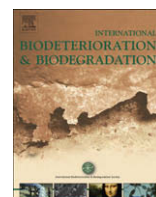




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Do the unique properties of nanometals affect leachability or efficacy against fungi and termites?

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ABSTRACT

Nanotechnology has the potential to affect the field of wood preservation through the creation of new and unique metal biocides with improved properties. This study evaluated leachability and efficacy of southern yellow pine wood treated with copper, zinc, or boron nanoparticles against mould fungi, decay fungi, and Eastern subterranean termites. Results showed that nanocopper with and without surfactant, nanozinc, and nanozinc plus silver with surfactant resisted leaching compared with metal oxide controls. Nearly all nanoboron and boric acid was released from the treated wood specimens during leaching. Mould fungi were moderately inhibited by nanozinc oxide with surfactant, but the other nanometal preparations did not significantly inhibit mould fungi. Mass loss from *Gloeophyllum trabeum* was significantly inhibited by all copper preparations, while *Antrodia* sp. was not inhibited by nanometal treatments. Nanometals imparted high resistance in southern yellow pine to the white-rot fungus, *Trametes versicolor*. Unleached specimens treated with nanoboron or nanozinc plus surfactant caused 100% and 31% mortality, respectively. All specimens treated with nanozinc or nanozinc plus silver inhibited termite feeding, but the copper treatments were less effective against termites. Nanozinc possessed the most favorable properties: leach resistance, termite mortality, and inhibition of termite feeding and decay by the white-rot fungus.

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1. Introduction

Nanotechnology has been defined as development and application of materials, devices and systems in the size range of 1–100 nm with fundamentally new properties and functions because of their structure (Siegel et al., 1999). Nanomaterials possess unique properties and may behave in unpredictable ways (Roco, 2006). Indeed, nanometal characteristics may be totally different from the characteristics of the elemental metals and may, in turn, potentially perform in an unusual manner. Nanometal preparations have several characteristics (e.g. size and charge), that may improve their performance in wood protection applications (Clausen, 2007). First, nanometals are created with controlled particle size. Precisely controlled particle size in the 1–100 nm range may improve penetrability of chemical into wood relative to micronized formulations. The commercial use of micronized copper preservatives is currently limited to easily treated pine species because of difficulties in obtaining adequate penetration in

other species. A preparation of nanometer metal particles also essentially increases the effective surface area of the metal in an evenly dispersed layer. If the particle size is smaller than the diameter of the wood window pit (<10,000 nm) or the opening of the bordered pit (400–600 nm), complete penetration and uniform distribution would be expected (Freeman and McIntyre, 2008). Secondly, nanoparticles demonstrate high dispersion stability. In a study on microdistribution of a micronized copper wood preservative (10–700 nm), Matsunaga et al. (2007) saw numerous particle deposits of copper in ray tracheids and pit lumens within the wood. They reported that these deposits created a different microdistribution pattern in wood treated with the micronized copper than was observed in wood treated with other copper-based preservatives. Fixation of micronized copper is believed to occur primarily through deposition in pit chambers, and on tertiary cell wall layers rather than via chemical reaction (Freeman and McIntyre, 2008). The addition of a surfactant can further increase dispersion stability by enabling liquid dispersion of higher concentrations of nanometals. Finally, nanometal preparations have a low viscosity. Combined, these properties present the potential for greater penetration and protection from more uniform particulate distribution over a wood surface.

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Table 1
Chemicals tested

Designation	Test chemical ^a	Stock solution before dilution (%)	Aqueous test solution (%) ^b
A	Nanocopper	50 + surfactant	1
B	Nanocopper	10	1
C	CuSO ₄	N/A	1
D	Nanozinc	50 + surfactant	1
E	Nanozinc	10	1
F	Nanozinc and silver	50 + surfactant	1
G	Nanozinc and silver	10	1
H	ZnSO ₄	N/A	1
I	Nanoboron	N/A	1
J	B(OH) ₃	N/A	1

^a CuSO₄, ZnSO₄, and B(OH)₃ contain Cu, Zn, and boron equivalent to the nano counterparts.

