# Effectiveness of Micronizing Zinc Borate to Improve Its Fungicidal Properties

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The effectiveness was evaluated for an established wood preservative, zinc borate, prepared in an agitating bead mill to extensively reduce its particle size. The generated micro- or nano-particles of zinc borate were characterized by X-ray powder diffraction, and the particle size distribution was determined to evaluate the effect of milling. Then the fungicidal effectiveness of the zinc borate of both milled and unmilled samples were assayed against brown- and white-rot fungi, on culture medium and on conifer and hardwood as substrates. Treated wood samples were subjected to leaching tests. Scanning electron microscopic images of wood samples were examined to analyze the distribution of zinc borate within the wood. The micronized zinc product kept its crystal structure intact, and it increased the proportion of particles with diameters below 100 nm by 25% when compared to the unmilled product. Malt extract-agar medium supplemented with 2.5% of w/w milled and unmilled zinc borate inhibited fungal growth tested. Both milled and unmilled zinc borate protected the wood when not subjected to leaching. The milled sample of zinc borate improved resistance to leaching, which would allow its application in environments of high moisture content; however, it did not improve the fungicidal action against decay fungi.

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## INTRODUCTION

The composition and physicochemical properties of wood make it susceptible to deterioration by microorganisms and abiotic agents. For more than a century, fungicidal and pesticide-based treatments have been used for preventing wood decay.

Among waterborne preservatives, inorganic compounds have shown long-term effectiveness against a broad spectrum of species (Eaton and Hale 1993); zinc, boron, and zinc borate have been widely used (Stirling and Temiz 2014). In particular, zinc borate has been used as an additive for lacquers, paints, polymeric materials, and in flame retardant formulations for wood impregnation. The zinc borate has also been used to improve the properties of wood composites and particleboard against wood-rotting fungi and as a fire retardant (Tascioglu *et al*. 2013, 2014, 2017).

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Within the past decade, numerous products for wood impregnation have been developed, including micronized particles and/or nanoparticles (Freeman and McIntyre 2008; Kartal *et al*. 2009; Clausen *et al*. 2009, 2010; Akhtari and Nicholas 2013; Civardi *et al*. 2015a). Micronized particles are produced by mechanical milling of insoluble metal compounds (copper, zinc, silver *etc.*) to a particle size below 1000 nm in at least 90% of the bulk material, using dispersing and wetting agents in aqueous or oily media. The particle size of nanometric products is below 100 nm in at least one dimension, and their behavior differs markedly from that of their constituent metal elements (European Commission 2011).

The enhanced biocidal effectiveness and leaching resistance of nanoparticles stems from their ability to be transported through the wood ultrastructure, where pore size can be well below 1000 nm. For example, the membrane openings of bordered pits are usually between 400 nm and 600 nm and provide the potential for high metal retention in the wood, therefore resulting in higher durability of wood (Freeman and McIntyre 2008; Kartal *et al*. 2009). Nanometric metals also increase the effective superficial area of the metal in an evenly dispersed layer (Freeman and McIntyre 2008). This nanoparticle film on the wood surface can provide resistance to leaching (Clausen *et al*. 2011). The biocidal profile of nanoparticles against xylophages differs from that induced by the same metals in ionic form. In addition, nano-particulate metals do not induce lignocellulosic enzyme activity (Shah *et al*. 2010).

Zinc- and boron-based nanomaterials dominate the topics of current research in this area. Nano-zinc has been reported to be more resistant to leaching than soluble metal oxides (Kartal *et al.* 2009). Nano-zinc oxide is effective against termites (Clausen *et al.* 2011; Lykidis *et al.* 2016 a, b) and it improves heat transfer within the wood matrix, allowing temperatures to reach 185 °C during heat treatment, thereby increasing resistance to biological attack (Ghorbani-Kookandeh *et al*. 2014). The nano-zinc borate has been shown to be effective against pine decay fungi, brown rot fungi, and termites (Lykidis *et al* 2013, 2016a, 2018).

The effect of micronized copper is well known (Freeman and McIntyre 2008; Civardi *et al*. 2015b); when applied to the wood, one part reacts with its components and the other remains in the form of copper particles that give a reservoir effect (Xue *et al*. 2014). No studies have reported on the use of micrometric zinc, boron, or zinc borate for wood preservation.

Nevertheless, most of the studies available in the literature were carried out using pure, analytical grade materials with known and sometimes uniform particle size distributions. Thus far, few studies have addressed the preservative potential of materials containing both micro- and nano-sized particles.

Therefore, the objective of this study was to obtain a readily available micronized zinc borate to be used as wood preservative. After milling the product as to improve its penetration in wood, and the characteristics of the milled product as wood preservative were compared to those of the unmilled one. It was hypothesized that an increase in the content of nanometric fraction of particles in the preservative would increase the effectiveness against decay fungi and leaching resistance. Both milled and unmilled products were assayed for their ability to protect *Pinus taeda* L., *Eucalyptus grandis* Hill ex Maiden and *Populus sp*. L. wood against white and brown rot fungi. The leaching resistance of the impregnated products was also tested.

