

Analysis of Green Wood Preservation Chemicals

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Abstract—Wood decay is addressed continuously within the wood industry through use and development of wood preservatives. The increasing awareness on the negative effects of many chemicals towards the environment is causing political restrictions in their use and creating more urgent need for research on green alternatives. This paper discusses some of the possible natural extracts for wood preserving applications and compares the analytical methods available for testing their behavior and efficiency against decay fungi. The results indicate that natural extracts have interesting chemical constituents that delay fungal growth but vary in efficiency depending on the chemical concentration and substrate used. Results also suggest that presence and redistribution of preservatives in wood during exposure trials can be assessed by spectral imaging methods although standardized methods are not available. This study concludes that, in addition to the many standard methods available, there is a need to develop new faster methods for screening potential preservative formulation while maintaining the comparability and relevance of results.

Keywords—Analytics, methods, preservatives, wood decay.

I. INTRODUCTION

WOOD for construction and other outdoor uses competes continuously with other materials. In recent years, the weight of sustainability and use of renewables has brought wood back to foreground in support structure and cladding uses while its popularity in terraces, fences and non-residential buildings has persisted. Nevertheless, even if renewability of wood is one of its most positive characteristics, it is also a challenge, as exposure to certain conditions make wood prone for decay. After all, biodegradation in a wet environment is a feature of wood to recycle it, in a timely way, back to the basic building blocks of carbon dioxide and water through biological, thermal, aqueous, photochemical and chemi-mechanical means.

The most relevant factors causing wood decay are the presence of decay fungi, their access to cell-wall polysaccharides and the moisture uptake of wood that sustains a suitable environment for biological degradation. Industrial wood preservation aims to slow down this natural decay by introducing biocidal components into wood [1], [2].

With a greater awareness of the persistence of some of these wood preservation chemicals in nature and the concern for our

ecosystem, less toxic or narrow spectra toxins have been explored and developed into commercial products. The severe toxicity for soil and water due to leaching of chemicals and the proven health implications from exposure and disposal issues of heavily treated wood have ceased or limited the use of chromated-copper-arsenate (CCA) [3], creosote [4], and other, as such effective, means to preserve wood [5].

Due to these limitations, several researchers have focused their efforts on finding environmentally benign substitutes. For instance, Anttila et al. [6] successfully tested conifer tannins against wood degrading fungi and Lu et al. [7] found that stilbenes inhibit the wood decay caused by these fungi. Other successful chemicals extracted from biomass side-streams include monoterpenes [8], different bark extracts [9] and thermochemical biomass distillates [10].

In addition, pyrolysis oils or distillate fractions from thermal decomposition of biomass have been tested [11]. These oils have been found to be successful wood decay inhibitors when produced from wood or forest and agricultural residues or side streams, such as bark [12] or pineapple waste [13]. Pyrolysis oils have also been reported to have variable effects based on different process and feedstock related factors [14].

There are ongoing investigations with several feedstocks such as coffee processing residues. Coffee beans are known to be rich in chemical composition [15] and they have been tested as fungi growth inhibitors [16]. Their potential in wood preserving applications have not been thoroughly explored.

Since the testing of novel wood preservative formulations in real outdoor conditions takes several years and demands significant volume of preserved wood material, it does not promote efficient product development. Standardized laboratory tests provide cheaper and faster initial screening of potential preservatives.

The most common screening test of chemical compounds is the antifungal test in petri dish, where fungi are grown in contact with the chemical that is expected to be antifungal, and the growth inhibition is estimated by comparing the growth in the same media without the chemical additive [17], [18]. This test is inexpensive and fast even if large amounts of replicates are needed due to high variability in fungal growth. Analyses of samples may consume a lot of time, leading to errors caused by fatigue and carelessness of seemingly endless repetition, so researchers have focused their efforts in finding automated ways to measure fungal growth in petri dish [19]. However, the most serious weakness of this method is the absence of wood material. When the chemical is integrated into wooden substrate, the fungi may be able to degrade the wood in spite of high concentration of the specific compound [20].

