0 mol/dm³ borax decahydrate (Lach-Ner S.R.O.) and 1.0 mol/dm³ zinc nitrate hexahydrate (Lach-Ner S.R.O.) at 70 °C with a 2-h reaction time (Gönen 2009). With this method, zinc-borate nanoparticles with an average size of 100 nm to 200 nm were prepared. The concentration of the basic zinc-borate nano-suspension was 2% (m/m).

Silver nanocubes were prepared in an aqueous solution with polyvinyl-pyrrolidone (PVP) (VWR Chemicals, Radnor, PA, USA) as a protecting agent. Silver nitrate (Scharlab, Debrecen, Hungary) precursor was added to deionized water containing PVP to obtain silver colloid at a 2% (m/m) Ag concentration. Then, the aqueous solution of L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) with an AgNO₃/C₆H₈O₆ molar ratio of 1.2 was added dropwise into the silver nitrate solution and stirred for 1 h (Zielińska *et al.* 2009). With this method, Ag nanoparticles with an average size of 80 nm to 100 nm were prepared. The concentration of the basic silver nano-suspension was 2% (m/m).

For the synthesis of the copper nanoparticles, an aqueous solution with 0.4 M L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) and 0.8 M PVP (VWR Chemicals, Radnor, PA, USA) was directly mixed by stirring with another aqueous solution containing 0.01 M copper (II) nitrate (anhydrous) (Acros Organics, Geel, Belgium) and 0.8 M PVP. Then, the mixture was kept at a constant 45 °C without any inert gas protection. After 1 h, the light blue initial precursor solution changed to a red colloidal slurry, which indicated the formation of copper nanoparticles. After 3 h, there was no further color change and the process was stopped (Wu *et al.* 2006). With this method, copper nanoparticles with an average size of 2 nm to 4 nm were prepared. The concentration of the basic copper nano-suspension was 2% (m/m).

Two molar anhydrous copper (II) nitrate (Acros Organics, Geel, Belgium) and 0.1 M borax decahydrate (Lach-Ner S.R.O.) water-based solutions were mixed at a borax/copper ratio of 1:4 to prepare nano-sized copper-borate. Dilute Cu(NO₃)₂ and borax solutions were mixed at 70 °C to prevent particle growth and agglomeration, as well as to obtain nano-sized copper-borate particles. The copper (II) solutions were added dropwise to the borax solutions, which were stirred at 450 rpm using a magnetic stirrer (MS2DHS, Witeg, Wertheim, Germany) (Alp *et al.* 2014). With this method, copper-borate nanoparticles were formed in planar geometry with the average dimensions of 2 µm (length) × 1 µm (width) × 100 nm (thickness). The concentration of the basic copper-borate nano-suspension was 2% (m/m).

After having prepared the nanosuspensions described above, they were divided into three parts to prepare three different concentrations for the tests. One part remained at the original concentration, one part was diluted in case of colloidal zinc-oxide nanoparticles

with methanol to a 2% (m/m) concentration, and one part to a 1% (m/m) concentration. In case of zinc-borate, copper and copper-borate nanoparticles and silver nanocubes, remained at the original concentration as well, and one part was diluted with distilled water to a 1% (m/m) concentration, and one part to a 0.5% (m/m) concentration.

Preparation of the samples

Impregnation of all samples was done according to the standard MSZ EN 113 (2001). For each concentration, test specimens that were kept dry and had a known mass (m_0) were impregnated with the nano-suspensions. Impregnation was done in order of concentration, starting with the highest concentration (2% or 5% m/m) and ending with the diluted nano-suspension (0.5% or 1% m/m). The impregnation process consisted of a 15-min vacuum at 0.7 kPa, followed by the introduction of a nanoparticle suspension to the vessel and keeping the specimens at atmospheric pressure in the suspension for 2 h. Following this impregnation treatment, the test specimens were immediately weighed to ascertain the mass after impregnation (m_1). According to the weight data, the retention value (kg/m^3) was calculated. After the impregnation procedure, the samples were climatized for 4 weeks at 20 °C and a 65% relative humidity.

Leaching Test

The leaching procedure has been done as described in MSZ EN 84 (2002). Steps of the procedure:

- impregnation of the samples with distilled water (4 kPa vacuum for 20 min., followed by leaving the samples in the distilled water at atmospheric pressure for 2 hours)
- changing the water on the samples after the impregnation step immediately, and after the first and second day of immersion
- changing the water on the samples further 7 times during the remaining 12 days at intervals of not less than 1 day and not more than 3 days

After the leaching procedure, the samples were climatized for 4 weeks at 20 °C and a 65% relative humidity.