## EXPERIMENTAL

### Preparation and Characterization of Milled Zinc Borate

Zinc borate, produced from commodities in a low-cost process, without using pressure or high temperature, was purchased from Perrín S. A. (Montevideo, Uruguay). A 34% (w/w) zinc borate suspension in water was prepared, to which traces of dispersant, moistener, surfactant, and defoamer were added (0.01 %; w/w) (Hydropalat 3204, 1080, from BASF Corp., Ludwigshafen, Germany; Bentone LT, Elementis Specialties, London). The mixture was then fed to a continuously agitating bead mill designed by Pinturas Inca, S. A. (Montevideo, Uruguay). The agitating period was of nine hours. Zirconium beads 1.2 mm in diameter were used as the milling medium.

X-ray powder diffraction (XRPD) data was collected for the milled and unmilled zinc borate on a Rigaku Ultima IV (285 mm radius; Tokyo, Japan) powder diffractometer operating in Bragg-Brentano geometry. Monochromatized CuKα radiation ($\lambda = 1.5418$ Å) with a diffracted beam from a bent germanium crystal was used to collect data over the $2\theta$ range of 10° to 60° at a scanning rate (step size) of 0.02°, using a scintillation detector.

A Coulter LS 230 (Beckman Coulter, Inc., Brea, CA, USA) particle size analyzer was used to compare the particle size distribution of the milled and unmilled zinc borate samples, using water as fluid, at a run length of 90 s and an obscuration of 11%. Particle morphology was assessed by scanning electron microscopy (SEM) with a JEOL JCM-6000: Neoscope II Benchtop (JEOL USA Inc., Peabody, MA, USA), used in high vacuum mode with an accelerating voltage of 10 kV and SE detector.

The particle size distribution was determined for each sample by a laser diffraction pattern and mathematical particle volume calculation, considering the particles as non-porous spheres and using XRPD data as diameter measurements.

### Fungus Growth on Zinc Borate-Supplemented Culture Medium

Three species of white rot fungi [*Phanerochaete chrysosporium* (1556, DZM), *Punctularia atropurpurascens* (H2126 CCMFQ), and *Trametes versicolor* (H2140 CCMFQ)] and one species of brown rot fungi [*Gloeophyllum trabeum* (H2130 CCMFQ)] were obtained from the fungal collection of Cátedra de Microbiología, Facultad de Química, Universidad de la República, Uruguay.

The strains were maintained on a medium containing malt extract (12.5 g/L) and agar (20 g/L) (MA, malt extract-agar medium), sterilized by autoclaving at 121 °C for 15 min. Malt extract and agar were purchased from Oxoid Ltd. (Basingstoke, UK).

Plastic Petri dishes (85 mm inner diameter) containing MA, with and without zinc borate, were inoculated with a mycelial plug (5 mm diameter) obtained from actively growing mycelia. Dishes were incubated at 28 °C for 21 days.

The MA was supplemented with milled and unmilled zinc borate, respectively, to final concentrations of 2.50, 1.25, 1.00, 0.65, and 0.01 % (w/w) in each case. The solid was added while the medium was still liquid. The minimum growth inhibitory concentration (MIC) was defined as the lowest concentration at which growth inhibition was total (Borokhov and Rothenburger 2000). Mycelial growth was assessed according to the size (diameter measured in mm) of fungal colonies grown in micronized zinc borate-supplemented MA (Hatvani and Mécs 2003). Unsupplemented MA was used as the control medium.

For each species, three replicate samples were collected for each concentration of zinc borate in the culture medium (including three control samples). Initial growth rates (mm/d) were calculated according to incubation data from days 1 to 10.

## Specimens Preparation

Blocks of *Eucalyptus grandis*, *Populus sp.*, and *Pinus taeda* mature sapwood (50 mm [L] × 25 mm [T] × 15 mm [R], grain direction parallel to the 50 mm edge) free of defects and with no visible evidence of fungal infection were prepared according to EN 113 (1996). The blocks were oven-dried at $103 \pm 2$ °C for 48 h and weighed (to nearest 0.001 g) for initial dry weight determinations. Test samples were selected so that their density did not differ from the mean oven-dried density value by over 15%: 0.44 $g/cm^3$ for *P. taeda*, 0.49 $g/cm^3$ for *E. grandis*, and 0.41 $g/cm^3$ for *Populus sp.*

All blocks were maintained at 20 °C and 65% relative humidity (RH) until equilibrium was reached at a moisture content of approximately 12%. Untreated blocks used as controls were not treated further.

## Wood Protecting Action and Leaching of Milled and Unmilled Zinc Borate Impregnated in Wood Substrates

Impregnation of wood blocks (30 of each wood species) was carried out using a zinc borate water suspension at two concentrations: 2.5% and 5.0% total solids, for both milled and unmilled products. The impregnation method was vacuum-to-vacuum. Samples were maintained in desiccators under vacuum (4 kPa) for 30 min prior to allowing zinc borate suspension into the desiccators. Samples remained submerged for an additional one hour. Zinc borate retained in samples after impregnation was then determined through the mass increase of each block (when compared to initial dry mass) per unit of wood volume. Treated and control samples were stored at $60 \pm 2$ °C for 36 h to provide same environment and to allow fixation.