^b Nanocopper and nanozinc solution concentrations were prepared based on metal oxides (CuO, ZnO).

Copper, zinc, and boron have played an integral part in the development of wood preservatives responsible for extending the service life of wood products against biodegrading organisms such as fungi and termites. The current trend is to reduce or eliminate the use of two common co-biocides, arsenic and chromium, for many wood protection applications because of 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 U.S. forest products industry (TAPPI, 2005). If nanoparticles of copper, zinc, or boron demonstrate unusual properties, they may be important in the development of the next generation of wood protection systems. The objective of this study was to evaluate preparations of 30-nm copper, zinc, and boron particles for leachability and the capacity to prevent mould growth, decay, and degradation by Eastern subterranean termites (*Reticulitermes flavipes* Kollar). This is the first study to examine resistance to leaching, mould fungi, decay fungi, and termites of nano-preparations of copper, zinc, and boron.

2. Materials and methods

2.1. Test chemicals

Chemicals used in the study are described in Table 1. Preparations comprised of 10% and 50% nanocopper (NanoArc), or nanozinc (NanoTek) were obtained from Nanophase Technologies Corporation, Romeoville, IL, USA. Fifty-percent solutions contained a surfactant to facilitate particle dispersion. One-percent final concentrations of the 50% stock solutions of those metals evaluated the effect of the surfactant compared to 1% final concentration of the 10% stock solutions that did not contain surfactant. The 1% solution concentrations were prepared based on the metal oxides (i.e. CuO, ZnO). Nanoboron solution was prepared from crystalline boron oxide nanopowder (American Elements, Los Angeles, CA, USA). All nanometal preparations were comprised of 30-nm particles. Copper sulfate and zinc sulfate were obtained from Mallinckrodt Chemicals Inc., St. Louis, MO, USA. Boric acid was obtained from National Borax, Cleveland, OH, USA.

2.2. Treatment

Test specimens were prepared from sapwood portions of southern yellow pine (SYP) for termite, mould and brown-rot decay

tests. For the white-rot decay tests, sweetgum sapwood specimens were used. All specimens were pre-weighed and conditioned at 20 °C and 65% RH (relative humidity) for 2 weeks prior to treatment. Specimen size varied for leaching, termite, mould, and decay tests according to American Wood Protection Association (AWPA) and American Standard for Testing and Material (ASTM) standard methods. Specimens were vacuum-treated (45 min vacuum at 550 mmHg) with 1% aqueous solutions of test chemicals. One-percent solution concentrations for treatment were based on the metal oxides (i.e. CuO, ZnO). Positive controls (CuSO₄ and ZnSO₄) were prepared to contain equivalent amounts of Cu and Zn for direct comparison with nanometals. Treated specimens were dried at 40 °C for 3 days, weighed, and reconditioned at 27 °C and 70% RH for 2 weeks. Some treated specimens were ground to pass a 30-mesh screen and analyzed for copper, zinc, or boron with inductively coupled plasma (ICP) emission spectroscopy (AWPA, 2007a) to determine chemical retention.

2.3. Chemical leaching

Leaching procedures were similar to AWPA E11-06 standard method (AWPA, 2007b). After conditioning, five specimens were placed into a 500-ml bottle, submerged in 100 ml of deionized (DI) water, and subjected to a vacuum to impregnate the specimens with the leaching solution. The sample bottles were then subjected to mild agitation for a total of 14 days, and leachates were collected after 6 h, and thereafter at 1-, 2-, 4-, 6-, 8-, 10-, 12-, and 14-day intervals. Leachates were analyzed for copper, boron, or zinc with ICP and expressed as mg g⁻¹ element in the wood compared to original retention.

2.4. Termite test

A no-choice termite resistance test with *Reticulitermes flavipes* Kollar (Eastern subterranean termites) was performed using five unleached test specimens (25 × 25 × 5 mm) for each treatment group. Termites were collected from Janesville, WI, USA. One specimen was placed in the bottom of an acrylic cylindrical container (90 mm diameter and 60 mm tall) with 1 g of *R. flavipes* and moist sand. The containers were maintained at 27 °C and 85% RH for 4 weeks based on AWPA E1-06 standard method (AWPA, 2007c). Tests were periodically checked for moisture and mortality. At the end of the test, wood specimens were oven dried, reconditioned at 27 °C and 70% RH, and reweighed to calculate mass losses. The termite mortality rate was estimated by visual inspection.