Decay Test

The decay test was performed according to MSZ EN 113 (2001). The test fungi for this study were the brown rot fungus *Coniophora puteana* and the white rot fungus *Coriolus versicolor*. The culture medium was a malt agar medium. Conditions during the test were 23°C temperature and 75% relative humidity.

The wood species used were pine sapwood (*Pinus sylvestris*) and beech (*Fagus sylvatica*), which are both susceptible to attack by fungi. The test material had been thoroughly impregnated by the nanoparticle suspensions. The dimensions of each specimen were 50 mm × 25 mm × 15 mm (L × T × R). The specimens were divided into three groups:

- Treated test specimens: impregnated specimens subjected to attack by the wood-destroying fungi. Five treated test specimens were used for each preservative concentration, timber species, and fungus species.
- Treated and leached test specimens: impregnated specimens subjected to attack by the wood-destroying fungi, after a leaching procedure that was done according to

MSZ EN 84 (2002). Five treated test specimens were used for each preservative concentration, timber species, and fungus species.

- Untreated test specimens: non-impregnated test specimens of the same wood species as that of the treated test specimens. They were placed in culture vessels next to the treated specimens and treated and leached specimens as a control.

Culture vessels (Kolle flask) containing the culture medium were inoculated by the test fungus. Introduction of the test specimens to the flasks was done after the mycelia covered the whole surface of the malt-agar medium. One inoculated culture vessel contained two treated specimens, two treated and leached specimens, and one untreated test specimen. After the introduction of the test specimens, the culture vessels were placed in a climate chamber at a constant temperature of 23 °C and relative humidity of 75% for 16 weeks. Following incubation, the specimens were removed from the culture vessels, cleaned from the mycelium, oven-dried (103 °C ± 2 °C) and weighed (m_2). The percentage mass loss was calculated according to the weight data (m_0 and m_2).

Equations

The chemical retention was calculated using Eq. 1,

$$R = \frac{m_1 - m_0}{V} \quad (1)$$

where R is the chemical retention of the nanoparticles for the wood specimens (kg/m³), m_1 is the sample weight after impregnation (kg), m_0 is the sample weight before impregnation (kg), and V is the volume of the specimen (m³).

When considering wood preservation through impregnation with the nanoparticle suspension, the percentage weight losses (PWL) were calculated using Eq. 2,

$$PWL = \frac{m_0 - m_2}{m_0} \times 100\% \quad (2)$$

where PWL is the percentage weight loss of the samples after 16 weeks of incubation in the fungi culture (%), m_2 is the sample weight after 16 weeks of incubation (g), and m_0 is the sample weight before impregnation (g).

Statistical analysis of the means was done with an analysis of variance ($\alpha = 0.05$) using Statistica 12.0 software (StatSoft, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Chemical Retention

The chemical retention values for the tested beech and pine specimens are shown in Table 1. There were remarkable differences in the chemical retention for the wood species and nanoparticle concentration. The pine samples showed higher retention values for all of the treatments (Table 1). There were no significant differences between the retention values of normal (impregnated) samples and samples prepared for the leaching procedure. Thus, the impregnation quality did not have an effect on the results of the leaching test. The retention increased proportionally with the nanoparticle concentration, according to the mean ratio of the nanoparticle retention levels (Table 1). This result complied with the nanoparticle concentrations ratio used during the experiments. This indicated that increasing the nanoparticles concentration did not negatively affect the

impregnation process within the investigated range; therefore, the decay specimens could effectively absorb the nanoparticle suspensions/colloids.