Half of the impregnated samples (15 blocks of each wood species) were subjected to leaching tests according to EN 84 (1997), which is an accelerated ageing test for samples treated with a preservative simulating long-term use condition. After the samples were maintained under vacuum (4 kPa) for 20 min, five times their volume of deionized water was allowed into the desiccator, until atmospheric pressure was reached. Then the samples were soaked for 2 h and finally maintained in fresh deionized water, changed daily, for 14 days.

Samples were tested for resistance to fungal decay according to standard method EN 113 (1996). *T. versicolor* and *G. trabeum* were inoculated onto solid MA medium in 600 mL flasks. When mycelial growth covered the flask bottom (c. 3 weeks culture), one control and two treated samples (previously sterilized at 121 °C for 15 min) were placed in each flask. The flasks were subsequently incubated in the dark at $23 \pm 2$ °C and 60% to 70% RH. Ten samples treated of each concentration were cultured as control without fungus.

After 16 weeks, the samples were oven-dried at $102 \pm 2$ °C and weighed for final dry weight determination. Fungicidal effectiveness was assessed in terms of dry mass weight loss percentage using Eq. 1,

$$\text{WL (\%)} = (w_1 - w_4) *100/w_1 \tag{1}$$

where $w_1$ is initial dry weight of the samples, and $w_4$ is the final dry weight of the samples after culture.

The distribution of impregnated zinc borate inside wood structure, was assessed using SEM images of treated and control wood samples. The SEM analysis was used in high vacuum mode at accelerating voltage of 10 kV and SE detector.

## Analysis of Boron and Zinc

*Acid digestion*

Five blocks of each wood species from each treatment were milled together in a horizontal blade mill (Marconi Modelo MA 680, San Pablo, Brazil) and sieved through a 30-mesh screen before being digested (Kurz *et al*. 1973; Hu and Qi 2014). The amounts of zinc and boron in the samples were determined by flame atomic absorption spectrometry and electrochemical techniques, respectively (Anzano and González 2000).

The digestion procedure was based on a modified method of AWPA A7-12 (2014). As microwave was not used for digestion, a previous assay was made in which wood samples were digested in different acidic mixtures ($HNO_3$-HCl, $HNO_3$-HCl, $HNO_3$-HCl-$H_2O_2$), selecting the one that allowed the highest zinc recovery.

About 0.5 mg of the milled wood sample was weighed and transferred to a flask and then heated in a stirring water bath, adding $HNO_3$ (65%, w/w) for *Eucalyptus*, and $HNO_3$ (65%, w/w) and HCl (35%, w/w), 50:50 for *Pinu*s until the temperature reached 80 °C, and then left for 120 min (Ramanathan and Ting 2015). It was later allowed to cool, filtered through a 0.45 mm filter, and made up to a volume of 100 mL with distilled water (Wilson 1958; Dawson *et al*. 1990).

*Quantification of boron and zinc elements*

The zinc (Zn) concentration was determined by using an atomic absorption spectrometer equipped with a 900T PinAAcle flame oven (Perkin Elmer, Kentucky, USA) (Anzano and González 2000), while the boron (B) concentration was determined by a potentiometric method, in which a complex between boric acid (or borates present in the sample) and mannitol (Dawson *et al.* 1990) was formed. This complex acidified the solution and was titrated using NaOH (0.025 M) solution. The valoration was made with a glass electrode and a Thermo Scientific Orion Stara 3215 (USA) potentiometer. The amount of B was equivalent to the amount of NaOH required to reach the initial pH of the sample (Wilson 1958).

## Statistical Analysis

Results were analyzed by STATISTICA software (version 7.1 2005, Statsoft Inc., Tulsa, OK, USA). Duncan´s test was used to assess the significant differences among treatments, with 95% confidence level.

## RESULTS AND DISCUSSION

## Characterization of Milled Zinc Borate

As homogeneous particle size distribution and the ability to penetrate the ultrastructure of wood are essential for the preservative effectiveness (Eaton and Hale 1993), prior to testing for fungicide effectiveness, the milled and unmilled zinc borate samples were analyzed in order to verify the effect of milling on the preservative effectiveness.

The XRPD analysis (Fig. 1) showed that both milled and unmilled zinc borate presented a $Zn_4O(BO_3)_2$ crystal structure. This crystal phase is described as a rhombohedral crystal system with space group R-3c, with a = 9.9115Å and c = 48.602Å according to Harrison *et al*. (1993).