2.5. Mould test

Unleached specimens (7 mm tangential × 20 mm radial by 7 cm long) were evaluated for resistance to mould fungi according to American Society for Testing and Material D4445-91 (ASTM, 1998). Three mould 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. *Aspergillus niger* and *Penicillium chrysogenum* were isolated and identified at Forest Products Laboratory, Madison, WI. A mixed spore suspension of the three test fungi was prepared by washing the surface of individual 2-week-old Petri plate cultures with 10–15 ml of sterile DI water. Washings were combined in a spray bottle and diluted to approximately 100 ml with DI water to yield approximately 3 × 10⁷ spores ml⁻¹. The spray bottle was adjusted to deliver 1 ml inoculum per spray. Treated and untreated wood specimens (5 specimens per treatment group) were sprayed with 1 ml of mixed mould spore suspension and incubated at 27 °C and 80% RH for 4 weeks. Following incubation, specimens were visually

Table 2
Chemical retention

Treatment group	Retention level (kg m ⁻³) ^a			
	Decay specimens		Termite specimens	
	Average	S.D. ^b	Average	S.D.
A	3.93	0.56	6.51	0.11
B	3.87	0.45	6.58	0.09
C	5.52	0.31	6.98	0.08
D	4.61	0.35	6.71	0.12
E	6.14	0.35	6.63	0.13
F	5.52	0.44	6.62	0.07
G	6.39	0.28	6.72	0.15
H	6.59	0.38	6.88	0.09
I	6.93	0.24	7.03	0.09
J	6.81	0.3	6.97	0.07

^a Retentions are expressed as metal oxide.^b S.D., standard deviation.

rated on a scale of 0–5 with 0 indicating the specimen was completely free of mould growth and 5 indicating the specimen was completely covered with mould growth.

2.6. Decay test

Southern yellow pine or sweet gum sapwood specimens (19 × 19 × 19 mm) were conditioned to 6% equilibrium moisture content and weighed. The specimens were then vacuum-treated as described in Section 2.2 with 1% aqueous solutions of individual test chemicals listed in Table 1. Five specimens per treatment group were weighed, dried at 25 °C overnight, conditioned for 2 weeks, and reweighed. Pine specimens were then subjected to *Antrodia* sp. TFFH-294 (formerly *Meruliporia incrassata* (Burk. & Curt.) Murr.) and *Gloeophyllum trabeum* (Pers.: Fries) Murr. (MAD 617), and

sweet gum specimens were subjected to *Trametes versicolor* (L. ex Fr.) Pilat (MAD 697) in a soil-block test following the guidelines of AWP Standard E10-01 (AWPA, 2007d). *Antrodia* was selected because of its high tolerance for copper. Following incubation, fungal mycelium was brushed from the specimens, specimens were oven dried, reconditioned, and reweighed. Percentage mass loss was calculated.

3. Results and discussion

3.1. Chemical retention

Chemical retentions for decay and termite specimens are shown in Table 2. There were notable differences in chemical retention based on specimen size and configuration. For example, thin termite specimens (25 × 25 × 5 mm) had a 40% higher retention of nanocopper and 21% higher retention of CuSO₄ than cubical decay specimens (19 × 19 × 19 mm). This was not true, however, for the zinc formulations. Nanozinc formulations plus surfactant (with and without silver) had higher chemical retentions (31 and 17%, respectively) than nanozinc without surfactant or ZnSO₄. There was no difference in the chemical retention of nanoboron or boric acid based on specimen configuration.

Within the decay group of specimens (19 × 19 × 19 mm), chemical retentions for nanocopper were 30% less than CuSO₄. Retentions were similar for zinc preparations without surfactant and ZnSO₄, but for treatments with surfactant, retentions were 30% and 16% lower than ZnSO₄ for nanozinc and nanozinc with Ag, respectively. Within the termite group of specimens (25 × 25 × 5 mm), chemical retentions were similar for all treatments. Likewise, the standard deviation was notably lower for termite specimens than for decay specimens, presumably because of differences in specimen size and configuration.