Table 1. Average Retention Levels of Beech and Pine Samples for Various Nanoparticle Suspension/Colloid Impregnations at Different Concentrations

Treatment Type	Average Retention Level (kg/m ³) and Ratio (in parentheses)							
	Beech				Pine			
	Normal		For leaching		Normal		For leaching	
Zinc-oxide 1% (m/m)	4.21	(1.00)	4.15	(1.00)	4.60	(1.00)	5.02	(1.00)
Zinc-oxide 2% (m/m)	8.38	(1.99)	8.31	(2.00)	9.82	(2.14)	9.10	(1.81)
Zinc-oxide 5% (m/m)	22.00	(5.23)	22.63	(5.46)	27.79	(6.04)	25.83	(5.15)
Zinc-borate 0.5%(m/m)	2.83	(1.00)	2.80	(1.00)	3.40	(1.00)	3.38	(1.00)
Zinc-borate 1%(m/m)	5.66	(2.00)	5.59	(2.00)	6.58	(1.94)	6.35	(1.88)
Zinc-borate 2%(m/m)	11.59	(4.10)	11.47	(4.10)	13.69	(4.03)	13.73	(4.06)
Silver-colloid 0.5%(m/m)	2.82	(1.00)	2.82	(1.00)	3.21	(1.00)	3.22	(1.00)
Silver-colloid 1%(m/m)	5.73	(2.03)	5.66	(2.01)	6.23	(1.94)	6.77	(2.10)
Silver-colloid 2%(m/m)	11.45	(4.06)	11.34	(4.03)	12.50	(3.9)	12.58	(3.91)
Copper 0.5%(m/m)	2.85	(1.00)	2.84	(1.00)	3.58	(1.00)	3.51	(1.00)
Copper 1%(m/m)	5.68	(1.99)	5.76	(2.02)	6.77	(1.89)	6.85	(1.95)
Copper 2%(m/m)	10.97	(3.85)	11.28	(3.97)	12.77	(3.57)	12.13	(3.46)
Copper-borate 0.5%(m/m)	2.80	(1.00)	2.82	(1.00)	3.44	(1.00)	3.26	(1.00)
Copper-borate 1%(m/m)	5.63	(2.01)	5.62	(1.99)	6.46	(1.89)	6.57	(2.01)
Copper-borate 2%(m/m)	11.34	(4.05)	11.53	(4.09)	14.08	(4.09)	12.74	(3.91)

The average retention ratios based on the lowest concentration are given in parentheses.

Decay Test

In general, the investigated nanoparticle treatments provided higher resistance against *Coriolus versicolor* (Fig. 2) compared with *Coniophora puteana* (Fig. 1). However, Terzi *et al.* (2016) and Lykidis *et al.* (2016) reported no inhibition against *Trametes versicolor* using zinc-oxide nanoparticles. In the current study, these nanoparticles were found effective against both white rot and brown rot fungus. This result shows the importance of nanoparticle formulations, particle size, and treatment method on the efficiency on decay resistance. Zinc-oxide showed a strong protection against both *Coniophora puteana* and *Coriolus versicolor*, even at the lowest concentration used (1% m/m). Depending on the concentration, wood species, and fungus species, the PWL was only 0.2% to 3% in the case of the zinc-oxide nanoparticle treatment. However, the copper nanoparticles did not provide effective protection or only low protection against the investigated wood-decaying fungi, with an improving trend related to the nanoparticle concentration. This meant that the PWL was found to be 52% and 61% lower for beech and pine, respectively, in the case of *Coniophora puteana* at the highest investigated concentration (2% m/m) compared with that of the control. For *Coriolus versicolor* at the highest concentration, only a 4% to 8% PWL was found, which was 69% and 77% lower for beech and pine, respectively, compared with that of the control, and there was a significantly decreasing trend related to the copper nanoparticle concentration. The reverse proportionality between the PWL and nanoparticle concentration showed that it might be possible to also reach strong protection levels using higher concentrations of copper nanoparticles. Earlier studies reported that the use of Cu-based nanoparticles instead of bulk Cu results in an improved durability (Akhtari *et al.* 2013; Cookson *et al.* 2010; Kartal *et al.* 2009; McIntyre and Freeman 2009). The reasons for this phenomenon are:

- high reactivity because of the increased effective specific surface area of Cu (Chen *et al.* 2006),
- less viscous formulations compared to bulk ones,
- a reservoir effect that allows a continuous protection over longer time (Freeman and McIntyre 2013; Xue *et al.* 2014).

Due to these properties, it is possible to use Cu-based nanoparticles with lower concentrations of the metal agent with similar or even better efficacy than conventional water-based Cu formulations (Kartal *et al.* 2009).