The performance of a chemical as a wood preservative can

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be tested *in vitro* by exposing impregnated wood to decay fungi in accordance with European norm EN 113 [21]. The performance of the chemical after 16-week exposure is defined by comparing the mass loss of treated wood, caused by the 3-4 known fungus strains, to mass loss of non-treated wood. In this method, complementing visual inspection, mass loss is the only parameter to measure the decay rate of small samples. In such tests, the decay level may be underestimated due to the presence of hyphae inside the wood, leading to errors that are not usually taken into consideration [22]. The weakness of *in vitro* decay tests is the controlled and simplified performance environment.

More realistic performance indicators of preservatives can be obtained by soil contact tests [23], [24] or above ground durability tests [25], in which the wood is exposed to the interaction of different microorganisms and abiotic factors.

The main objective of this study is to test different natural chemicals, as tannins, pyrolysis oils and spent coffee, as wood rotting fungi inhibitors and to compare the different analytical methods for assessing their performance and interaction with wood as a substrate. This study also presents first results of a near-infrared spectroscopy (NIR) that can be used for checking the distribution of chemicals in wood after it has been treated with preservatives and used for field trials.

II. MATERIALS AND METHODS

A. Wood Material

Commercial timber of Scots pine (*Pinus sylvestris*) from Kerimäki sawmill, Finland, was used as wood substrate. The small specimens of 5×40×10 mm³ (radial x longitudinal x tangential) for decay tests *in vitro* were analyzed under UV light (313 nm) to include only pure sapwood pieces to impregnation while rejecting pieces containing hardwood. Larger pieces for soil contact trials (20×340×20 mm³) and NIR analyses (20×30×40 mm³) included both heartwood and sapwood.

B. Decay Fungus

The decay fungus used was the brown rotting *Gloeophyllum trabeum* (strain BAM 115). A brown rot species was chosen due to their relevance in the decay of softwood timber, the most common timber in constructions and outdoors uses. The fungus was grown in petri dish, in 5% malt and 2% agar culture medium, at 22±2 °C and 30±5% relative humidity. When the fungus covered the whole surface of the petri dish, about 0.275 cm² plugs were used for preparing new colonies or they were stored in a fridge (10 °C) for further use. The fungi were taken back to the growing chamber 2 days before using them in the antifungal or wood decay test.

1. Bio-Based Preservatives Tested

As case examples, the wood specimens were treated with organic preservatives, namely tannin, pyrolysis distillate of bark (bio-oil) and coffee residue extract, while commercial copper preservative was used as reference (Table I).

The bio-oil from slow pyrolysis of Norway spruce (*Picea abies*) bark was provided by the Department of Applied

Physics of the University of Eastern Finland. This distillate fraction was the more viscous black tar-like fluid from 350 °C. Compositional analysis of the oil fraction was obtained using Bruker Avance-III HD 600 MHz NMR system.

The commercial tannin Colatan GT10, extracted from Quebracho (*Schinopsis Lorenzii*) was provided by Haarla Ltd (Tampere, Finland) (Fig. 1). Its composition was measured with Bruker BioApeX IV Fourier-transform ion cyclotron resonance (FR-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) and the data acquired was further analyzed with Data Analysis 4.0 SP5 software (Bruker Daltonik GmbH).



Fig. 1 Colatan GT10 tannin powder

TABLE I
 SUMMARY OF PRESERVATIVE COMPOSITION USED

Preservative	Abbreviation	Primary ingredients (unit)
Pyrolysis distillate used as 1%	Bio-oil	Acetic acid (1.6 M)
		Propanoic acid (0.14 M)
		Methanol (0.12 M)
Quebracho tannin used as 0.5 - 6%	Tannin, GT10	Carboxamide thioether (-)
		benzenesulfonic acid (-)
		Benzenesulfonic acid derivatives (-)
		Palmitic acid (-)
Spent coffee used as 1%	Spent coffee	Chlorogenic acid (102 mg/L)
		Caffeine (31 mg/L)
		Neochlorogenic acid (65 mg/L)
Celcure C4 used as 1.6%	Cu preservative	Copper(II) carbonate (17%)
		Ethanolamine (< 35%)
		Benzalkonium chloride (4.75%)

