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Evaluation of mold, decay and termite resistance of pine wood treated with zinc- and copper-based nanocompounds

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ABSTRACT

In this work, the resistance of black pine wood (*Pinus nigra* L.) vacuum-treated with zinc oxide, zinc borate and copper oxide nanoparticles against mold and decay fungi and the subterranean termites was evaluated. Some of the nanocompounds tested were forced with acrylic emulsions to avoid leaching. Results showed that mold fungi were slightly inhibited by nanozinc borate, while the other nanometal preparations did not inhibit mold fungi. Mass loss from fungal attack by *Trametes versicolor* was significantly inhibited by the zinc-based preparations, while the brown-rot fungus, *Tyromyces palustris* was not inhibited by the nanometal treatments. Notably, nanozinc borate plus acrylic emulsion imparted very high resistance in pine wood to the white-rot fungus, *T. versicolor* with a mass loss of 1.8%. Following leaching, all pine specimens treated with nanozinc borate, with or without acrylic emulsion, strongly inhibited termite feeding, i.e. mass losses varying at 5.2–5.4%. In contrast, the copper-based treatments were much less effective against the subterranean termites, *Coptotermes formosanus*. In general, nanozinc borate possessed favorable properties, that is, inhibition of termite feeding and decay by *T. versicolor*.

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1. Introduction

Wood is a heterogeneous and hygroscopic material, which is of lignocellulosic nature and thus is susceptible to biological degradation by fungi, insects and termites. One promising wood protection treatment which has attracted considerable interest from the scientific community during the last decade, is the impregnation of cell wall with nanocompounds (nanometals) with or without emulsions (Clausen, 2007; Matsunaga et al., 2007; Clausen et al., 2010, 2011; Akhtari and Nicholas, 2013; Lykidis et al., 2013). The benefits of applying nanotechnology to wood treatments include the ability of the compounds to have a far greater degree of penetration into the wood (Mantanis and Jones, 2012). It is known that nanoparticles of metals can increase the surface area when evenly dispersed in a layer (Freeman and McIntyre, 2008). In addition, if the particle size is smaller than the diameter of the wood window-like pits (<10,000 nm) or the smallest openings in the bordered pits, i.e. in the margo (400–600 nm) as in the case of pines, complete penetration should be expected and a uniform distribution (Freeman and McIntyre, 2008; Kartal et al., 2009).

In general, copper-, zinc- and silver- based nanocompounds have been used in recent times to enhance the resistance of wood against fungi and termites (Green and Arango, 2007; Cooper and Ung, 2008; Freeman and McIntyre, 2008; Németh et al., 2012; Akhtari and Nicholas, 2013; Lykidis et al., 2013). Clausen et al. (2011) reported a considerable durability enhancement of wood against termites, when impregnated with nanozinc oxide. Németh et al. (2012) showed that spruce, beech and poplar wood impregnated with nanozinc oxide, exhibited a high biological resistance against the brown-rot fungus *Rhodonia placenta*, a particularly tolerant fungus to zinc compounds.

As a matter of fact, nanomaterials possess unique properties and can behave in unpredictable ways (Roco, 2006). Thus, their preparations have several characteristics, e.g. size and charge that may improve their performance in wood protection applications (Clausen, 2007; Mantanis and Jones, 2012). Notably, the commercial use of micronized copper preservatives is currently limited to easily treated pine species because of difficulties in obtaining sufficient penetration in other species. Secondly, the nanoparticles demonstrate high dispersion stability. Fixation of micronized copper is thought to occur mainly through deposition in pit chambers

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and on tertiary cell wall layers rather than via chemical reactions (Freeman and McIntyre, 2008). The addition of a surfactant can further increase dispersion stability by enabling liquid dispersion of higher concentrations of nanometals. Furthermore, the addition of a water-borne acrylic emulsion binder in the compound may increase its affinity to wood polymers and subsequently decrease leaching (Lykidis et al., 2013). In addition, such nanometal preparations have a very low viscosity. Combined, these properties enhance the potential for a much greater penetration into the cell wall as well as a higher protection as a result of the more uniform distribution over the wood surface area.