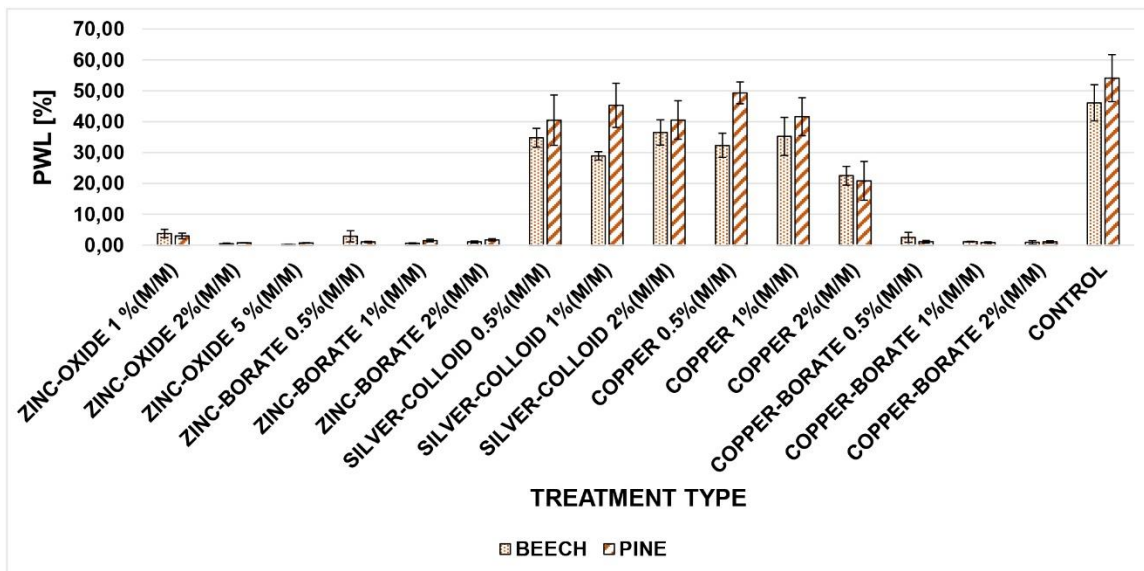


Fig. 1. PWL values of the nanoparticle-treated and control samples of the beech and pine sapwood caused by the brown rot fungus *Coniophora puteana*

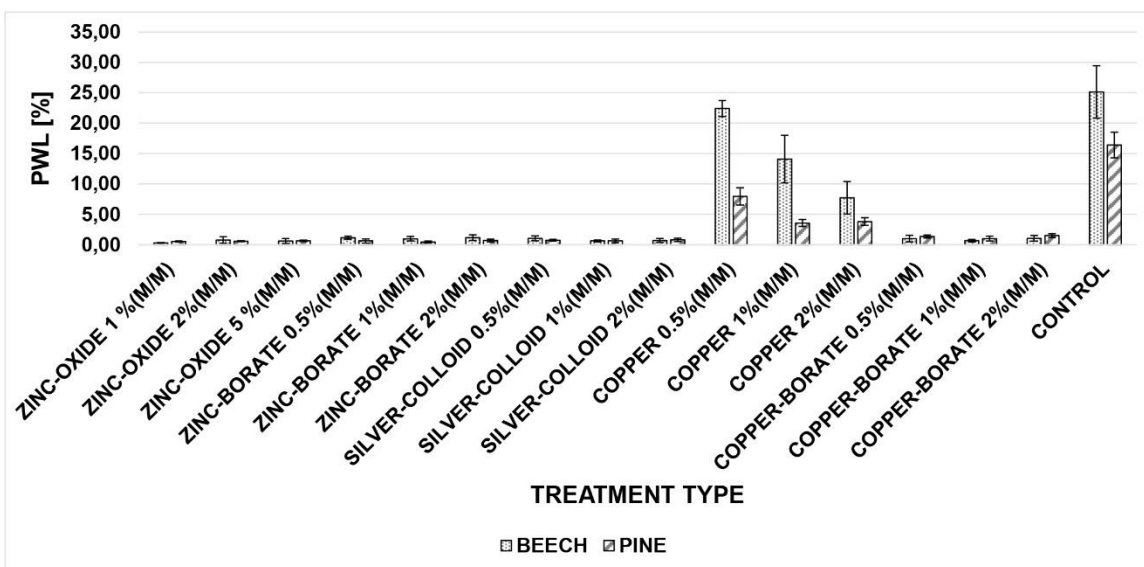


Fig. 2. PWL values of the nanoparticle-treated and control samples of the beech and pine sapwood caused by the white rot fungus *Coriolus versicolor*

Copper tolerance is usually associated with brown-rot fungi (Young 1961; Green and Clausen 2005). Guillén and Machuca (2008) evaluated the effect of copper on growth of different fungi. The use of copper concentration of minimum 3 mM led to complete growth inhibition in the case of the most white-rot fungi, and *Trametes versicolor* was strongly suppressed. In contrast, two of three brown-rot fungi showed a higher growth rate. The results of the present study confirm the previous findings by other authors, that a copper concentration lower than 3 g/L can improve the durability of wood even against brown-rot fungus, but the minimum of 3 g/L is necessary to reach good protection level.