Spent coffee grounds (100% *Coffea arabica*, middle roast) were collected from a commercial restaurant. Extractives from spent coffee were obtained after 45 min hot water extraction of 50 g of used coffee grounds in 1L MilliQ water (100 °C), and removing the solid residue by filtration (30-µm pore size). This coffee solution was condensed in 100 °C until the colloidal solids concentration was 1%. The main constituents were identified based on the retention time and UV spectrum of the compounds and literature with HP 1100 series LC-system (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector (DAD), where the reverse-phase separation was performed on a Hypersil ODS 75×4.6 mm i.d. RP C18 column. In total 70 components were observed, most prominent of which were chlorogenic acid and its derivatives, such as hydroxycinnamates, and ferulic acid, caffeic and p-hydroxycinnamic acid, and caffeine.

Commercial copper-based AB-class preservative (Celcure C4 at 1.6%, Koppers Inc., USA) was used.

2. Assessment of Antifungal Activity

The growth media for fungi was prepared by mixing the preservative chemicals with malt and agar, a modified method based on Belt [18]. A mix of 4% malt powder, 2% agar, and either bio oil or coffee in 1% mass based concentration was prepared in MilliQ water. The pH of malt-agar solution was fixed to 6 with 1M NaOH to allow its later solidification. Afterwards, the solution was leveled up, autoclaved (120 °C, 15 min) and while kept in sterile conditions, 20 ml of the mix was poured in each petri dish.

Following the same procedure, malt-agar mixes were prepared with bio-oil, tannin, spent coffee extract, commercial copper preservative (commercial reference) and a growth media with only malt and agar (control). For the growing media with tannins, the same preparation process of bio-oil and coffee was followed, but tannins were added when the solution with malt and agar was cooled down to about 50 °C after autoclaving, to avoid breaking down the tannins due to excessive heating.

To inoculate the amended plates with the fungus, a plug of roughly 0.275 cm² of mycelium from an actively grown colony was positioned on the malt-agar media. The plates were then sealed and incubated in a chamber with no light, at 22±2 °C and 30±5% relative humidity until the mycelium reached the petri dish edge. Inhibition was measured with a modified formula of the one used by Chang et al. [26]:

$$\text{Inhibition} = (1 - (\text{AT-IA})/(\text{AC-IA})) \cdot 100 \quad (1)$$

where AT is the area of the experimental plate, AC is the area of the control plate, and IA is the size of the inoculated plug.

For each chemical, commercial reference and control 10 replicates were measured, and based on their growth variability, the mean inhibitions were calculated (Fig. 2).

The statistical analyses were done by running a LSD test with IBM SPSS Statistics 23.

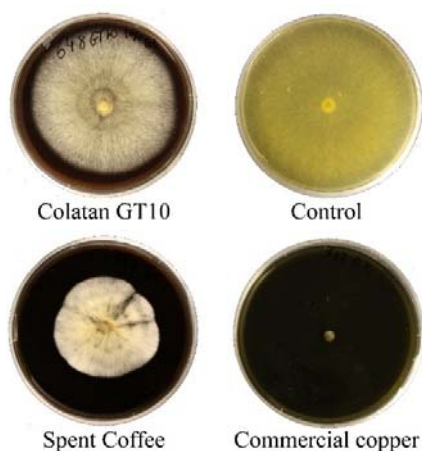


Fig. 2 Comparison of growth of *G. trabeum* in growth media with different chemicals after 13 days

3. Laboratory Assessment of Chemical Preservative Efficiency in Wood

Wood decay test was carried out by following a modified version of EN 113 test described by Lu et al. [7] using the sample size of 10×40×5 mm³ (radial × longitudinal × tangential). Same bio-oil and tannin as in the antifungal test were prepared in mass concentrations of 2%. For spent coffee samples, the concentration was 1%. Each of these chemicals was used to impregnate 32 sapwood specimens (Fig. 3).