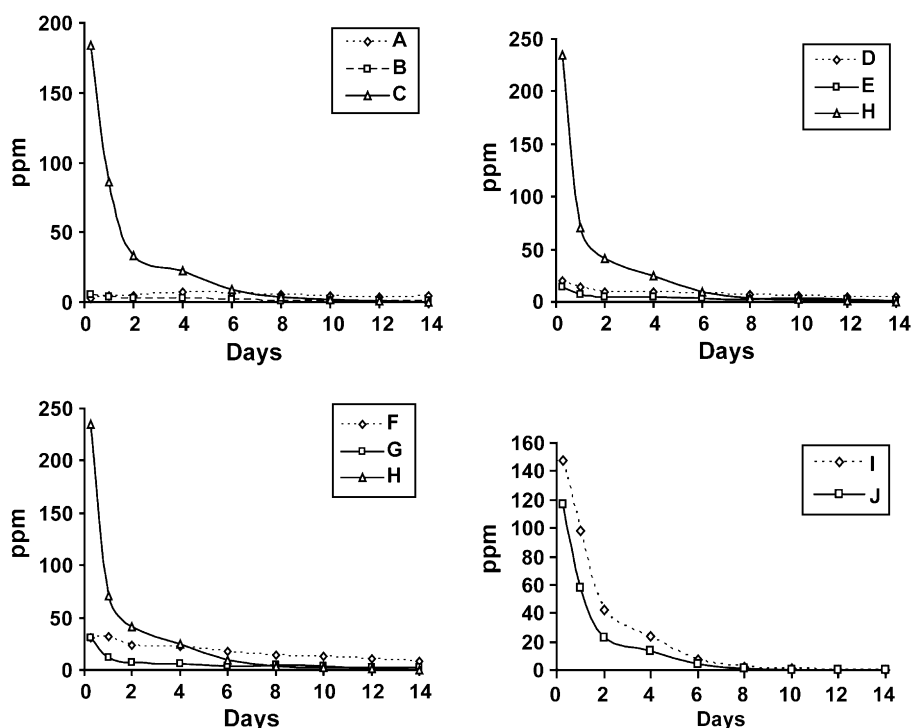


Fig. 1. Leach rate of nanometals from vacuum-treated southern yellow pine: (A) nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc; (H) zinc sulfate; (I) nanoboron; (J) boric acid. Data points represent results for leachates collected after 6 h, 1, 2, 4, 6, 8, 10, 12, and 14 days.

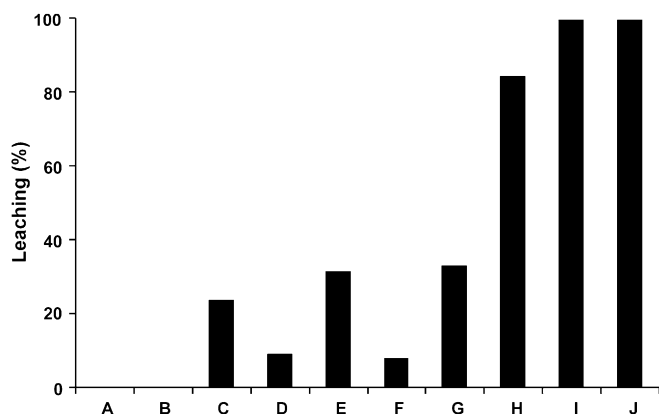


Fig. 2. Summary of percentage treatment leached from southern yellow pine vacuum treated with nanometals and respective controls: (A) nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc/silver; (H) zinc sulfate; (I) nanoboron; (J) boric acid.

