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Wolfiporia cocos – A potential agent for composting or bioprocessing Douglas-fir wood treated with copper-based preservatives

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Inhalt · Contents · Contenu

Kurzmitteilungen / Short Notes

Wolfiporia cocos - A potential agent for composting or bioprocessing Douglas-fir wood treated with copper-based preservatives[1]

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K e y w o r d s: Copper preservative, wood, decay, *Poria, Wolfiporia, Postia,* fungi, composting, bioremediation

1. Introduction

Interest in wood-inhabiting fungi that can tolerate high levels of copper has generally focused on their capabilities to decay preservative-treated wood in service. The potential for utilizing copper-tolerant fungi in bioprocessing of spent wood materials that are treated with copper-based wood preservatives has received little attention. The objective of this study was to examine the potential of *Wolfiporia cocos* and other decay fungi with a known tolerance for copper, as agents for composting Douglas-fir wood treated with copper-based wood preservatives or as agents for sequestering copper from treated-wood products.

2. Background

Compared with other microbial groups, fungi can be extremely tolerant of toxic metals (G. M. GADD, 1993; O. SCHMIDT and B. ZIEMER, 1976).

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Several brown-rot wood decay fungi in the genera *Serpula* ([Pers.] Gray) and some species that were once included within the genus *Poria* (Pers. ex Gray) are tolerant of copper (O. COLLETT, 1992; O. SCHMIDT and U. MORETH, 1996; H. LEITHOFF *et al.*, 1995; J. D. THORNTON and M.A. TIGHE, 1987; K. TSUNODA *et al.*, 1997; *G.* R. WILLIAMS and R. F. FOX, 1994; R. A. ZABEL, 1954).

It is generally recognized that microorganisms with tolerance to copper are a part of the natural environment (G. R. WILLIAMS and R. F FOX, 1994). Indeed, the first inhabitants of sandy areas in northern Florida and the coastal, southeastern United States referred to sclerotia of *Wolfiporia cocos* (Schw syn. *Poria cocos*) as tuckahoes (G. F. WEBER, 1929). Most studies of microbial populations used agar medium to isolate microorganisms from natural environments that are challenged with high loadings of metals. Results from such studies have led to the development of a general (C. A. FLEMMING and J. T. TREVORS, 1989; G. M. GADD, 1993; L. E. HALLAS and J. J. COONEY, 1981; M. D. JONES and T. C. HUTCHINSON, 1986) perception that introducing additional metals to a soil reduces the diversity of microbial species with a resultant increased relative abundance of constitutively metal-tolerant, indigenous microorganisms (H. K. YAMAMOTO *et al.*, 1985). Sometimes this is associated with increases in populations of microorganisms that colonize remains of microfauna that succumb to the additional metals in their environment (A. NORDGREN *et* al., 1985). Arguments for constitutive tolerance in populations of organisms isolated from metal contaminated soils are based on the isolation of tolerant microflora from unaffected sites. Evidence for adaptive development of metal-tolerance is not observed in population-oriented studies but in laboratory studies with pure cultures of *Antrodia vaillantii* in a “choice test”. *A. vaillantii* grew preferably towards wood treated with a copper-containing preservative (H. LEITHOFF *et al.*, 1995).

The few direct comparisons, which can be made from published literature, on relative tolerance of specific cultures of decay fungi to copper when assayed in agar media and wood substrates indicate a greater tolerance of copper by fungi in wood than in agar (E. W. B. DA COSTA and R. M. KERRUISH, 1964) and show a general (K. TSUNODA *et al.*, 1997) correlation of results between the two methods. Most fungal species that tolerated high percentages of (divalent) copper in agar adjusted to pH 2 with sulfuric acid (G. A. YOUNG, 1961) also decayed pine sapwood treated to a retention of 0.175 lb/ft^3 (2.8 kg/m^3) (C. G. DUNCAN, 1958).

I. STEPHAN *et al.* (1995) investigated the use of brown rot causing fungi *Antrodia vaillantii* and other, *Poria* species to convert chemically fixed inorganic preservative complexes from impregnated wood waste into water-soluble components. Results indicated that large amounts of oxalic acid and the resulting high acidity of the wood substrate caused water solubility of chromium and arsenic after fungal attack. The leachability of copper was at its maximum when the pH of the leachate was 3.2. Under some experimental conditions, more than 90% of chromium and more than 80% of the arsenic was leached from the wood, whereas copper reacted with oxalic acid to form copper oxalate with limited water solubility. Consequently, copper was leached to less than 7%. No major difference was detected among strains of *A. vaillantii* in its ability to induce leaching of metals from chromium-copper-boron-(CCB-)treated wood chips.

Comparative laboratory tests (J. D. **THORNTON** and M. A. **TIGHE,** 1987; J. WAZNY and J. D. THORNTON, 1986) of strains of *Serpula lacrymans* (Schum. ex Fr.) S. F. Gray have demonstrated that the ability of some fungi to decay wood is stimulated by low levels of copper in the wood and that the relative tolerance of strains to copper-containing preservatives varies with the formulation of the preservative. In those studies, the same strains of *S. lacrymans* were used in agar plate assays against copper chromate arsenate (CCA) and agar block tests against copper naphthenate. There was a difference in relative tolerance of the isolates against the two preservatives, but the differences in methods and potential for confounding effects of chromium and arsenic in one study does not allow detailed analysis of these data.