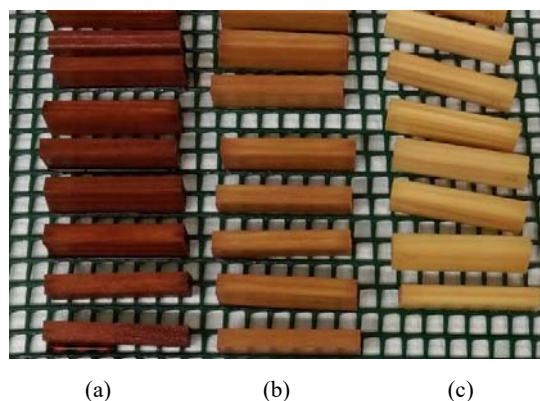


Fig. 3 Wood samples treated with tannins (a), spent coffee (b) and bio oil (c)

The Bethel process used for impregnation was as follows: First, a vacuum phase of 30 minutes in 0.4 bar. Afterwards, pressure was increased to 9 bars, held for 1 hour and then released without any final vacuum. Temperature was always kept in 21±1 °C.

The treated specimens were tagged and oven dried at 50 °C until they reached constant mass. Sterilization of dry specimen with Gamma radiation (30 kGy) was conducted by Synergy Health GmbH (Germany).

Petri dish (Ø 90 mm and 15 mm height) was prepared with 30 ml of 4% malt powder and 2% agar growing media. A plug of 0.275 cm² was cut from fresh *G. trabeum* colony and placed in the center of the petri dish, and kept in a growing chamber at 22±2 °C and 70±5% relative humidity until the fungi covered the whole surface of the petri dish. Then, a stainless steel mesh (50% of the surface area perforated) was placed in each petri dish, and four wood pieces treated with the same impregnation chemical were equidistantly positioned in the petri dish (Fig. 4).

For each impregnation chemical, eight petri dishes were done, with a total of 32 sapwood specimens per treatment. The petri dish was then incubated in a growing chamber (22±2 °C and 70±5% relative humidity). From each petri dish, one specimen was harvested after 4 weeks, the second one after 8 weeks, the third after 12 weeks and the fourth after 16 weeks to find out the temporal development of fungal degradation.

After harvesting, mycelium was brushed off the blocks (Fig. 5) and the dry weight was recorded after drying at 50 °C until constant mass. Then, the mass loss was calculated by subtracting the mass after decay from the mass before decay.

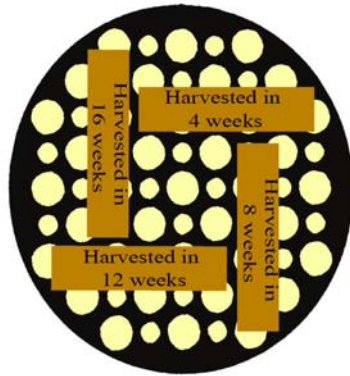


Fig. 4 Schematic of the up inside the petri dish and the harvesting days of the different wood pieces. The black figure represents the stainless steel mesh over the fungus (light yellow)



Fig. 5 Wood sample which has just been harvested from the petri dish, where the mycelium has not still been brushed off

4. Accelerated Soil Contact Test

An accelerated soil contact test was set up following the soil-box method described by Venäläinen et al. [27]. In this soil-box, the middle of $20 \times 340 \times 20$ mm³ sized specimen is exposed under warm and humid conditions to the microbes inhabiting fertile garden soil (Fig. 6). Compared to the normative ENV 807 [24] test, this method enables the monitoring of strength loss. For the long-term durability monitoring, also the normative EN 252 [23] test was set up in the field.



Fig. 6 Setting wood samples to the soil-box used for accelerated soil contact durability testing. The specimen penetrating the box will be entirely covered with moist soil mimicking the ground contact tests for several weeks

The first harvest of specimens from the soil-box test will be carried out 4 months after the initiation period. Thus, the results are not available when writing this report.

5. Assessment of Chemical Presence in Wood

Pinus sylvestris samples of $20 \times 30 \times 40$ mm³ (radial \times longitudinal \times tangential) were impregnated with the Bethel process mentioned previously and with tannin solutions of 0.5%, 1%, 2%, 4% and 6%, ten samples per concentration. A reference group of ten samples was impregnated with distilled water. After impregnation, the four narrow edges were sealed with tape and all the samples were subsequently dried at 50 °C, forcing the moisture to evaporate from the two large 30×40 mm² surfaces.