Nanometal elements like copper and zinc, as well as boron, have played an integral part in the growth of new-generation preservatives responsible for extending the service life of wood products against biological organisms like fungi and termites. The current trend is to diminish or eliminate the use of two common biocides namely arsenic (As) and chromium (Cr) for several wood protection applications because of the potential environmental issues. Copper-based preservatives continue to dominate the market, but even copper as a biocide has come under scrutiny in some countries. Utilizing nanomaterials to create a new generation of novel cost-effective products is a key issue identified by the United States forest products industry (TAPPI, 2005).

The objective of this work was to evaluate the resistance of pine wood vacuum-treated with preparations of zinc- and copper-based nanoparticles, against mold growth, fungal decay by *Trametes versicolor* and *Tyromyces palustris*, and degradation by the subterranean termites, *Coptotermes formosanus*.

2. Materials and methods

2.1. Test chemicals

Chemicals used in the work are shown in Table 1. Three types of nanocompounds were used, namely zinc oxide, zinc borate, and copper oxide, all in combination with two different emulsion binders. The emulsions A and B used are water-borne acrylic polymer emulsions and their characteristics are shown in Table 1. The nanocompounds tested are proprietary formulas, developed by the nanotech company NanoPhos SA (Lavrio, Greece). Two-percent concentrations were prepared based on the metal oxides (i.e., ZnO, CuO). All nanometal preparations were comprised of ∼80 nm particles; their specific size distribution was not available.

2.2. Treatment

A black pine (*Pinus nigra* L.) tree was harvested from a mountainous area of Drama, east Macedonia, Greece, on March 2011, for the research. All test specimens were prepared from the black pine tree's sapwood portions having approx. 5–7 annual rings per cm. The specimens were free of knots and other defects and had no visible signs of infection by wood-destroying organisms. All specimens were pre-weighed and conditioned at 20 °C and 65% RH (relative humidity) for 6 weeks prior to treatment. The average air-dry density of black pine wood was 0.568 g/cm^3. The specimen size varied for leaching, decay, and termite tests according to the AWPA (American Wood Protection Association) E11-97 (AWPA, 2010) and E10-06 methods (AWPA, 2007b), and the Japanese Industrial Standard JIS K 1571 method (JIS, 2010), respectively. Decay and termite specimens were treated with 2% aqueous nanodispersions of test chemicals, in three steps: i) initial 30 min vacuum at 550 mmHg, ii) 5 min vacuum treatment of specimens under vacuum at 550 mmHg, and iii) final 15 min immersion of specimens into the dispersion, under normal climatic conditions. For mold tests, wood specimens were immersed in the nanocompound solutions for a total time of 20 s (ASTM, 1998). Treated specimens were dried at 40 °C for 3 days, weighed, and reconditioned in a conditioning room at 20 °C and 65% RH for two weeks. Differences between the specimen air dry weights (at ∼12% moisture content level), before and after the vacuum treatments were used in order to determine the chemical retention, taking into account the solids content of the nanopreparations.

Table 1
Chemicals tested.

Designation	Test chemical	Type of aqueous acrylic emulsion[a]	Aqueous test solution (%)
A	Zinc oxide	–	2
B	Zinc oxide	Emulsion A	2
C	Zinc oxide	Emulsion B	2
D	Zinc borate	–	2
E	Zinc borate	Emulsion A	2
F	Copper oxide	–	2
G	Copper oxide	Emulsion A	2
H	Copper oxide	Emulsion B	2

Emulsion A: *SurfaPore W* emulsion formulation (NanoPhos SA, Greece)
[a] Emulsion B: Linear acrylic emulsion with TiO_2 (NanoPhos SA, Greece).