There was no significant difference in either peak position or intensity between the milled and unmilled zinc borate samples. Analysis of full width at half maximum (FWHM) shows that the size of the crystalline domain is around 100 nm.
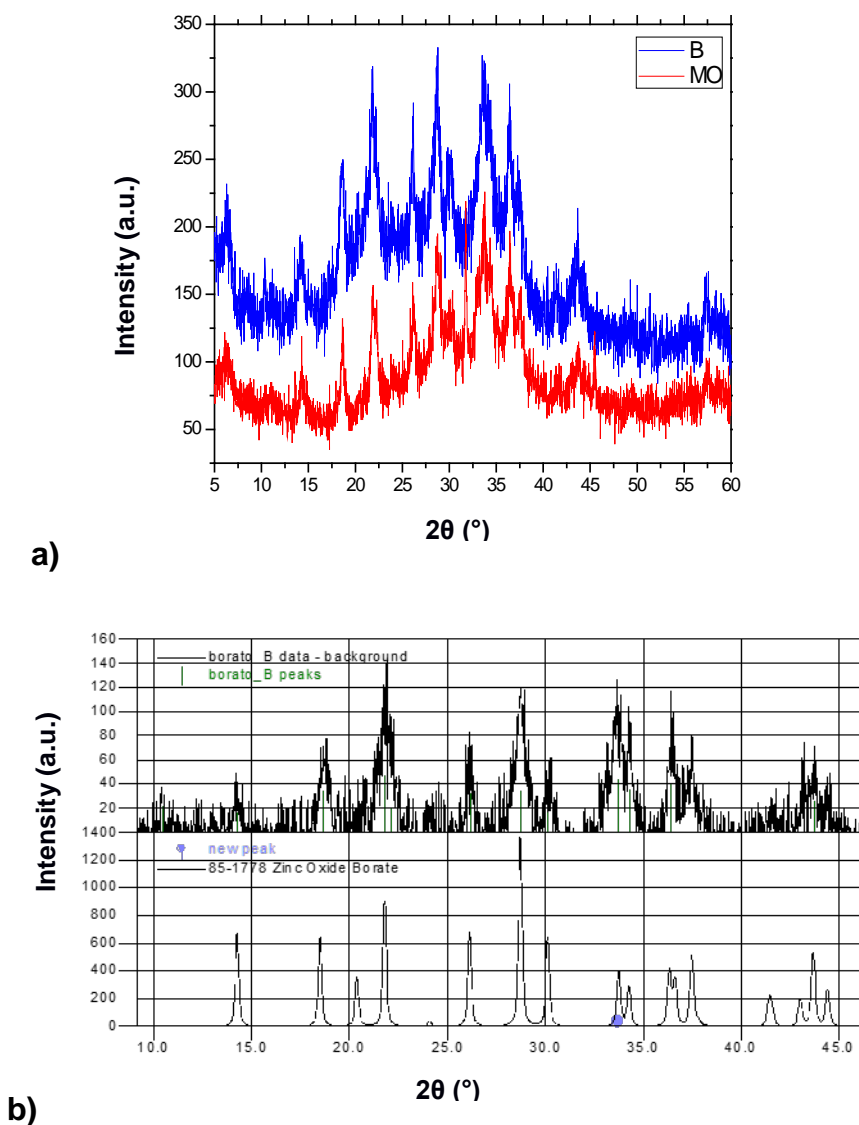


**a)**



**b)**

**Fig. 1.** a) X-ray powder diffraction analysis of milled and unmilled zinc borate; and b) peak details.

Milling effectiveness was assessed according to the particle size distribution of the milled zinc borate, which in turn reflects the percentage of particles that can penetrate and be transported throughout the wood matrix. Figure 2 shows the particle volume distribution of the suspension. Milling was found to increase by less than 25% the proportion of smallest-sized particles with diameters below 100 nm. This demonstrated that the nanometric content varied very little between the milled and unmilled products. However,

the milling decreased the fraction of particles larger than 250 nm (0.25 µm), as well as increased the fraction of particles sized between 150 nm and 250 nm (0.15 µm and 0.25 µm). Therefore, the milled zinc borate should be capable of penetrating the nanocapillary network of the 1 µm to 10 µm thick lignified cell wall (Fujino and Itoh 1998), in which the membrane openings in a bordered pit are typically 400 nm to 600 nm, and the window pit diameter is typically of 10,000 nm. It should be noted that the milled sample of zinc borate does not include particles larger than 25,000 nm, which would occlude some mesopores and clog tracheids, preventing the preservative from penetrating the wood (Freeman and McIntyre 2008). At the same time the product adherence to the cell wall caused increased leaching resistance, despite the improved distribution of particles and penetration into wood associated with particle diameters below 1000 nm (Zhang *et al*. 2012).
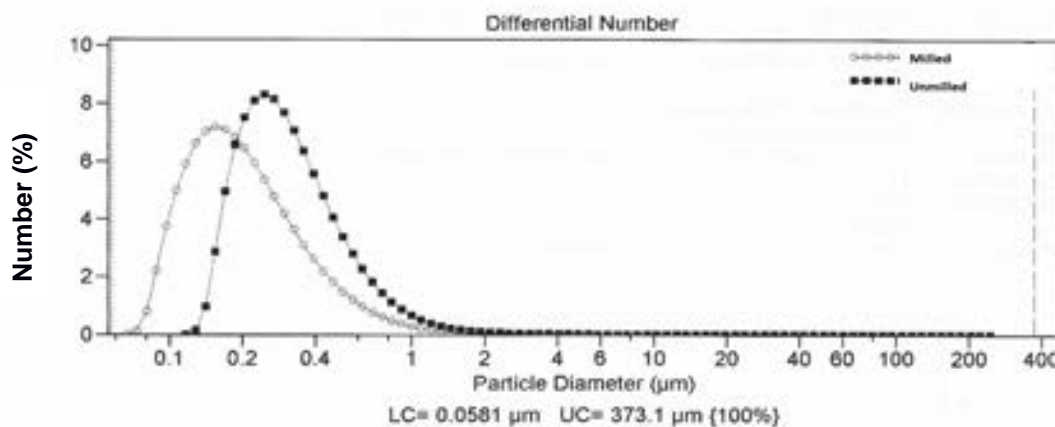