The dried samples were cut longitudinally and across to reveal internal surfaces with different orientations. The cut samples were scanned with a 320-pixel near-infrared line scanner sensitive in the 900-2500 nm wavelength range and the reflection at 256 wavelengths were recorded at an optical resolution of 0.5 mm/pixel. The raw spectra were treated with a standard normal variate filter, and then smoothed with the Savitzky-Golay filter, and finally the second derivative was determined.

A principal component analysis was done to get an overview of the variations present in the samples. Subsequently a PLS-model for the individual pixel spectra were calibrated within each concentration group. This model was then used to predict the concentration of the impregnating fluid for each pixel in the NIR-images, thus revealing eventual tannin gradients within samples and differences between samples.

III. RESULTS AND DISCUSSION

A. Assessment of Antifungal Activity

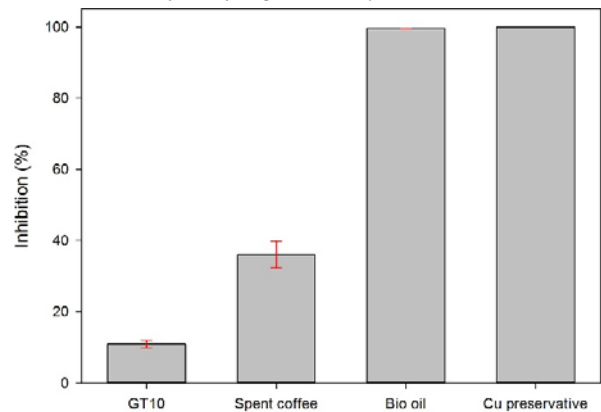


Fig. 7 The effect of natural chemicals and one commercial wood preservative to the growth of brown rot fungus *G. trabeum* (N = 6 to 10)

The results of the antifungal test are presented in Fig. 7. The results present high inhibition caused by bio oil. Previous investigations noted that some pyrolysis oils can inhibit wood decaying fungi or delay wood decay [12], [13], [28], [29], which agrees with our findings. This was the only chemical

with a similar inhibition to the commercial copper-based preservative.

The commercial Colatan GT10 tannin inhibited the growth of the wood decaying fungus too, but the inhibition was rather low ($10.8 \pm 1.0\%$). Anttila et al. [6] outline the effect of the extraction method, the stage of enrichment and purity of the fraction. This may be the reason of the low effect of our tannin mix, while presence of impurities, especially sugars, may have hindered its antifungal properties.

Spent coffee showed an inhibition of $36.0 \pm 3.7\%$ in the growth of the wood decaying fungus, the second best chemical after the bio-oil. Arora & Ohlan [16] demonstrated that coffee has antifungal effects against decay fungus. The paper at hand agrees with those findings, and proves that some of the antifungal chemicals which remain after the coffee beans are used for preparation of coffee beverages.

B. Assessment of Chemical Preservative Efficiency in Wood

The results of the wood decay test in petri dish after 16 weeks exposure are presented in Fig. 8. The average mass loss of samples treated with bio-oil and spent coffee did not differ from that of the untreated control samples.

Colatan GT10 inhibited the wood decay best of all the natural chemicals. Tannins have been known for a long time as potential wood preserving agents, and research has been done to check how to fix them to wood [30], [31], and the present work agreed with the previous findings.

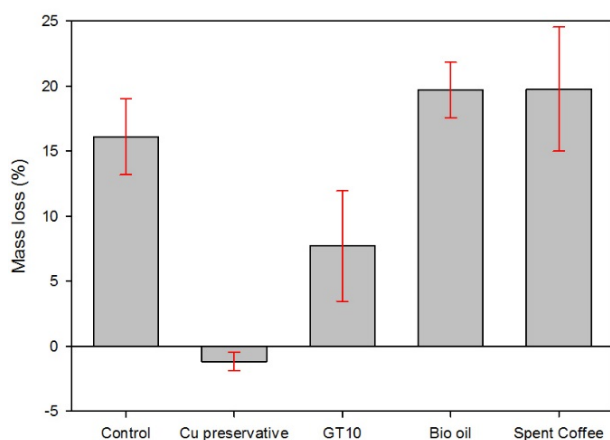


Fig. 8 Mass loss caused by *G. trabeum* in 16 weeks in the treated wood specimens. (N = 6 to 10)

None of the natural chemicals reached the inhibition effect of the synthetic copper-based preservative.