The silver nanocubes (in colloidal form) only resulted in slight protection against *Coniophora puteana*. Depending on the nanoparticle concentration, the PWL was 25% to 29% and 17% to 26% lower for beech and pine sapwood, respectively, compared with that of the control. In contrast, the white rot *Coriolus versicolor* treatment with colloidal silver nanocubes was effective even at the lowest concentration (0.5% m/m), as the PWL, depending on the nanoparticle concentration, was only 0.6% to 1% and 0.6% to 0.8% for beech and pine, respectively, which were lower compared with the values of 25% and 17% for the control beech and pine samples, respectively. This result showed the different tolerances of the tested fungi against colloidal silver nanocubes. Moya *et al.* (2017) found similar result by applying silver nanoparticles to nine tropical species. The treatments improved their durability and classifies them highly resistant to white-rot fungus *Trametes versicolor*. However, resistance to the brown rot fungus *L. acuta* was found lower. This result was attributed to low particle concentration. Dorau *et al.* (2004) tested ionic silver-based biocides (1% Ag solution) and found it not effective to protect southern yellow pine wood to brown rot fungi. In the study of Pařil *et al.* (2017), silver nanoparticles inhibited the tested white rot fungus *Trametes versicolor*, whereas brown rot fungus *Poria placenta* was only suppressed that was shown by the lower mass loss in comparison to control specimens.

Among metallic elements, silver shows high toxicity for microorganisms, coupled with the lowest toxicity for animal cells at the same time (Rai *et al.* 2009; Pulit *et al.* 2013, Golubovich and Rabotnova 1974). Silver ions in solution are able to inhibit the activity of the cellulase enzymes of the decay fungi (Dorau *et al.* 2004). Additionally, silver nanoparticles act as reservoir for the antifungal effect, as metallic silver oxidizes in the presence of water that results in the continuous release of silver ions. Silver ions are responsible for the antifungal effect. Silver oxidation is a slow reaction, therefore the size of the particles is an important factor in the growth inhibition of fungi. Smaller particle size means larger specific surface area, which results in more effective oxidation. Particle size of less than 100 nm is necessary to reach proper protection against decay fungi through the continuous release of silver ions. The main advantages of silver nanoparticles compared to organic biocides are that they are non-volatile and non-degradable over time, are odorless, and ensure long term efficacy (Moya *et al.* 2017).

Zinc-borate and copper-borate nanoparticles provided effective protection against both of the investigated decay fungi, even at low concentrations (0.5% m/m). This result showed that combinations of different elements could provide much more effective preservation than using them individually. In contrast, the well-known efficiency of boron as a preservative is shown with these results as well. Mantanis *et al.* (2014) investigated the effect of zinc oxide, zinc borate and copper nanoparticles against decay fungi *Trametes versicolor* and found that mass losses were remarkably inhibited by the zinc-based formulations. Especially, zinc borate nanoparticles in acrylic emulsion revealed very high resistance to *Trametes versicolor*.

Overall, the results of the efficiency of the investigated nanoparticles against the brown and white rot fungi were diverse. It was concluded that one of the investigated fungi showed a significantly higher tolerance to the investigated nanoparticles in some cases. Thus, these treatments are not recommended for general use, and only for use at higher concentrations. The generally effective nanoparticle treatments were the combinations containing borate in its chemical composition, and zinc-oxide.