**Fig. 2**. Particle volume distribution of unmilled (black squares) and milled (white circles) zinc borate

The SEM images of zinc borate particles (Fig. 3) indicated no changes in the appearance of the solids (aside from particle size) once zinc borate was milled, despite the temperature rise during the milling process. At the same time, it also showed how unmilled solid particles tend to join together, which does occur to a lesser extent with the milled solid, perhaps due to the addition of milling dispersants.
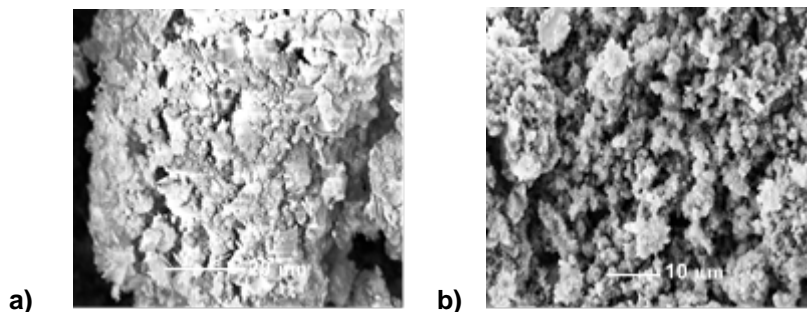


**Fig. 3.** SEM images of zinc borate particles: a) unmilled; and b) milled.

## Fungus Growth on Zinc Borate-supplemented Culture Medium

Zinc borate suspensions were prepared to determine the fungicidal effectiveness (on culture medium and wood) for milled and unmilled products, respectively, at a pH of

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8.32 and 8.22, and an average decanting time of 28:19 min and 38:11 min. In order to maintain its homogeneity and low decanting time, the suspensions were kept in agitation mode.

The effect of milled and unmilled zinc borate samples on the initial growth rate of three white-rot and one brown-rot fungal species was studied. Figure 4 shows that the MIC value for the assayed fungus. The brown-rot fungus was the most sensitive —*i.e.*, having the lowest initial growth rate— to milled zinc borate in the culture medium. For *P. atropurpurascens* and *G. trabeum*, the MIC values for milled and unmilled zinc borate samples was 1.25% (w/w), and for *T. versicolor* and *P. chrysosporium* it was 2.5 % (w/w) —*i.e.*, the highest concentration tested. The growth curves were analogous for milled and unmilled products, with no significant differences at a significance level of 0.05. This result showed that the fungicide capacity of zinc borate was not affected by the particle size.
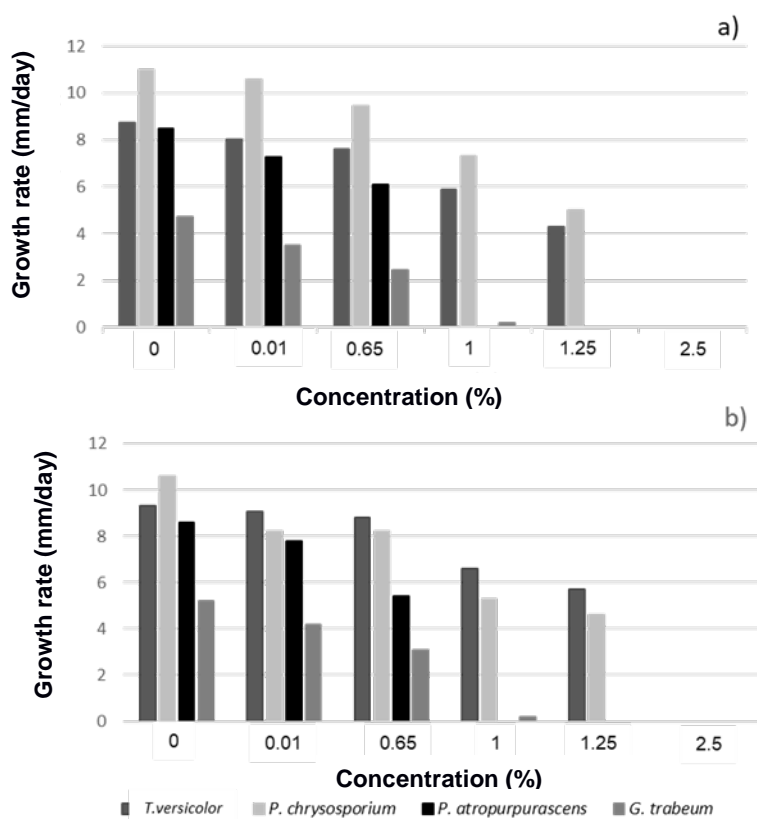


**Fig. 4.** Inhibitory effect of zinc borate on the initial growth rate of white- and brown-rot fungi; initial growth rate of test species on culture medium supplemented with 0, 0.01%, 0.65%, 1.00%, 1.25%, and 2.5% zinc borate: a) unmilled, b) milled

## Wood Protecting Action and Leaching of Milled and Unmilled Zinc Borate Samples Impregnated in Wood Substrates

Table 1 shows the zinc borate retentions in wood and the mass losses of the wood specimens treated with both milled and unmilled zinc borate after being exposed to fungi.