The performance of different chemicals in the tests A and B was very different. The antifungal test without wood substrate is fast and simple, but it obviously provides only preliminary indication of the effect of the chemical as a wood preservative.

As such, our results are in line with the work done by Loman [20], where he considered that results of tests in malt agar media cannot be extrapolated to the natural media. However, it is the most common way to screen possible wood preserving agents [32]. After the screening, decay tests *in vitro* with impregnated wood samples are the next necessary step

towards a wood preservation product.

In both tests, the commercial wood preservative had the best performance. However, within the natural preservatives, bio-oil was the best in the antifungal test, with a very similar performance to the commercial preservative, but had no significant effect in the wood decay test, while Colatan GT10 showed a poor performance in the antifungal test but was the best one delaying decay in the wood decay test.

One reason for the variability in their performance may be the acidity, as suggested for liquefied wood by Hrastnik et al. [33]. However, in the present work, the acidity of bio-oils was neutralized in the antifungal test but not in the wood decay test, which would suggest that more acidic bio-oil performed poorly against decaying fungi.

Another reason may be related to the fact that some chemicals may get chemically bonded to wood. Furthermore, it ignores the mass of the fungus in the measured dry mass, which can cause significant errors [22].

Additionally, the retention of the chemicals in wood are not known in this study, while we know the original concentration of the chemical used for treating the wood. This makes the comparison challenging between both methods.

1. Assessment of Chemical Presence in Wood

The NIR PLS-model not only shows the tannin level differences between samples, but also a tannin concentration gradient that develops within each tannin impregnated sample because of the drying process (Fig. 9).

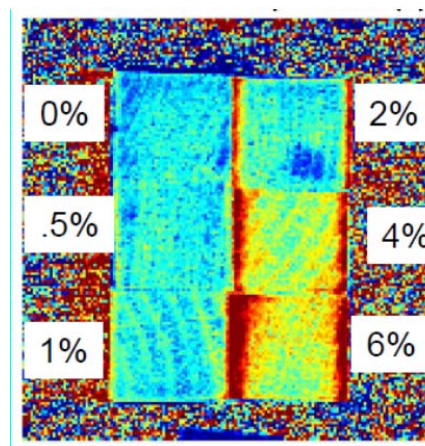


Fig. 9 Tannin concentration differences within samples and between samples and groups as detected by NIR. One crosscut sample per group is shown. The drying process concentrates the water-soluble tannins at the evaporation surfaces

This result shows that water-soluble chemicals redistribute themselves inside wood rather than solidifying immediately after impregnation, creating an increasing concentration gradient towards the surface and leaving the core at a much lower concentration than intended.

It is estimated that the surface can be at up to twice the expected concentration if no redistribution occurred, while the core is at approximately half the expected concentration. The sealing method used during the drying process was not ideal,

so the actual concentration levels would possibly have been different. However, the general conclusion that the drying process leads to a concentration gradient is valid.

A more detailed study is needed to predict the actual tannin concentrations in the wood with NIR. However, the analysis method was useful and holds potential to study the redistribution of chemicals during field trials.

IV. CONCLUSIONS

The laboratory methods used for testing wood preservatives gave somewhat conflicting results. Based on the antifungal test, the bio-oil and spent coffee are the best performing green chemical mixes.

Results of wood decay test show that bio-oil and spent coffee cause no reduction in the mass loss compared to control, while GT10 has a positive effect. This study highlights some of the limitations associated with conventional screening methods. Comparison of the results and their interpretation might be challenging due to differences in the test setup and variables and leading to a different and sometimes misleading outcome.

It can be concluded that further testing methods, additionally to standardized laboratory methods, are necessary to the identification of potential wood preservatives. Additionally, efforts should focus on finding a fast but also reliable method for identifying wood decay and testing wood preservatives.

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