Brown rot fungi showed higher resistance against copper and silver nanoparticles during the present study. This phenomenon is related to the ability of fungus to influence the pH of the substrate. Brown rot fungi are able to remarkably reduce the pH of the substrate by oxalic acid production, even in first stage of decay (Humar *et al.* 2001, 2005). The production of chelating compounds (*e.g.* oxalic acid) reduces toxicity of metals by the formation of insoluble compounds, and thus biologically unavailable forms of oxalate (Hastrup *et al.* 2005; Gadd 1999). Additionally, other mechanisms might be in the background as well that are used by microorganisms to avoid toxicity of metallic compounds (Guggenbichler *et al.* 1997), *e.g.* biomethylation; development of efflux pumps; binding of metal ions to cell surfaces; or the removal of metal ions by precipitating. However, in this study, the decay resistance against *Coniophora puteana* and *Trametes versicolor* was noticeably improved by zinc-oxide nanoparticles and the borate containing nanoparticles.

Unfortunately, the zinc-borate and copper-borate treated samples showed low resistance to leaching because after leaching, the treated samples showed similar or only slightly lower PWL values compared with those of the control for both the brown and white rot fungi (Figs. 3 and 4).

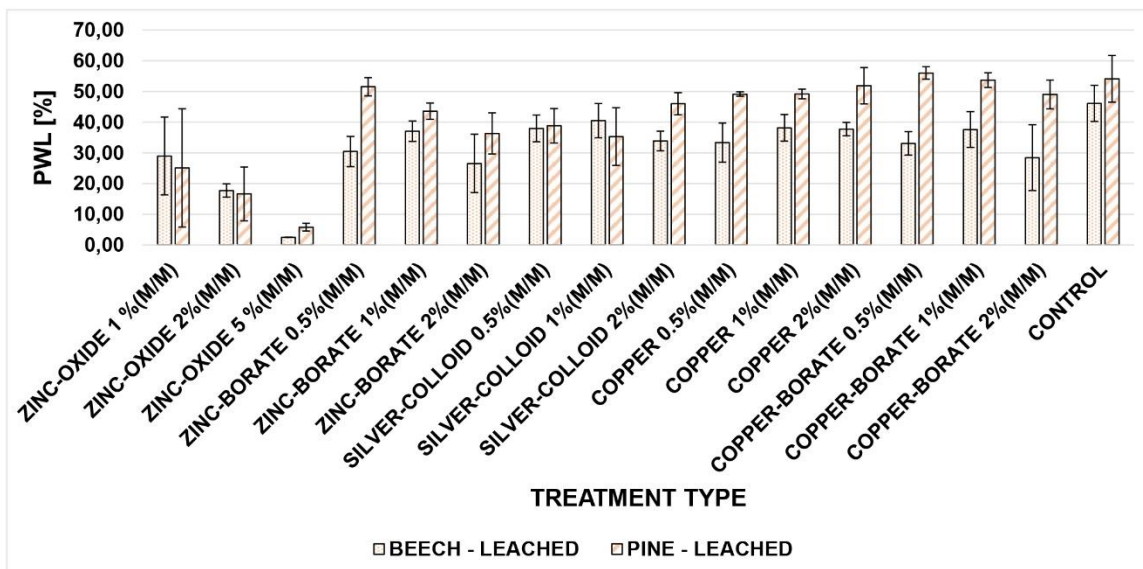


Fig. 3. PWL values of the nanoparticle-treated and control samples of the beech and pine sapwood after the leaching procedure caused by the brown rot fungus *Coniophora puteana*

The copper nanoparticle-treated samples showed similar PWL values compared with that of the control for *Coniophora puteana*; meanwhile, in the case of *Coriolus versicolor*, they only showed a slightly lower PWL at lower concentrations, but a significantly lower PWL at higher concentrations. These results were identical with the PWL results of the non-leached samples, which indicated a strong leaching resistance of

the copper nanoparticles. With the result of the reverse proportionality between the PWL and nanoparticle concentration, this showed that it might be possible to reach strong protection levels using higher concentrations of copper nanoparticles. In spite of the results of the recent study, Pařil *et al.* (2017) reported low fixation of copper nanoparticles that led to remove of their substantial part from wood.