2.3. Leaching tests

The leaching procedures were similar to the AWPA standard method E11-97 (AWPA, 2010). One replicate set of five specimens was obtained from each treatment group. Each set of five specimens was placed into a 250 ml bottle, submerged in deionized water, and subjected to a vacuum to impregnate the blocks with the leaching solution. The sample bottles were subjected to mild agitation for a total of 336 h (14 days) and renewed with deionized water. Some unleached and leached wood specimens were ground to pass a 30-mesh screen and analyzed for zinc and copper, as well as boron, with inductively coupled plasma (ICP) emission spectroscopy AWPA A21-08 (AWPA, 2007a) to determine retention of nanometals after the leach test.

2.4. No-choice termite resistance tests

A no-choice termite resistance test with the subterranean termites, *C. formosanus* (Shiraki) was performed. Untreated and treated test specimens (20 × 20 × 10 mm) were exposed to *C. formosanus* worker and soldier termites according to the JIS K 1571 standard method (JIS, 2010). An acrylic cylinder (80 mm diameter, 60 mm height), the lower end of which was sealed with a 5 mm thick hard dental plaster (GC New Plastone, Dental Stone; G-C Dental Industrial Corp., Tokyo, Japan), was used as a container. A test specimen was placed at the centre of the plaster bottom of the test container. A total of 150 worker termites collected from a laboratory colony at RISH, Kyoto University, Japan were introduced into each test container together with 15 termite soldiers. The assembled containers were set on damp cotton pads to supply water to the specimens and kept at 28±2 °C and >85% RH in darkness for 3 weeks. The mass loss of the specimens as a result of termite attack was calculated on the basis of the differences in the initial and final dried (60 °C, 3 days) weights of the specimens after cleaning off the debris from the termite attack. Three leached and unleached specimens were tested for each treatment group. The termite mortality rate was calculated by counting the living termites at the end of the tests.

2.5. Mold resistance test

Unleached wood specimens (7 mm tangential × 20 mm radial ×7 cm long) were evaluated for resistance to mold fungi according to the American Society for Testing and Material D4445-91 (ASTM, 1998). Three mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 were grown and maintained on 2% malt agar (Difco, Detroit, MI, USA) at 27 °C and 80% RH. *A. niger* and *P. chrysogenum* were isolated and identified at the Forest Products Laboratory, Madison, Wisconsin, USA. A mixed spore suspension of the three test fungi were prepared by washing the surface of individual 2-week-old Petri plate cultures with 10–15 ml of sterile deionized water. Washings were combined in a spray bottle and diluted to approximately 100 ml with deionized water to yield approximately 3×10^7 spores ml^{-1}. The spray bottles were adjusted to deliver 1 ml inoculum per spray. Wood specimens (5 specimens per group) were sprayed with 1 ml of mixed mold spore suspension and incubated at 27 °C and 80% RH for 4 weeks. Following incubation, specimens were visually rated on a scale of 0–5, with 0 indicating the specimen is completely free of mold growth, and 5 indicating the specimen is completely covered with mold growth.

2.6. *Decay resistance test*

Black pine sapwood specimens (19 × 19 × 19 mm) were conditioned to 12% equilibrium moisture content and weighed. The specimens were then vacuum-treated as described in Section 2.2 with 2% aqueous solutions of individual test chemicals listed in Table 1. Ten specimens per treatment group were weighed, dried at 25 °C overnight, conditioned for two weeks, and reweighed. Pine specimens were then subjected to *T. versicolor* (L. ex Fr.) Pilát (MAD-697) and *T. palustris* (TYP-6137) in a soil-block test following guidelines of the AWPA standard E10-06 (AWPA, 2007b). Following 12-week incubation, fungal mycelium was brushed from the specimens, specimens were oven dried, reconditioned, and reweighed. Percentage mass losses occurred in the specimens were then calculated.

2.7. *Statistical analysis*

Statistical analysis was conducted using the SPSS program in conjunction with analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to test statistical significance at $\alpha = 0.05$ level.