3. Methods

3.1 Fungi

Isolates of two decay fungi known to be tolerant to copper were obtained from the Center for Forest Mycology Research at the USDA Forest Service, Forest Products Laboratory, and used in this study. One was *Postia placenta* (Fr.) M. J. Larsen & Lombard (M698), which is one decay fungus referenced in standard decay tests (ASTM, 1996; AWPA, 1997b). The other was an isolate of *Wolfiporia cocos* (MD 104), which was demonstrated to have a higher tolerance for copper and a faster growth rate when cultured on copper-containing agar media than other isolates in that species (B. **WOODWARD** and R. C. **DEGROOT, 1999).**

3.2 Bioprocessing

In this study, we utilized mill-run wood from second growth forests of Coastal Douglas-fir. Wood was experimentally treated with copper-based, waterborne preservatives (Table 1). The waterborne preservative ACZA is used extensively for treatment of Douglas-fir. Two other newly developed waterborne preservatives are just entering the commercial sector. The oilborne preservative CuN is a system of long standing that has received renewed interest in response to environmental concerns. Treated materials were challenged with assay fungi in standard, soil jar methods (ASTM, 1996; AWPA, 1997c) that utilize loss of weight in wood blocks as an indication of decay caused by the fungus.

Table 1: Copper-based preservatives used for treatment of Douglas-fir.

Preservative	Active Ingredients	
Water		
Ammoniacal copper zinc arsenate (ACZA)	copper as CuO	50.0 %
	zinc as ZnO	25.0 %
	arsenic as AS_2O_5	25.0 %
Ammoniacal copper quat, Type B (ACQ-B)	copper as CuO	66.7 %
	Quat as didecyldimethyl-ammonium chloride	33.3 %
Ammoniacal copper citrate (CC)	copper as CuO	62.3 %
	citric acid	35.8 %
Oilborne		
Copper naphthenate (CuN)	active ingredient is copper naphthenate	

In our study, a major difference from standard procedures was in the preparation of wood substrates for assay In the standard procedures, wood blocks are prepared directly from an untreated wood source. In this work, wood blocks were prepared from treated stakes as described herein. This study is part of a larger program (R. C. **De Groot,** 1996) in which we are evaluating alternative preservatives in a variety of wood species. The cardinal test unit in the entire program is a 19- by 19- by 450-mm wood member. Quantities of these members are being used in field evaluations, and we structured the laboratory experiments to match those trials.

Wood members equilibrated to a constant weight were arranged prior to treatment by weight into groups of 30 replicates. Groups were developed in such a manner that each group had equivalent mean and variation in density. Because the total program encompassed a host of wood species, the retention series (dose) for all preservatives in all woods was accomplished by treating, as a batch, groups of 30 wood members from each species with the same gradient series of percentage active ingredients and allowing the resultant retention (weight/volume of wood) to be distributed as determined by the variability in density of wood materials being treated (Table 2). Treatment was by the full-cell process, To simulate an empty cell process, as would be used with oilborne treatments in commerce, oilborne solutions of copper naphthenate were formulated with the oil (#2 diesel fuel) constituting about 30% by weight of each of the treating solutions. The balance of the solvent was toluene. Preservative retention levels were determined on the basis of weight gain at time of treatment and through chemical analysis of wood from the entire cross section of selected stakes. Chemical analysis of preservative was by means of atomic absorption spectroscopy (AWPA, 1997, 1997a).

The identity of each individual stake was maintained throughout the entire experimentation. Prom each group of 30 replicate stakes, 10 were randomly selected and withdrawn for use in laboratory decay tests and chemical analysis. The remaining 20 stakes in each group were exposed in field plots. Ultimately, results from groups of stakes in both laboratory and field experiments will be displayed

T a b 1 e 2: Concentration of copper in Douglas-fir that was treated with copper-based preservative.

		Concentration of copper prior to decay trials		Concentration (weight %) of copper[c] after decay by			
				W. cocos		*P. placenta*	
Preservative	% Active ingredient	Retention of total preservative (kg/m^3)[a]	Retention of copper (weight %)[b]	Leached	Not leached	Leached	Not leached
ACZA	0.37	2.59 (0.15)	0.44	0.54	0.32	0.47	0.33
				0.43	0.35	0.37	0.29
	0.75	5.47 (0.72)	0.71	1.05	0.53	0.71	0.54
				0.67	0.59	0.64	0.57
	1.50	10.66 0.56)	0.99	1.37	1.18	1.00	1.07
				0.90	0.91	0.84	0.99
	3.00	21.87 (1.14)	2.33	1.60	1.60	1.58	1.57
				1.77	1.83	1.74	1.95
ACQ-B	0.37	2.52 (0.16)	0.36	0.52	0.61	0.56	0.40
				0.49	0.47	0.39	0.42
	0.75	5.15 (0.26)	0.63	0.73	0.81	0.80	0.67
				0.74	0.74	0.66	0.61
	1.50	10.62 (0.64)	1.27	1.36	1.56	0.98	2.18
				1.34	1.46	1.28	2.01
	3.00	21.03 (1.04)	2.50	2.18	2.58	1.83	1.60
				2.01	2.35	2.18	2.23
CC	0.37	2.61 (0.15)	0.39	0.65	0.60	0.46	0.45
				0.59	0.58	0.55	0.43
	0.75	5.28 (0.50)	0.62	1.15	1.08	0.75	0.74
				0.86	0.95	0.71	0.66
	1.50	11.0 (0.63)	1.28	2.47	1.49	1.27	1.49
				1.67	1.25	1.04	1.25
	3.00	22.26 (1.59)	1.91	3.32	2.02	1.63	2.01
				2.69	2.37	1.78	1.83
CuN	0.31	1.35 (0.26)	0.08	0.32	0.27	0.22	0.27
				0.26	0.31	0.26	0.26
	0.62	2.73 (0.48)	0.55	0.43	0.43	0.41	0.62
				0.46	0.49	0.47	0.50
	1.25	4.92 (0.90)	0.73	0.68	0.74	0.73	0.93
				0.73	0.75	0.56	0.79
	2.50	11.00 (1.71)	1.60	1.58	1.69	0.92	1.43
				1.68	1.60	1.64	1.76