**Table 1.** Weight Loss of Wood Samples Treated with Milled and Unmilled Zinc Borate and Control Against Wood-decaying Fungi

| P. taeda | | Milled Zinc Borate | | | Unmilled Zinc Borate | | Control |
|---|---|---|---|---|---|---|---|
| | | Unleached | Leached | | Unleached | Leached | |
| | Retention | Weight loss | Weight loss | Retention | Weight loss | Weight loss | Weight loss |
| | kg/m$^3$ | % (sd) | % (sd) | kg/m$^3$ | % (sd) | % (sd) | % (sd) |
| T. versicolor | 17.6 | 0.1 (0) | 3.1 (0.7) | 7.7 | 1.3 (0.5) | 5.2 (1.2) | 29.6 (5.2) |
| | 30.2 | 3.2 (1.1) | 0.8 (0.3) | 23.8 | 3.5 (0.7) | 4.8 (1.4) | |
| G. trabeum | 17.6 | 0.2 (0.1) | 1.1 (0.9) | 7.7 | 1.2 (0.3) | 3.1 (0.8) | 24.3 (1.2) |
| | 30.2 | 2 (0.9) | 3.5 (1.9) | 23.8 | 3.5 (1.4) | 4.1 (0.9) | |
| E. grandis | | | | | | | |
| | Retention | Weight loss | Weight loss | Retention | Weight loss | Weight loss | Weight loss |
| | kg/m$^3$ | % (sd) | % (sd) | kg/m$^3$ | % (sd) | % (sd) | % (sd) |
| T. versicolor | 12.1 | 0.2 (0) | 2.2 (1.2) | 4.4 | 3.7 (1.1) | 5.9 (0.5) | 29.6 (3.3) |
| | 22.3 | 4.3 (0.8) | 3.1 (1.8) | 7.6 | 3.9 (0.5) | 6.1 (0.9) | |
| G. trabeum | 12.1 | 0 (0) | 3.5 (1.4) | 4.4 | 2.8 (0.9) | 4.2 (0.9) | 19.9 (3.2) |
| | 22.3 | 0 (0.3) | 3.1 (0.4) | 7.6 | 2.1 (1.1) | 3.9 (0.8) | |
| Populus sp. | | | | | | | |
| | Retention | Weight loss | Weight loss | Retention | Weight loss | Weight loss | Weight loss |
| | kg/m$^3$ | % (sd) | % (sd) | kg/m$^3$ | % (sd) | % (sd) | % (sd) |
| T. versicolor | 13.2 | 0 (0.1) | 2.1 (1.4) | 5.2 | 2.5 (0,6) | 4.5 (1.4) | 26.4 (4.1) |
| | 28.2 | 0 (0) | 9.7 (3.1) | 8.6 | 3.1 (1.4) | 5.6 (1.4) | |
| G. trabeum | 13.2 | 0.5 (0.2) | 0.2 (0.1) | 5.2 | 2.2 (0.7) | 7.4 (0.9) | 19.6 (3.5) |
| | 28.2 | 0 (0) | 0.6 (0.1) | 8.6 | 1.3 (1.2) | 6.6 (0.9) | |

"sd" denotes standard deviation.

Retention results for milled and unmilled zinc borate samples after vacuum treatment differed significantly according to wood species. As shown in Table 1, pine had a higher retention than eucalypt and poplar wood species. Varied retentions can be attributed to different pore-size distributions of the ultrastructure of various species studied, as similar behavior was reported for wood impregnated with nano-zinc (Németh *et al*. 2013). Retention values for pine wood were slightly lower than those reported by Lykidis *et al*. (2013) (17.9 kg/m$^3$) in a study using a vacuum-pressure-vacuum method with 3% w/w suspension and was larger than those obtained by Mantanis *et al*. (2014) (5.64 kg/m$^3$) using a vacuum-vacuum method with 2.5% w/w suspension. Both research groups worked with *Pinus nigra* L. wood. Those variations can be attributed to the concentration of the dispersions used and the different impregnating methods or differences between wood anatomy characteristics of pine species. However, when comparing the retention values in

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Table 1 with those reached with a micronized product (such as micronized copper azole (MCA) in rubberwood), the same retention increase was observed when the suspension concentration were increased. This was contrary to an earlier finding that retention values were lower (2.8 to 11.5 kg/m$^3$) Shukla *et al*. (2019).

Weight loss of untreated specimens consistently amounted to nearly or over 20%, except for *G. trabeum* colonizing *Populus sp*., thus validating the test conditions according to the EN113 (1996) standard. Mass loss of treated wood samples without fungus was reduced by less than 1% in all cases.

Table 1 shows that both milled and unmilled zinc borate protected the wood without leaching, as the wood is considered protected when the mass losses obtained is of less than 3%, according to EN 113 (1996). The exception was for *E. grandis* against *T. versicolor*, which presented a mass loss close to 4% at the higher value of zinc borate retention tested but still is considered protected.

*Populus* wood was protected by zinc borate against fungal action except against *T. versicolor*, with only negligible weight losses found for leached samples.