3. Results and discussion

3.1. *Chemical retention*

Chemical retentions for decay and termite specimens are shown in Table 2. There were significant differences in chemical retention based on specimen size and configuration. For decay resistance test specimens, the highest retention levels were obtained in the treatments with zinc oxide without acrylic emulsion (treatment A) and copper oxide with emulsion B (treatment H). Copper oxide without any emulsion (treatment F) resulted in the lowest retention in treated specimens. In most cases, chemical retention levels were much higher in termite resistance test specimens when compared to decay resistance test specimens. In this group, copper oxide without any emulsion (treatment F) resulted in highest retention in the specimens followed by zinc oxide plus emulsion B (treatment C). The lowest retention value was obtained in the wood specimens treated with zinc oxide without any emulsion. The reason for this phenomenon is not clear but size distribution of nanoparticles used might be effective.

Table 2
Chemical retention.

Treatment group	Retention level (kg m^{-3})[a]			
	Decay test specimens		Termite test specimens	
	Average	S.D.[b]	Average	S.D.
A	7.20	1.10	7.02	1.10
B	5.45	0.40	7.30	0.60
C	5.80	0.40	9.00	0.85
D	5.64	1.20	7.82	0.90
E	6.00	0.72	8.20	0.80
F	4.60	0.50	9.10	0.50
G	5.64	0.42	7.08	0.56
H	7.15	0.70	8.91	0.83

$n = 10$.
[a] Retentions are expressed as differences between *final* and *initial* air dry weights of specimens.
[b] S.D., standard deviation.

3.2. *Leaching*

A summary of the percentage treatment leached in the duration of the leach test is presented in Fig. 1. Nanopreparation of zinc oxide plus emulsion A (Fig. 1B) had a low percentage of leaching (9%), whereas, the same zinc preparations without emulsion and with emulsion B (Fig. 1A and C) showed a moderate percentage leaching (38% and 31%). Similarly, nanozinc borate plus emulsion A (Fig. 1E) considerably resisted leaching, i.e. 8%, as compared with the control nanozinc borate (Fig. 1D) which had a 31% leaching. Consequently, it appears that the addition of emulsion A (Figs. 1B, E) in the two nanometal systems improved dramatically the leach resistance of nanozinc. These findings are consistent with those of Kartal et al. (2009), who found that the addition of a surfactant decreases the leaching of nanozinc. In the nanozinc borate systems, 100% and 87% (Fig. 1D, E) of nanoboron were leached during the course of the leach test. This finding is in agreement with the literature (Kartal et al., 2009; Lykidis et al., 2013) which affirms the high

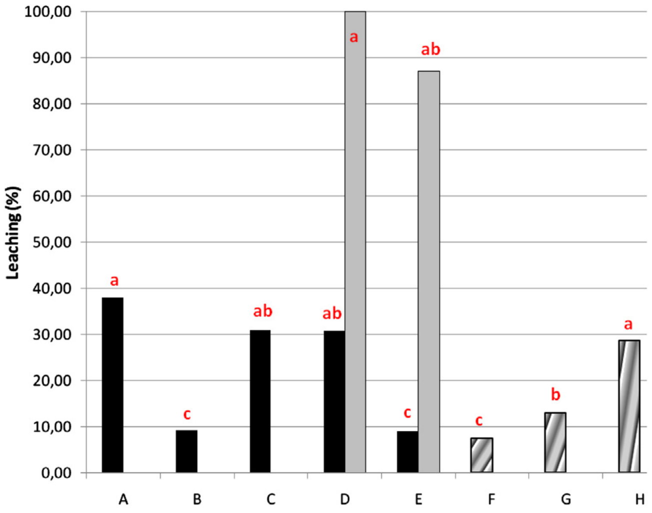

Fig. 1. Summary of percentage treatment leached from black pine wood vacuum-treated with nanometals: (A) nanozinc oxide; (B) nanozinc oxide plus acrylic emulsion A; (C) nanozinc oxide plus acrylic emulsion B; (D) nanozinc borate; (E) nanozinc borate plus acrylic emulsion A; (F) nanocopper oxide; (G) nanocopper oxide plus acrylic emulsion A; (H) nanocopper oxide plus acrylic emulsion B. Bar patterns designate nanometals: solid black, zinc; solid gray, boron; diagonal stripe, copper. The same letters in each bar indicated that there was no statistical difference among the treatment groups according to Duncan's multiple range test.

leachability of boron particles. Nanocopper oxide treated specimens (Fig. 1F) showed the lowest percentage of leaching (7.5%) in this study, while the same copper nanopreparations with the emulsions (Fig. 1G and H) had much higher percentage leaching effects, that is, 13% and 29%, respectively.