[a] Retention of total preservative based on gain in weight during pressure treatment. Average of 10 replicate determinations. Numbers in parentheses are standard deviation.
[b]Determined from chemical analysis of wood from cross section of stake prior to decay. Blocks of both thicknesses were cut from these stakes. Values shown are average of 10 replicate stakes.
[c]Determined from chemical analysis of wood residuals following decay. Values shown reflect one determination of a collated sample of 10 decayed blocks. Upper value is for 9-mm-thick blocks; lower value of each paired set of data is for the 19-mm-thick blocks.

on a computer-generated spreadsheet that relates results to preservative retention on an individual stake basis. Only the laboratory tests using Douglas-fir are described in this report.

Each of the ten 450-mm-long wood members that were selected from each set of 30 for laboratory tests was processed as follows: Four 19-mm-long blocks and four 9-mm lengths were cut from the midlength of each 450-mm member for use in soil block trials. Two blocks of each size group were leached with water prior to exposure to the decay fungus; the other two blocks were not. Standard leaching procedures were used (AWPA, 1997c). This created for each decay fungus 10 replicated leached and 10 replicated unleached blocks of each dimension per treatment (preservative by retention combination). Another 75-mm length was also removed from the midsection of each of the 450-mm members and processed for chemical analysis to determine the amount of copper that was actually in the wood that was being bioassayed. Uniformity of preservative penetration within the cross sections of stakes was visually determined by spraying a Chrome Azurol S indicator solution (APWA, 1995) for copper on the internal cut end of the residual portion of each stake selected for analysis.

We conducted decay studies with both a 19- and a g-mm-long block from each stake because H.-P SUTTER *et al.* (1983) in working with small wafers described increased ability of fungi to move copper out of wood in a longitudinal direction (parallel to the longitudinal axis of wood fibers). Thus, we wanted to compare block size because it might be an important factor to consider in comminuting furnish for bioprocessing. We compared the effects of leaching because it also might be an important step in industrial bioprocessing. All blocks were steam sterilized (100 °C) for 20 minutes prior to inserting them into the decay chambers. Nonparametric analysis of variance tests (D. C. MONTGOMERY, 1991) were conducted on weight loss data as the distributions of weight loss data within many sets of 10 replicates were skewed, with occasional extreme observations, rather than normally distributed.

After blocks were re-equilibrated to a constant weight and weighed to determine weight loss, blocks were ground for chemical analysis. After blocks had been decayed, the residual wood in each set of 10 replicates was grouped for analysis by atomic absorption spectroscopy. The concentration of copper in wood following decay was compared with that in wood prior to decay. Results from chemical analyses of blocks before and after decay were not analyzed statistically because the decayed wood had only one composite sample per set of 10 replicates.

4. Results

4.1 Chemical analysis

The array of preservative retention levels realized (Table 2) spanned the series of retention levels used in a variety of commercial products. Uniform penetration throughout the entire cross section of stakes was not achieved with all treatments. Instead, uniformity of penetration was correlated with retention.

In the residue of decayed wood, concentrations of copper that were greater than that of the undecayed wood occurred most frequently in wood treated with ammoniacal copper citrate (CC) (Table 2). With this treatment and others, these results occurred most frequently at the lower retention levels in thinner blocks. Elevated concentration levels of copper in the decayed residues were slightly more frequent in wood decayed by *W. cocos* than in wood decayed by *P. placenta*. At the highest retention levels, concentration levels of the residual wood were sometimes less than that of undecayed wood.

4.2 Weight loss caused by decay

Statistical analysis of weight loss data revealed significant effects of major variates as well as a complex of significant interactions among preservatives, fungi, leaching procedure, and block size. Major effects are described first, followed by a discussion of the interactions.

4.2.1 Major effects

The capacity of *P. placenta* to decay untreated (control) blocks, without regard to block size, was significantly greater than that of *W. cocos*. (Table 3). The reverse occurred in treated blocks. Overall, the median weight loss caused by *P. placenta* was severely reduced by treatments, but that of *W. cocos* was slightly greater for the whole set of treated blocks than it was in the untreated blocks.

Table 3: Median weight loss in treated and in untreated (control) blocks

Blocks (all sizes combined)	Fungus	Median % weight loss	Rank test[a]
Untreated	*P. placenta*	48.1	A
	W. cocos	20.5	B
Treated	*P. placenta*	5.3	B
	W. cocos	21.7	A

[a] Within the lots of treated or untreated blocks, separately, values with different letters are significantly different.