In all cases the mass losses of the samples treated with micronized zinc borate were lower compared to those treated with unmilled solid, which was attributed to a better distribution of the solid inside the wood because of the higher percentage of smaller sized particles. The micronized zinc borate at the lowest value of retention tested consistently led to smaller weight losses than it did at the highest retention value. No significant differences ($\alpha = 0.05$) were found between leached and non-leached samples of pine treated with milled zinc borate at the retention values tested. Protection against *T. versicolor* was consistent with the findings of Mantanis *et al*. (2014). However, in contrast with what these authors reported for *Tyromyces palustris*, the milled zinc borate used in this study showed fungicidal activity against *G. trabeum*.

Finally, it can be observed that the mass losses showed the same tendency reported by Clausen *et al*. (2011) and by Lykidis *et al* (2016a): there occurred greater leaching at a higher concentration of the suspension.

Prior to the study on Zn and B loss by leaching, different wood digestion conditions were assayed, in which different acidic mixtures were used: $HNO_3$, $HNO_3$-$HCl$, and $HNO_3$-$HCl$-$H_2O_2$. In each case, retrieved Zn concentrations were evaluated. For the three conditions tested, the highest Zn recovery was obtained for *E. grandis*. The best results were obtained with $HNO_3$ for eucalyptus, and $HNO_3$-$HCl$ of 50:50 ratio for pine.

**Table 2.** Zn (mg/L) Recovered from Wood Digestion Using Various Acidic Mixtures: $HNO_3$, $HNO_3$-$HCl$, and $HNO_3$-$HCl$-$H_2O_2$, for *E. grandis* and *P. taeda*

|  | Acidic Mixtures | Zn (mg/L) |
|---|---|---|
| *E. grandis* | $HNO_3$ | 17 |
|  | $HNO_3$-$HCl$ | 15 |
|  | $HNO_3$-$HCl$-$H_2O_2$ | 7 |
| *P. taeda* | $HNO_3$ | 6.7 |
|  | $HNO_3$-$HCl$ | 12 |
|  | $HNO_3$-$HCl$-$H_2O_2$ | 7.4 |

Table 3 shows the percentages of leached Zn and B, determined as the difference between initial quantities of Zn and B with those obtained after leaching according to EN

84 (1997). Both data were obtained from the acid digestion of the treated wood. These tests were done only for *P. taeda* and one of the hardwood species, *E. grandis*.

**Table 3.** Percentage of Zn and B Lost Through Leaching of Milled and Unmilled Zinc Borate

| | | *P. taeda* | | |
|---|---|---|---|---|
| | | $(kg/m^3)$ initial | $(kg/m^3)$ leached | % $(kg/m^3)$ lost |
| **Unmilled** | **Zn** | 1.94 | 1.01 | 52.1 |
| | **B** | 4.91 | 2.11 | 62.9 |
| **Milled** | **Zn** | 2.66 | 0.92 | 34.6 |
| | **B** | 2.91 | 2.15 | 73.9 |
| | | *E. grandis* | | |
| | | $(kg/m^3)$ initial | $(kg/m^3)$ leached | % $(kg/m^3)$ lost |
| **Unmilled** | **Zn** | 2.26 | 1.85 | 81.8 |
| | **B** | 4.73 | 4.44 | 93.9 |
| **Milled** | **Zn** | 1.08 | 0.66 | 61.1 |
| | **B** | 4.27 | 3.11 | 72.8 |

The wood treated with unmilled sample was more susceptible to leaching, leaving the wood unprotected for all the retentions tested and in all three-wood species studied (Tables 1 and 3).

The milled zinc borate used in this study does not leach easily compared with ammoniacal zinc borate (Ibáñez *et al.* 2014). The weight loss due to leaching was significant only for the hardwood species ($\alpha = 0.05$). This was in line with the insignificant leaching reported for pine wood impregnated with nanometric ZnO (70 nm particle size) (Clausen *et al.* 2011).

In Fig. 5, *E. grandis* SEM images are shown. Unmilled zinc borate was hardly distributed throughout the wood, instead accumulated as disperse and scarce crystals (arrow in Fig. 5b). On the other hand, wood treated with milled zinc borate showed white deposits on the internal walls within the wood tissue and accumulated in the cellular wall (arrow in Fig. 5c). However, after leaching the milled product accumulated in aggregates (smaller-sized particles joined together forming larger-sized particles), in the cellular lumen due to the presence of moisture (arrow in Fig. 5d) therefore preventing them from leaching out of the wood matrix. The same thing happened with copper compounds (Matsunaga *et al.* 2007, 2009; Leach and Zhang 2010); it is reported that leaching from MCQ copper (Alkaline Micronized Copper Quaternary) -treated wood amounts to half the amount leached from wood treated with ACQ (Alkaline Copper Quaternary) (McIntyre and Freeman 2009).