3.3. *Termite resistance*

Termite mortality for unleached wood specimens following the 4-week incubation in a no-choice termite test is summarized in Fig. 2. Both nanozinc borate control (Fig. 2D) and nanozinc borate plus emulsion A (Fig. 2E) caused 100% termite mortality, and resulted in considerably low mass losses, i.e. 3.3% and 2.3%, respectively. All nanozinc oxide formulations (Fig. 2A, B, C) caused low mortalities at 10%, 9% and 9%, respectively, while suffered significantly low mass losses at ~4%, 7% and 5%. Notably the three nanocopper oxide treatments (Fig. 2F, G, H) provided much lower protection against subterranean termites, that is, mass losses at 10%, 10.5% and 16% compared to 21% of the untreated controls. Green and Arango (2007) have reported in a recent study that nanocopper provided no protection against the subterranean termite, *Reticulitermes flavipes*.

Mass losses for both leached and unleached wood specimens after the termite test are shown in detail in Fig. 3. All leached and unleached specimens treated with the copper-based solutions and challenged with the subterranean termites, *C. formosanus* had mass losses higher than 10%, varying at 10–16%, compared with 21–23% for the untreated controls (Fig. 3). Although having the highest boron leaching, the two nanozinc borate treatments (Fig. 3D and E) exhibited significantly reduced mass losses (5.2–5.4%) yet in the leached specimens. Similarly low (5.5%) was also the mass loss of the zinc oxide treated specimens (Fig. 3A) in relation to the untreated controls (23%).

3.4. *Mold resistance*

Average ratings of unleached specimens for resistance to the mold fungi are shown in Fig. 4. All nanopreparations used in this work and respective controls (zinc oxide, zinc borate, copper oxide, and untreated) failed to provide adequate protection against mold growth. The control nanozinc borate preparation (Fig. 4D) only slightly inhibited mold growth showing the lowest average rating of 4.0 after 8 weeks of incubation; a rating of 4.0 is equivalent to 80% mold coverage of test specimen surfaces. In general, the nanometal preparations used in the work did not inhibit mold fungi.

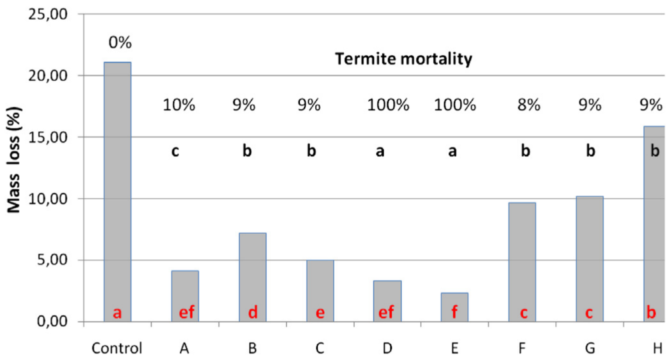

Fig. 2. Mass losses in the unleached specimens after termite resistance tests (% values represent termite mortality at the end of test): (A) nanozinc oxide; (B) nanozinc oxide plus acrylic emulsion A; (C) nanozinc oxide plus acrylic emulsion B; (D) nanozinc borate; (E) nanozinc borate plus acrylic emulsion A; (F) nanocopper oxide; (G) nanocopper oxide plus acrylic emulsion A; (H) nanocopper oxide plus acrylic emulsion B. The same letters in each bar indicated that there was no statistical difference among the treatment groups according to Duncan's multiple range test.