Fungi varied significantly in their tolerance to the preservatives (Table 4). With *C. cocos,* the weight loss at the lower retention levels of several preservatives was significantly greater than that which occurred in the untreated controls (Table 4, Fig. 1). Such significant stimulation of growth was not observed with *P.* placenta.

Table 4: Weight loss by fungus for all blocks treated with preservatives

Preservative	Median percentage weight loss[a]	
	P. placenta	*W. cocos*
Cu Cit	22.3 A	46.9 A
ACQ-B	7.5 B	24.5 B
CuNap	2.9 B	4.1 C
ACZA	0.9 B	4.7 C

[a] Within each column, values with common letter are not significantly different.

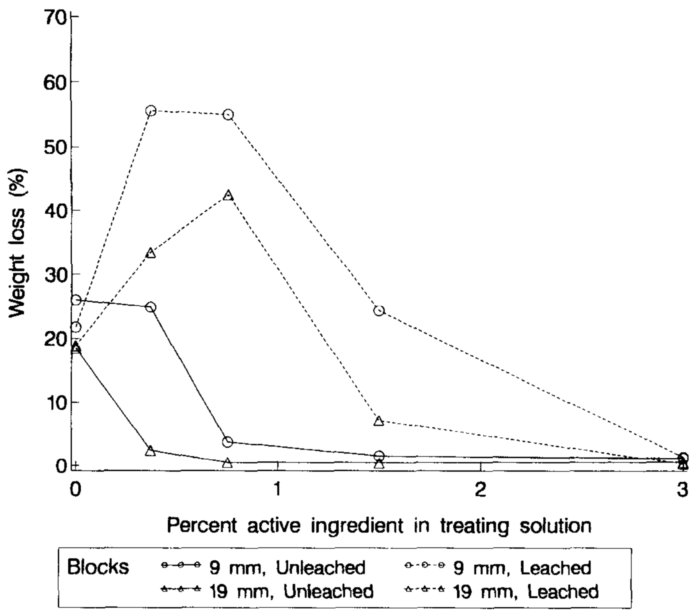

Figure 1a

Figure 1: Median percentage weight loss in blocks of Douglas-fir. See Table 1 for description of preservative composition. (a) ACZA - *W. cocos,* (b) ACZA - *P. placenta,* (c) ACQ-B - *W. cocos,* (d) ACQ-B - *P. placenta,* (e) CC - *W. cocos,* (f) CC - *P. placenta,* (g) CuN - *W. cocos,* (h) CuN - *P. placenta.* See Table 2 for data on actual retention levels that were achieved with the different preservatives.

Overall, significantly greater median percentage weight losses occurred in leached treated blocks than in unleached treated blocks. The relative difference between leached and unleached was greater for *P. placenta* than it was for *W. cocos* (Table 5).

Table 5: Weight loss in leached and unleached treated blocks.

Processing scheme	Median percentage weight loss[a]	
	P. placenta	*W. cocos*
Leached	10.2 A	26.8 A
Not leached	4.3 B	18.6 B

[a] Within each column, values with common letter are not significantly different.

Block size had a significant effect on decay capacity of both fungi in treated and untreated control blocks. With both fungi, the percentage weight loss in the g-mm-thick blocks was significantly greater than in the 19-mm-thick blocks. (Table 6). With *W. cocos*, the overall effects of block size and leaching were generally similar. With *P. placenta,* leaching was more important. In the untreated controls, block size was more important than leaching for both fungi.

Table 6: Weight loss in 9- and 19-mm thick treated and untreated blocks

Block thickness (mm)	Treatment	Fungus	Median % weight loss	Rank test[a]
9	none (control)	*P. placenta*	50.9	A
19	none (control)	*P. placenta*	44.3	B
9	none (control)	*W. cocos*	25.0	A
19	none (control)	*W. cocos*	18.8	B
9	treated	*P. placenta*	8.6	A
19	treated	*P. placenta*	3.2	B
9	treated	*W. cocos*	26.2	A
19	treated	*W. cocos*	15.9	B

[a] Within each pair, values with different letters are significantly different.

4.2.2 Interactions

Overall, the experimental variate with the most influence on the resultant weight loss in treated wood blocks was the choice of preservative (Fig. 2). Within those blocks treated by an individual preservative, the species of fungus deployed accounted for the largest difference in weight loss. Thereafter, the effects of preservative retention, leaching, and block size depended somewhat on the specific combinations within which they were assessed.

Preservative	Experimental Variates
ACZA	retention > leaching > size > fungi
ACQ-B	fungi • *W. cocos*: interactive effects • *P. placenta* – retention > leaching > size
CC	fungi • *W. cocos:* size > leaching • *P. placenta:* retention > leaching > size
CuN	retention > (small, but measurable effects)
control	fungi • *W. cocos:* size > leach • *P. placenta:* size > leach

Figure 2: Diagrammatic presentation of relative importance of interactive experimental variates, by preservative treatment.

The details of interactive effects (see Fig. 2) are reported by preservative with subordinate consideration given to fungi and the remaining variates within that grouping.