Zinc borate was observed to behave differently in pine wood. Milled zinc borate was detected as large white clusters both in the cellar walls and in the lumens (as indicated with an arrow in Fig. 6b). However, after leaching, the cellar lumens were left empty. For the micronized and particulate copper present in MCA (micronized copper azole) and ACQ

in southern pine, Feng *et al.* (2019) identify the parenchyma cells associated with the radial and axial resin channel network as major copper deposition sites, with different distribution in the wood.
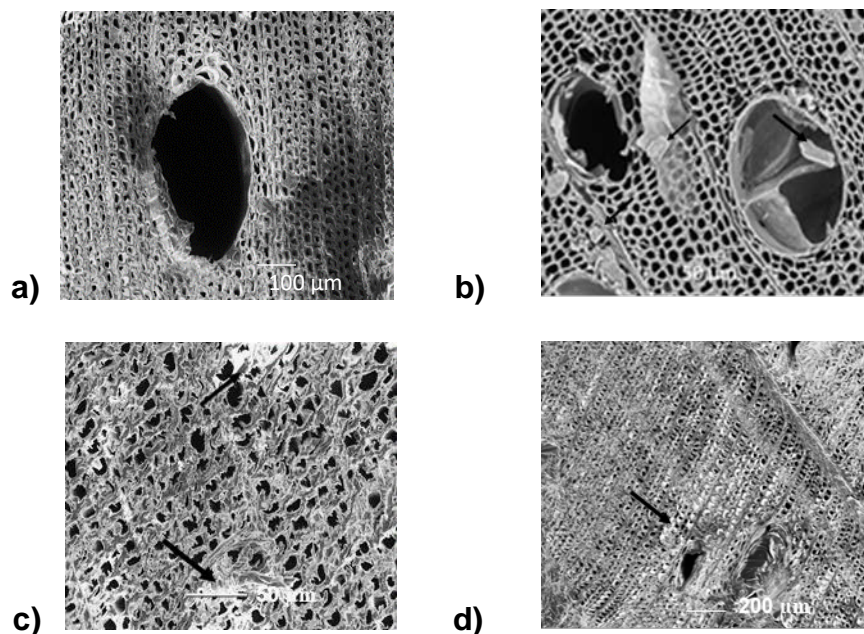


**Fig. 5.** SEM images of *Eucalyptus grandis*: a) untreated, b) with unmilled ZnB, c) with milled ZnB, d) leached wood, previously treated with milled ZnB
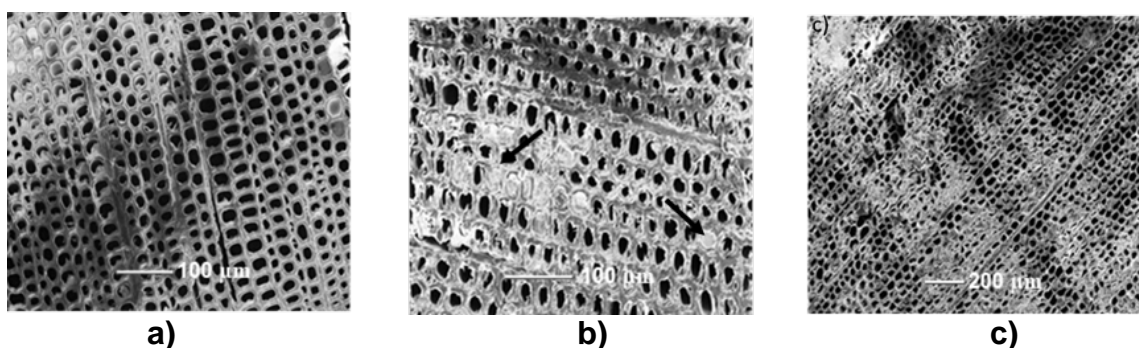


**Fig. 6.** SEM images of *Pinus taeda*: a) untreated, b) with milled ZnB, and c) b extended

The SEM images show that both milled and unmilled zinc borate were not distributed homogeneously section-wise; nor was it found to form aggregates or deposits in a consistent pattern that could have led to the identification of preferential deposition zones, such as the middle lamella or the secondary wall. In some sections, no deposits were found. In the following studies, the apparent greater presence of milled zinc borate within the wood (when compared to the one treated with the unmilled product) will be verified by energy-dispersive analysis of X-rays (EDX).

The results can be considered as promising, since it will be verified that a reduction in the particle size leads to an improvement in wood protection, despite the Zn and B elemental loss percentage being similar to those presented by wood treated with the

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unmilled product. The improvement when using the milled product can be attributed partly to a more homogeneous distribution within the porous microstructure of the wood (Matsunaga *et al.* 2008), which at the same time depends on particle size and increased penetration of those micronized particles with diameters smaller than the bordered pits (Shukla *et al.* 2019). However, this neither improves nor decreases the fungicidal properties of the product.

This study thus revealed that the enhancement in wood protection can be achieved at a very low cost, because the impregnating material was synthetized from commodities by application of a low energetic cost process. This is in contrast, for instance, to the synthesis of nano- or micro-sized materials that require expensive catalysts. Further studies are required to provide a more comprehensive knowledge on how to reduce leaching even more by incorporating additives in the formulation.

## CONCLUSIONS

1.  In this work, zinc borate was milled and compared with the unmilled material, against wood rotting fungi. Both solids are able to protect the wood when they are not subjected to leaching.

2.  The milled zinc borate material tested, although did not improve the fungicidal action against brown- and white-rot fungi, did prevent leaching, which would allow its application in environments of high moisture content.

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