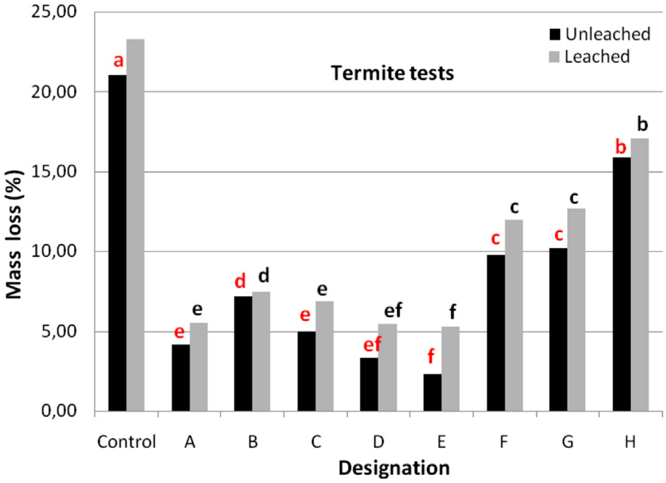

Fig. 3. Mean mass losses following termite resistance tests for unleached and leached treated black pine specimens: (A) nanozinc oxide; (B) nanozinc oxide plus acrylic emulsion A; (C) nanozinc oxide plus acrylic emulsion B; (D) nanozinc borate; (E) nanozinc borate plus acrylic emulsion A; (F) nanocopper oxide; (G) nanocopper oxide plus acrylic emulsion A; (H) nanocopper oxide plus acrylic emulsion B. The same letters in each bar indicated that there was no statistical difference among the treatment groups according to Duncan's multiple range test.

3.5. *Decay resistance*

Mean mass losses following soil-block decay tests for unleached and leached treated pine specimens are shown in Fig. 5. The leaching effect significantly affected the decay resistance against both fungi in the case of specimens treated with nanozinc borate (Fig. 5D, E). Both leached and unleached copper-treated specimens had the highest average mass losses (Fig. 5G, H). Mass loss by *T. versicolor* was significantly inhibited by the zinc-based preparations used. It should be noted that nanozinc borate plus an acrylic emulsion (Fig. 5E) imparted very high resistance in pine wood to the white-rot fungus, *T. versicolor*, i.e. a mass loss of 1.8%. After

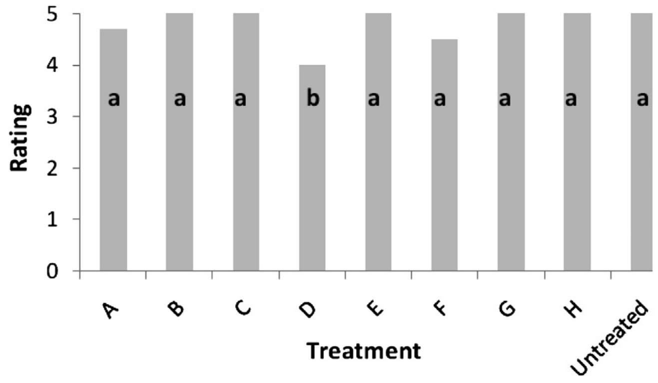

Fig. 4. Average mold ratings for black pine treated with nanometals (after 8 weeks): 0 indicates no mold growth, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% mold coverage. (A) nanozinc oxide; (B) nanozinc oxide plus acrylic emulsion A; (C) nanozinc oxide plus acrylic emulsion B; (D) nanozinc borate; (E) nanozinc borate plus acrylic emulsion A; (F) nanocopper oxide; (G) nanocopper oxide plus acrylic emulsion A; (H) nanocopper oxide plus acrylic emulsion B. The same letters in each bar indicated that there was no statistical difference among the treatment groups according to Duncan's multiple range test.