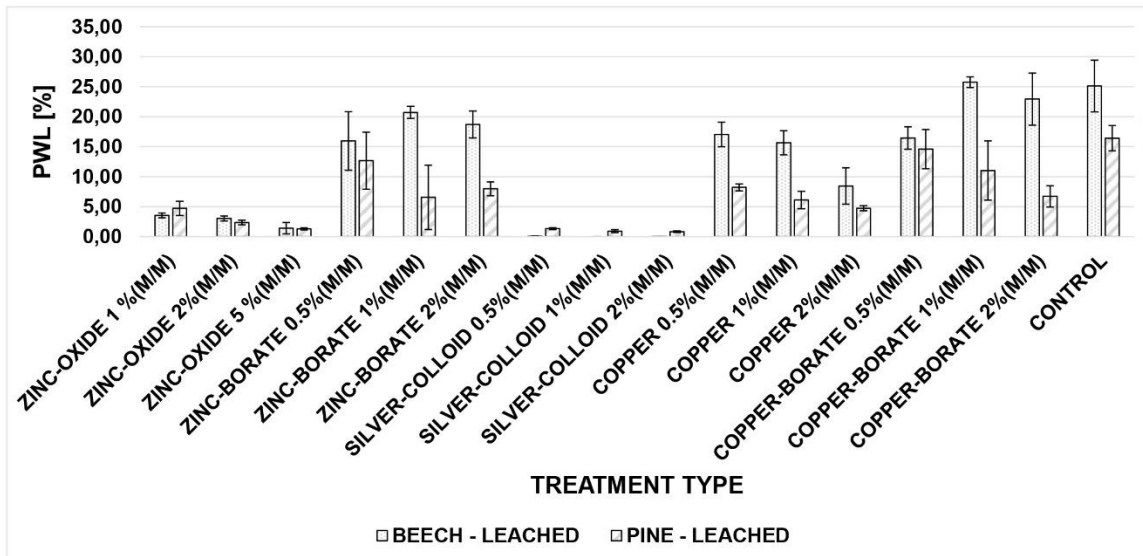


Fig. 4. PWL values of the nanoparticle-treated and control samples of the beech and pine sapwood after the leaching procedure caused by the white rot fungus *Coriolus versicolor*

The silver nanoparticle-treated samples showed a strong resistance to leaching, as the PWL values were identical to the results of the samples without leaching for all of the variations in the wood and fungi species. Unfortunately, this treatment was only effective against *Coriolus versicolor* and it only showed a slight efficiency against *Coniophora puteana*, as this fungus showed a high tolerance against silver nanocubes during this test. Pařil *et al.* (2017) reported better leaching resistance of silver nanoparticles, compared to copper nanoparticles, beside that silver nanoparticles only reduced fungal activity in case of brown rot fungus and inhibited it in case of white-rot fungus. In this study, leaching resistance of silver and copper nanoparticles were found to be similar.

The zinc-oxide nanoparticles showed remarkable resistance against leaching as well. However, there was an increase in the PWL for all of the variations in the wood and fungi species. The treatment remained effective against *Coniophora puteana* only at the highest investigated concentration (5% m/m). Against *Coriolus versicolor*, there was only a slight increase in the PWL values as a result of leaching. This indicated the higher tolerance of *Coniophora puteana* against the zinc-oxide nanoparticles compared with that of *Coriolus versicolor*, which was similar to the results of the silver nanocubes and copper nanoparticles.

However, higher retention values were found for the pine sapwood for all of the sample groups (Table 1) compared with those of the beech sapwood. Also, the pine sapwood did not show lower PWL values in all of the cases. In general, the PWL was lower for the beech with *Coniophora puteana*, and for the pine with *Coriolus versicolor*.

Earlier studies have shown the low resistance of nanoparticle treatments against leaching as well. After leaching the efficiency of treatment was usually significantly

reduced. Especially, decay caused by brown rot fungus to nanoparticle treated specimens is comparable with control specimens (Terzi *et al.* 2016; Pařil *et al.* 2017).

CONCLUSIONS

1. In some cases, one of the investigated fungi showed a significantly higher tolerance to the investigated nanoparticles; thus, these treatments are not recommended for general use, and only for use at higher concentrations (zinc-oxide, silver nanocubes, and copper). The generally effective nanoparticle treatments were the combinations containing borate in its chemical composition, and zinc-oxide.
2. Only the zinc-oxide, copper, and silver nanoparticles showed a remarkable leaching resistance. The zinc-borate and copper-borate nanoparticles provided the greatest efficiency against the decaying fungi, but they were not resistant to leaching.
3. The best results were found with the zinc-oxide nanoparticle treatment, as it showed a low PWL for both fungi and a strong leaching resistance. Also, the copper nanoparticles showed potential as an effective treatment at higher concentrations.
4. The investigated nanoparticle treatments provided greater resistance against *Corioliolus versicolor* compared with *Coniophora puteana*.

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