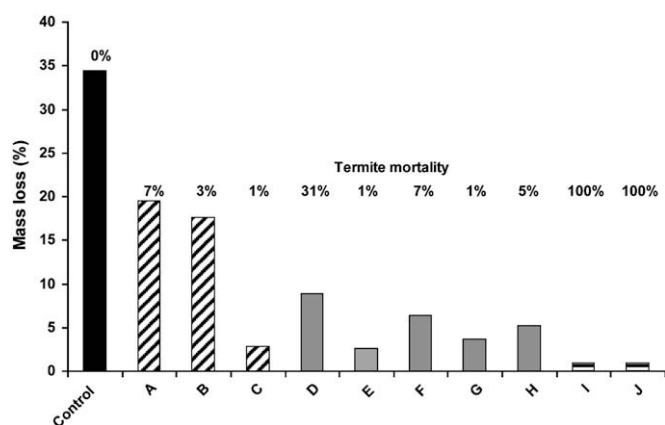


Fig. 3. Mass losses in the unleached specimens after termite resistance tests (% values represent termite mortality at the end of test): (A) nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc/silver; (H) zinc sulfate; (I) nanoboron; (J) boric acid. Bar patterns designate treatment group: diagonal stripe, copper; solid gray, zinc; horizontal stripe, boron.

3.2. Leach results

Leach rates for all test solutions are presented in Fig. 1. Data represent leachates for nine time points for each treatment. Copper in leachates from wood treated with nanocopper was barely detectable, but the rate of leaching for copper sulfate (C) was rapid through day 2. Cooper and Ung (2008) also reported low leach rates for micronized copper with the AWP A E-11 leach test. Nano preparations of zinc (Fig. 1D and E) and zinc with silver (Fig. 1F and G) showed low leaching of the nanometals compared with the rate of leaching for zinc sulfate (Fig. 1H), which was rapid through day 2. Changes in charge and Van der Waals forces may account for the low leaching of nanometals. The rate of leaching for both nanoboron (Fig. 1I) and boric acid (Fig. 1J) treatments were similar and rapid through day 4, with nanoboron leach rate exceeding that of boric acid until day 6. A summary of the percentage treatment leached for the duration of the leach test is shown in Fig. 2. Nanocopper, both with (Fig. 2A) and without (Fig. 2B) surfactant greatly resisted leaching compared with copper sulfate (24%). Nano preparations of zinc plus surfactant (Fig. 2D) and zinc with silver plus surfactant (Fig. 2F) had a low percentage of leaching (9% and 8%, respectively), whereas the same zinc preparations without surfactant (Fig. 2E and G) showed a moderate percentage leaching (31% and 33%) compared with zinc sulfate (84%). Ninety-nine percent of nanoboron (Fig. 2I) and boric acid (Fig. 2J) leached during the course of this test. Leach resistance is a desirable and unexpected characteristic of the nanocopper and nanozinc preparations. It appears that the addition of a surfactant improved the leach resistance of nanozinc.

3.3. Termite results

Termite mortality for unleached specimens following the 4-week incubation in a no-choice termite test is summarized in Fig. 3. Both nanoboron and boric acid control caused 100% mortality. Nanozinc with surfactant caused moderate mortality at 31% but also suffered the greatest mass loss of any zinc formulation. All remaining treatments resulted in low mortality rates of 1–7%. Mass losses following termite testing and comparative mortality rates are shown in Fig. 3. In a related study, Green and Arango (2007) reported that 0.5% nanozinc with and without silver inhibited termite feeding and caused 70–76% mortality, whereas