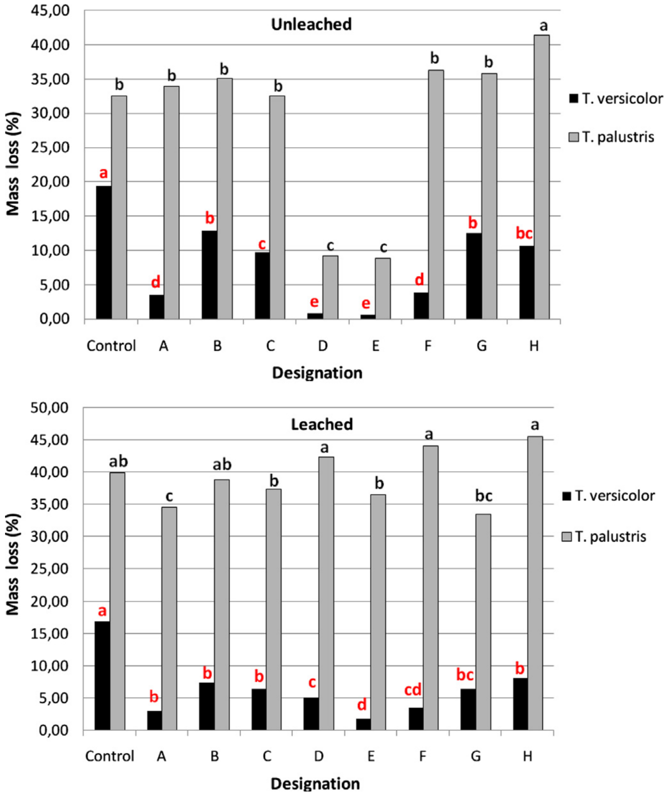

Fig. 5. Mean mass losses following soil-block decay test for unleached and leached treated black pine specimens: (A) nanozinc oxide; (B) nanozinc oxide plus acrylic emulsion A; (C) nanozinc oxide plus acrylic emulsion B; (D) nanozinc borate; (E) nanozinc borate plus acrylic emulsion A; (F) nanocopper oxide; (G) nanocopper oxide plus acrylic emulsion A; (H) nanocopper oxide plus acrylic emulsion B. The same letters in each bar indicated that there was no statistical difference among the treatment groups according to Duncan's multiple range test.

leaching, all pine specimens treated with nanozinc- oxide or borate, with or without acrylic emulsions, strongly inhibited fungal decay of wood, i.e. the mass losses varied at 5.3–7.5%.

Noticeably, the brown-rot fungus *T. palustris* was not inhibited by the nanometal treatments used in the work. This finding is similar with that of Lykidis et al. (2013) relating to the zinc-oxide treatments of wood tested with the brown-rot fungus, *Coniophora puteana*. Only in the unleached specimens (Fig. 5D, E) treated with both nanozinc boron solutions, the mass losses were lower than 10% as compared with 32% mass loss for the untreated controls. This result differs significantly from that of Lykidis et al. who found that pine wood impregnated with nanozinc borate exhibited high resistance against the brown-rot fungus, *C. puteana*. This finding can be explained from the fact that the specimens used in the study of Lykidis et al. were actually unleached, having also higher retention levels, i.e. >17.9 kg/m^3, as compared with those used in this work (i.e. <6.0 kg/m^3).

4. Conclusions

This study reported on the resistance of pine wood vacuum-treated with zinc oxide, zinc borate and copper oxide nanoparticles against mold and decay fungi and the subterranean termites. Results showed that the mold fungi were negligibly inhibited by nanozinc borate, while the other nanometal preparations did not inhibit the mold growth in the specimens. Mass loss by the white-rot fungus, *T. versicolor* was significantly inhibited by the zinc- and copper-based preparations used, while the brown-rot fungus, *T. palustris* was not inhibited by the nanometal treatments used in this work. It should be noted that the very high leach resistance of nanozinc oxide and nanozinc borate, forced with an acrylic emulsion, was a very promising result found in this work. In general, nanozinc borate possessed favorable properties, that is, inhibition of termite feeding and decay by *T. versicolor.*

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