ACZA – With both fungi, leaching contributed significantly to enhanced weight loss. In the unleached blocks, the weight loss caused by *W. cocos* at all retention levels, except the lowest retention in the 9-mm-thick blocks, was significantly less than that which occurred in the untreated controls. The exception was equivalent to the control. Leaching accounted for greater weight losses in the blocks than did block size or reduced retention levels. In blocks of both sizes, the amount of weight loss at each of the three lower retention levels was significantly greater in leached blocks than in the respective unleached blocks. In leached 9-mm-thick blocks, the amount of weight loss at the three lower retention levels was also significantly greater than that of the controls (see Fig. la).

Growth of *P. placenta*, as determined by weight loss in the wood blocks, was suppressed at all retention levels. Weight losses as a result of attack by *P. placenta* were always significantly less than the controls. Leaching of the blocks accounted for more change in amount of weight loss caused by *P. placenta* than did block size or retention level. Block size was of relatively little importance, with the largest difference at the lowest retention level where more weight loss occurred in the 9-mm blocks (see Fig. lb).

ACQ-B – With *W. cocos*, all the experimental variates seemed to be interactive. Weight losses that were significantly greater than the control did occur at the lower retention levels in blocks that were 9 mm thick (see Fig. 1c).

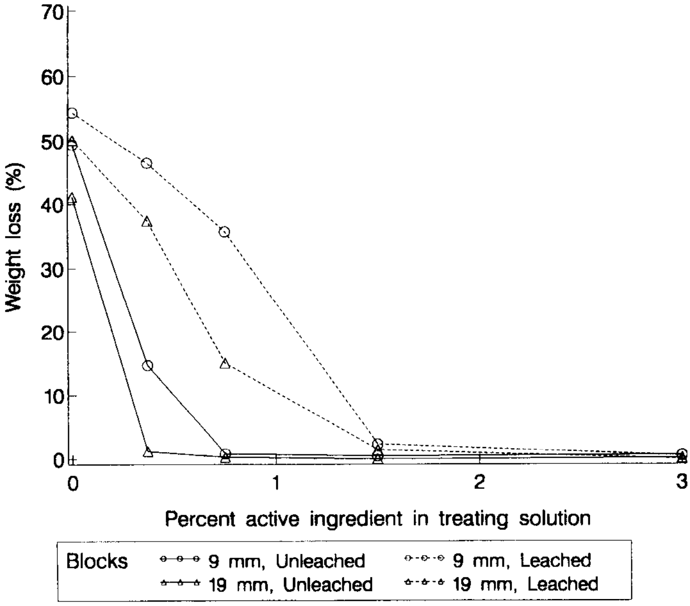

Figure 1b

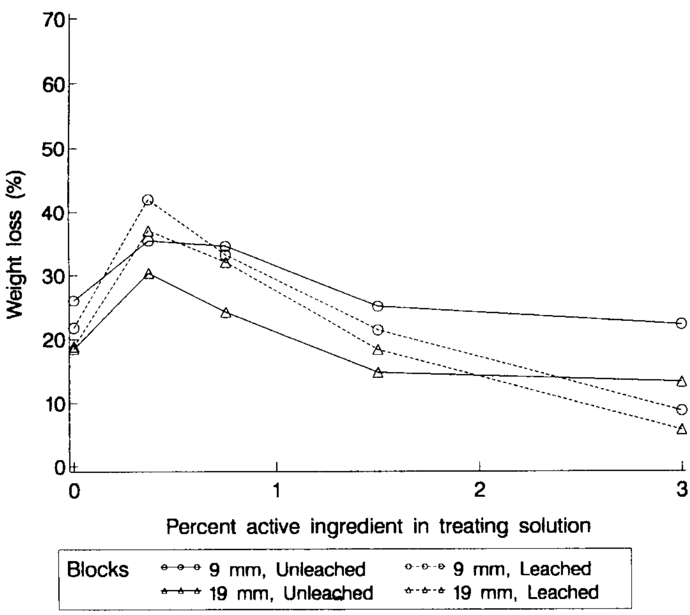

Figure 1c

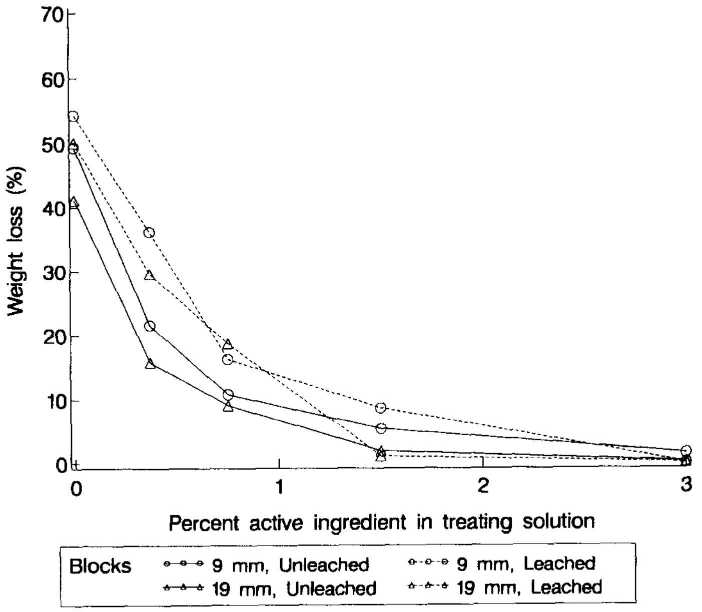

Figure 1d

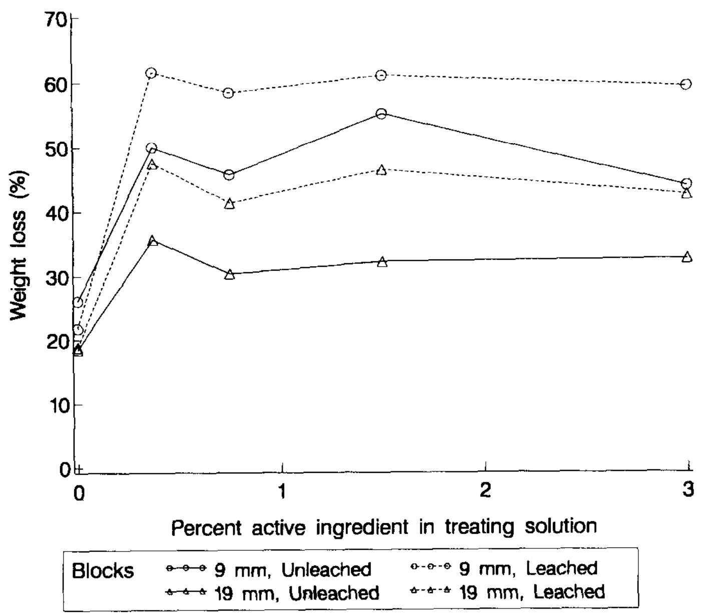

Figure 1e

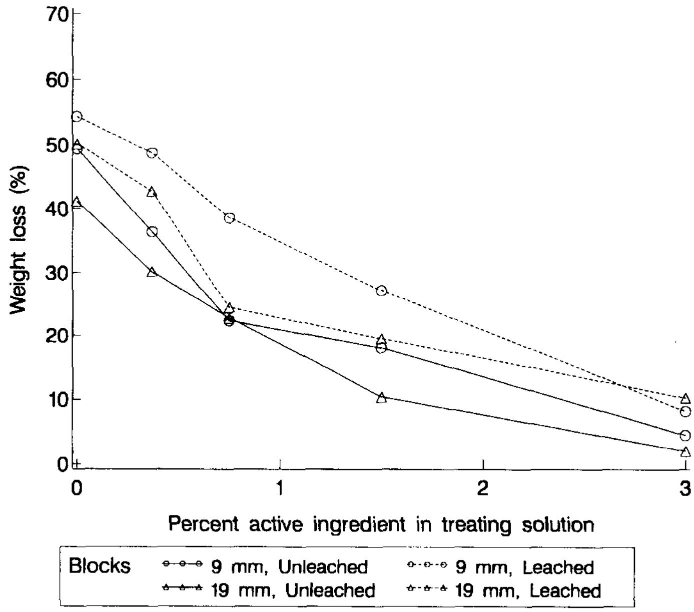

Figure 1f

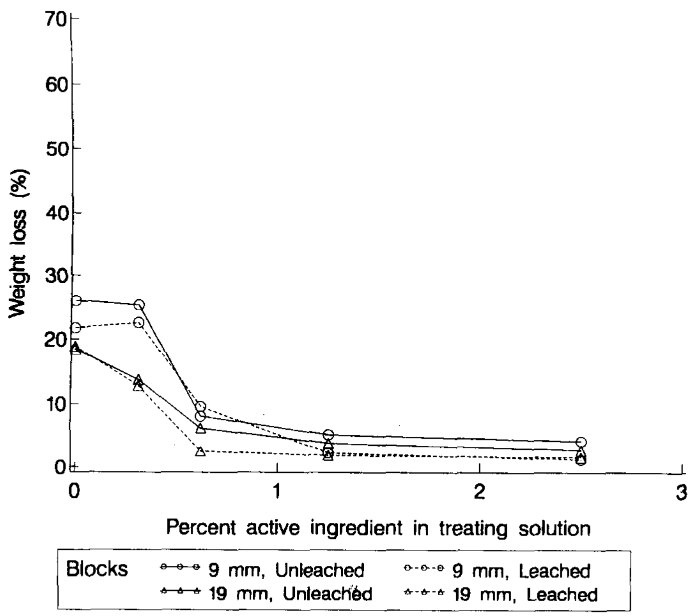

Figure 1g

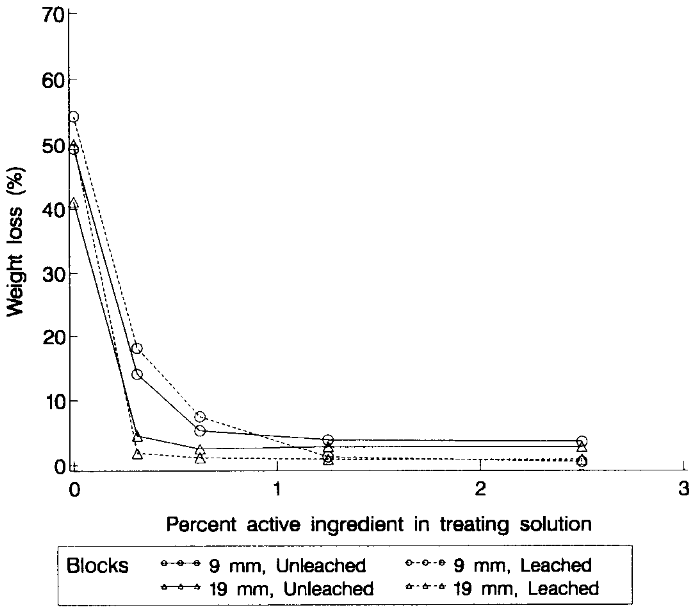

Figure 1h

With *P. placenta,* retention level had a dramatic effect (Fig. 1d). With *P. placenta,* significantly more weight loss occurred at the lower two retention levels in blocks of both thicknesses than in the higher retention levels. This probably reflects not only an increased amount of biocide per unit of mass, but also a greater internal distribution of preservative throughout the blocks as retention levels increased. None of the treated blocks subjected to *P. placenta* had weight losses significantly greater than in the untreated controls.