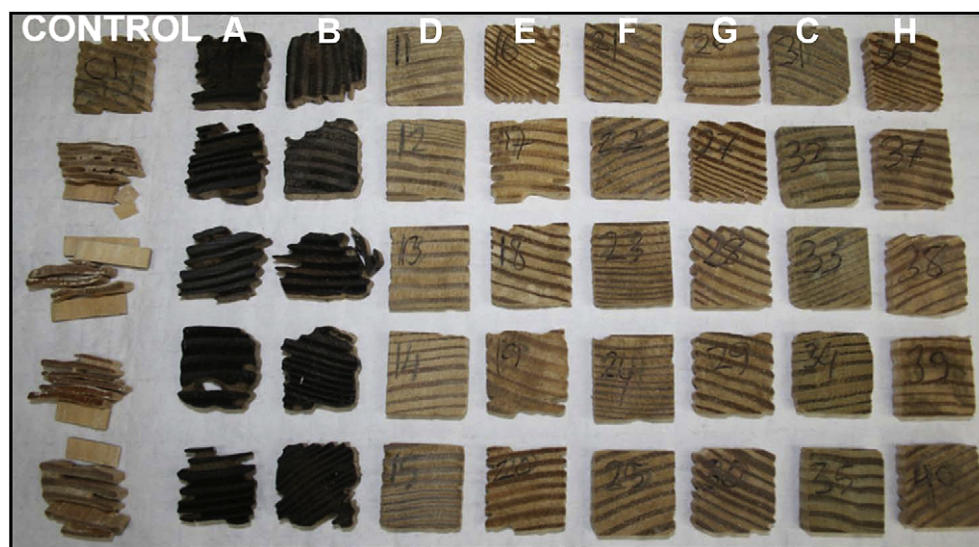


Fig. 4. Appearance of specimens after termite resistance tests: (A) nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc/silver; (H) zinc sulfate.

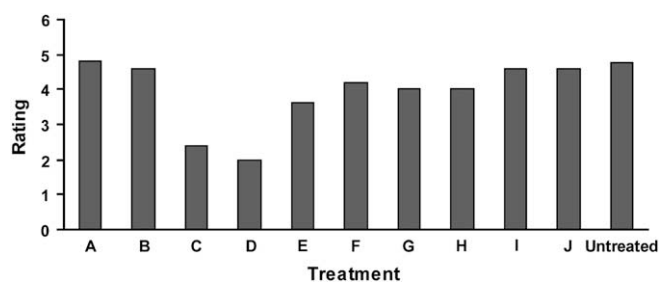


Fig. 5. Average mould ratings for southern yellow pine vacuum treated with nanometals: 0 indicates no mould growth, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% mould coverage. (A) Nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc/silver; (H) zinc sulfate; (I) nanoboron; (J) boric acid.

nanocopper provided no protection against subterranean termites. In this study, mass losses for leached nanoboron and boric acid-treated specimens (40%) were not statistically different than mass losses of untreated control specimens according to Duncan's multiple range test ($P < 0.05$) (data not shown). In other words, because 100% nanoboron and boric acid leached from the treated samples, leached specimens were essentially the same as untreated controls. Therefore, only unleached nanoboron specimens were tested for mould and decay resistance. Mass losses for unleached nanoboron and boric acid specimens were less than 5%. The appearance of treated wood specimens following the laboratory termite test is shown in Fig. 4A–H. In general, mass losses decrease from left to right.

3.4. Mould results

Average ratings of unleached specimens for resistance to mould fungi are shown in Fig. 5. All nanometal preparations and respective controls (copper sulfate, zinc sulfate, boric acid, and untreated) failed to provide adequate protection against mould growth. The nanozinc preparation with surfactant had the lowest average rating of 2.0 after 6 weeks of incubation; a rating of 2.0 is equivalent to 40% mould coverage of test specimen surfaces.

3.5. Decay results

For brown-rot decay fungi, copper-treated specimens exposed to *Antrodia* sp. showed high mass losses (19–33%). Unleached copper sulfate-treated specimens had the lowest average mass loss at 19%. For *Antrodia* sp., there was no evidence that resistance to leaching of nanocopper or nanozinc improved decay resistance. Mass loss was greatly reduced in specimens treated with nanocopper or copper sulfate (3–15%) that were exposed to *G. trabeum* compared with untreated controls (65%) (Fig. 6); i.e. nanocopper formulations were efficacious against *G. trabeum*. Except for the leached copper sulfate-treated specimens (15%), all mass losses were <10% for all copper solutions challenged with *G. trabeum*, indicating copper sensitivity. All leached and unleached specimens treated with copper solutions and challenged with the white-rot fungus *T. versicolor* had mass losses less than 10% compared with 31% for untreated controls (Fig. 6). It does not appear that the higher chemical retention rate for copper from CuSO_4 affected the decay results.