CC – Growth of *W. cocos* was stimulated by presence of CC in the wood to the extent that the amount of decay in most blocks treated with copper citrate was significantly greater than in the controls (Fig. 1e, Table 5). The amount of weight loss in the 9-mm blocks was significantly greater than that which occurred in the 19-mm blocks. The effect of leaching was also significant, but less so than the effect of block size.

Preservative retention had the most effect on suppression of *P. placenta* by CC (Fig. 1 f) and resultant weight losses. The largest weight loss was at the lowest retention level. The second most important factor was the leaching of the blocks, resulting in greatest gains in weight loss of blocks at midrange of the preservative retention levels. With unleached materials, block size had little effect. Slightly more weight loss occurred in the leached 9-mm blocks than in the leached 19-mm blocks. Size of block was apparently important only as it contributed to the extent of leaching, not to metal transport by the fungus.

Table 5: Weight loss caused by *W. cocos* in treated blocks (all sizes and leaching regimes combined).

Concentration of active ingredient in waterborne preservative	Preservative (median % weight loss)[a]			
	ACZA	ACQ	CC	CuN
0.0	20.5 B	20.0 B	20.5 B	20.5 A
0.37	29.6 A	35.5 A	48.5 A	18.9 A
0.75	18.5 C	32.4 A	45.9 A	6.0 B
1.5	5.5 D	21.1 B	50.0 A	3.1 C
3.0	3.0 E	11.2 C	43.3 A	2.5 C

[a] Medians with same letter are not significantly different. Those followed by different letters are significantly different.
[b] Concentrations of copper naphthenate were slightly different from those shown for waterborne preservatives. See Table 2 for actual retention levels obtained with each preservative.

CuN – The least amount of weight loss and variation among retention levels was in blocks treated with copper naphthenate (Fig. 1g, h) Statistically significant differences associated with leaching, being important at the high retention levels and in block size at the lower retention levels, were detected. With the low amount of weight loss, however, these differences are probably not of practical importance.

5. Discussion

A growing, public awareness of needs for product management on a life cycle basis is expanding the list of criteria to be considered as materials are selected. Post-consumer disposition of construction materials is one concern that is receiving more attention in that selection process. Whether some form of bioprocessing is deployed in the conversion of spent, treated-wood products remains to be determined, but these results indicate that for some systems, the potential does exist. It must be recognized that the transition from laboratory-scale experiments to an industrial operation involves many challenges. Potential biological purification processes must be evaluated critically in a sequence of laboratory-scale and larger scale phase-up trials before their suitability can be confirmed (H. LEITHOFF and R.-D. PEEK, 1997).

A commercial lot of spent, treated Douglas-fir wood will contain a mix of both treated and untreated fiber. Douglas-fir has refractory heartwood. In commercial practice, timbers of Douglas-fir are not treated throughout. Protection from preservative treatment is derived from a "shell" of preservative around the periphery of the product. Thus, the presence of both treated and untreated heartwood, as occurred in blocks of some stakes, is representative of wood in commerce. Most waterborne preservative systems used on Douglas-fir are typically ammoniacal, which enhances preservative penetration into the wood. Because the alkalinity of the treating solution is somewhat linked to the concentration of the active ingredient, the uniformity of cross sectional penetration of waterborne systems in this study was also linked with retention.

H.-P SUTTER *et al.* (1983) postulated that the oil component physically prevents movement of precipitated copper oxalate to the exterior of the wood surface. This principle may, in some way, have contributed to the reduced weight loss as a result of decay in blocks treated at all retention levels with copper naphthenate.

The potential for use of copper-tolerant fungi in composting operations was demonstrated, but potential for bioconcentration of metals was less well demonstrated in this work. A mass balance calculation was not possible because no determination was made of the copper content of the mycelium removed from the blocks when they were withdrawn from the decay jars, and the amount of copper that may have leached or translocated to the supporting feeder strip and soil was not determined. The reduced density or selective decay of less treated wood in individual blocks may also have contributed to apparent results. The same amount of copper in a less dense residual wood or in wood where the untreated component was disproportionately decomposed would yield an apparent increased concentration of copper.

The importance of leaching seems to be inversely related to fungal tolerance of the preservative-treated wood. The fact that concentration of metals during decay was most pronounced in wood treated to low retention levels and in the thinner blocks suggests that a chemical extraction may be needed at some time in commercial operations targeted at recycling metals from treated wood.