Brown-rot decay results for protection of SYP treated with nanozinc solutions, both with and without silver and zinc oxide were not notably different from untreated controls. Zinc-treated specimens exposed to the white-rot fungus had significantly reduced mass losses (4–21%) compared to the untreated controls (31%). Only specimens treated with nanozinc without surfactant,

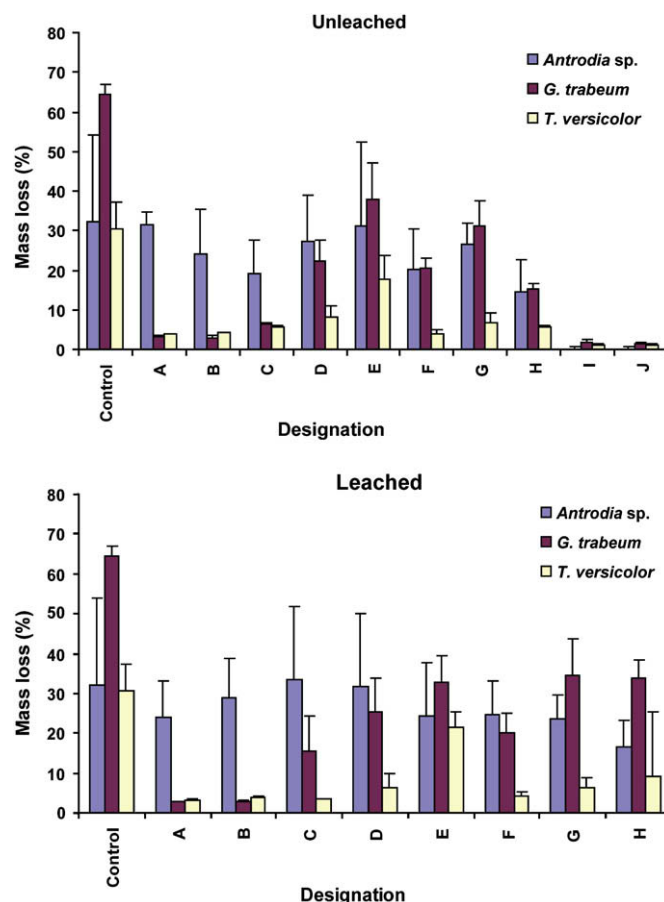


Fig. 6. Mean mass losses following soil block decay test for unleached and leached treated southern pine or sweet gum specimens; (A) nanocopper plus surfactant; (B) nanocopper; (C) copper sulfate; (D) nanozinc plus surfactant; (E) nanozinc; (F) nanozinc/silver plus surfactant; (G) nanozinc/silver; (H) zinc sulfate; (I) nanoboron; (J) boric acid.

both leached and unleached, had an average mass loss greater than 10% (Fig. 6), suggesting that the surfactant in the nanozinc solution may possess antifungal properties. Recall that nanozinc formulations with surfactant also had lower chemical retentions than those without surfactant. Because of rapid leaching of nanoboron and boric acid, only unleached specimens were tested for decay resistance. Decay results showed that both nanoboron and boric acid effectively inhibited all decay fungi (Fig. 6).

4. Conclusions

The nanoform of metals typically used in the wood preservation field were evaluated for leachability and resistance to mould growth and biodegrading organisms. Nanocopper and nanozinc showed favorable leach resistance compared to their soluble metal oxides. Nanozinc also inhibited termite feeding and caused moderate termite mortality. Nanometals evaluated in this study generally inhibited the white-rot test fungus and were approximately as effective as their soluble counterparts at inhibiting the brown-rot test fungi. However, there is some indication that the nanocopper and nanozinc may not have been quite as effective as the soluble forms of metals against brown-rot fungi, and this relationship may need further exploration. None of the chemicals evaluated in this study effectively inhibited the mould test fungi and as such, would be unsuitable for mould protection at 1% concentration. Overall, leach resistance of nanozinc and nanocopper was the most promising and unexpected result. Nanozinc

also possessed other unique properties: increased termite mortality, inhibition of termite feeding and degradation by the white-rot fungus.

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