It might be questioned whether decay fungi, such as *W. cocos,* which are significantly stimulated by presence of copper in wood, should be included as assay fungi in standardized evaluations of copper-based preservatives. It has been demonstrated in laboratory trials with treated and untreated plywood veneers that proximity of treated wood to untreated wood can protect the untreated wood from attack by certain decay fungi (E. W. B. **Da Costa** and L. D. **Bezemer,** 1973). However, that effect does not seem to be universal-witness the stimulatory effect that presence of copper had on the decay capacity of *W. cocos.* Extrapolation of these results to ultimate durability of treated wood in field applications is difficult. As mentioned, we have stakes treated with these same systems and installed in field plots. We are not observing massive failures of these systems, even at the low retention levels, within a few years of installation in field plots. However, examination of data from a 30-year field plot (R. C. De **Groot,** 1988) revealed that copper-naphthenate-treated stakes that performed as well as reference stakes during the first decade of testing did less well during the next two decades. It may be speculated that metal leaching from a source (treated-wood product) would on a microscale cause the same change in soil microflora and fauna that occurs in larger sites. If copper-tolerant, wood-degrading fungi were present in the surrounding soil, they would ultimately be preferentially selected. Depending on the concentration of copper building up or eluting from the soil proximal to the product and the species of fungi present, decomposition could occur rapidly, when the concentration of copper decreased below subthreshold levels for fungi that are stimulated by presence of copper.

6. Conclusions

Results of this study indicate that certain fungi either adapt or are stimulated by copper in wood and that these fungi have potential for bioprocessing copper-containing wood. Using only two fungi and four preservatives, results also indicate that the potential for commercial bioprocessing of spent wood that has been treated with copper-based preservatives is (a) greater for some preservatives than others, (b) could be optimized through careful paring of specific fungi for individual preservatives, and (c) could be enhanced by developing leaching procedures to remove high concentrations of preservative prior to bioprocessing.

Relative to future commercial bioprocessing schemes, results from these two fungi indicate that particle size and leaching are important variates. This suggests that comminution processes should be keyed to the optimization of extraction and biodegradation processes.

7. Summary

In laboratory experiments, Douglas-fir wood blocks that were treated with copper-based wood preservatives were challenged with two wood decay fungi known to be tolerant of copper. Factors influencing the amount of decay, as determined by loss of weight in the test blocks, were preservative, then fungus. Within those combinations, the relative importance of preservative retention, block thickness, or leaching depended upon the specific pairings of preservative and fungus. Low retention levels of some preservatives stimulated decay by *Wolfiporia cocos* but not *Postia placenta*. We conclude that the potential for commercial bioprocessing of spent wood that has been treated with copper-based preservatives is greater for some preservatives than others. Optimization of the process can be achieved through selection of specific fungi, comminuting wood furnish to a small particle size, and developing preprocessing leaching procedures.

Zusammenfassung

Wolfiporia cocos – ein möglicher Organismus zur Kompostierung und biologischen Aufbereitung von Douglasienholz, das mit kupferhaltigen Schutzmitteln behandelt wurde

In Laborversuchen wurde mit kupferhaltigen Schutzmitteln behandeltes Douglasienholz mit zwei kupfertoleranten Pilzen geprüft. Der Einfluß des Schutzmittels und der Pilzart auf das Verrotten von Holz wurde anhand des Gewichtsverlustes der Prüfklötzchen bestimmt. Der relative Einfluß der Schutzmittelaufnahme, der Klötzchengröße oder der Auswaschung auf die einzelnen Pilz-Schutzmittel-Kombinationen wurde untersucht. Geringe Mengen einiger Schutzmittel stimulierten den Befall durch *Wolfiporia cocos,* nicht jedoch den durch *Postia placenta.* Aus den Ergebnissen wird geschlossen, daß eine kommerzielle biologische Aufbereitung von Altholz, das mit kupferhaltigen Schutzmitteln behandelt war, bei einigen Schutzmitteln aussichtsreicher als bei anderen ist. Das Verfahren kann durch die Auswahl spezifischer Pilze, durch die Zerkleinerung des Holzes und durch die Entwicklung geeigneter Auschwaschverfahren optimiert werden.

Résumé

Wolfporia cocos, un agent potentiel pour le composte ou pour le traitement des bois préservés avec des produits à base de cuivre

Au cours d'expériences en laboratoire, des échantillons de bois traités avec des agents de préservation à base de cuivre ont été soumis à deux champignons de pourriture connu pour tolérer le cuivre. Les facteurs qui influencent la pourriture, déterminés par la perte de poids des échantillons de bois, ont été l'agent de préservation et le champignon. Dans ces différentes combinaisons, l'importance relative de la rétention de l'agent de préservation, de l'épaisseur des échantillons de bois, ou du lessivage dépend de l'association agent de préservation et champignon. Les taux de rétention bas de certains agents de preservation stimulent la pourriture par *Wolfiporia cocos* mais pas par *Postia placenta.* Nous avons conclu que le potentie1 de commercialiser le bioprocédé pour des bois qui ont été traités avec des agents de cuivre est plus important pour certains agents de preservation que pour d'autres. L'optimisation du procédé peut être achevée en sélectionnant des champignons spécifiques, réduisant le bois en particules de petite taille, et en développant des procédés de prélessivages.

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