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Changes of the pH value of impregnated wood during exposure to wood-rotting fungi

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Changes of the pH-value of impregnated and unimpregnated wood caused by wood rotting fungi were studied. Wood, impregnated with two aqueous solutions of copper(II) sulfate and of copper(II) octanoate with ethanolamine, was exposed to different wood rotting fungi for 2, 4 and 12 weeks. Two of them were white-rot fungi (*Trametes versicolor* and *Schizophyllum commune*). The third species was the brown-rot fungi *Antrodia vaillantii*, using three different strains of this species. *Antrodia vaillantii* substantially decreased the pH-value of impregnated and unimpregnated wood before any mass loss appeared. On the other hand, the white-rot fungus *T. versicolor* and *S. commune* caused a slight increase of the pH of impregnated and unimpregnated wood. It is suggested that a decrease of pH of wood may indicate early stages of decay by brown rot fungi.

Änderungen des pH in imprägnierten Hölzern während des Pilzabbaus

Änderungen des pH in imprägnierten und unbehandelten Holzproben während des Pilzabbaus wurden untersucht. Zur Imprägnierung wurden zwei wässrige Lösungen von Kupfer(II)sulfat und Kupfer(II)octanoat mit Ethanolamin verwendet. Die Proben wurden einem Pilzabbau über 2, 4 und 12 Wochen ausgesetzt. Zwei Pilzstämme waren Weissfäuleerreger (*Trametes versicolor* und *Schizophyllum commune*). Die dritte Art war vom Braunfäuletyp (*Antrodia vaillantii*), wovon drei verschiedene Stämme eingesetzt wurden. *Antrodia vaillantii* senkte den pH deutlich in imprägnierten und unbehandelten Proben, noch bevor ein Massenverlust auftrat. Die beiden Weissfäuleerreger *T. versicolor* und *Sch. commune* ließen den pH dagegen ansteigen. Anhand des pH-Abfalls konnte man schon frühe Stadien eines Braunfäuleabbaus erkennen.

1 Introduction

Wood decay can be detected visually through discolourations, changes in structure, changes in figure and the like. However, in order to inactivate fungi in infested wood before they can cause substantial damage, it is very important to detect them early, in the first stages of attack and decay. The first stages of decay are exhibited by strength loss, accumulation of ions, decreased pH-value of wood etc. (e.g. Shortle 1990). Changes were observed of the pH of infested wood in vitro long before mass loss could be detected, and the results of this research are reported herein.

Ions accumulating in wood infected by fungi can be associated with a markedly increased acidity of wood (drop in pH) at all stages of decay (Shortle 1990). A substantial reduction of the pH-values caused by fungi was observed both in liquid cultures and in solid wood substrates (e.g. Jellison 1992). Especially brown-rot fungi were able to cause a significant reduction of pH of wood. White-rot fungi were also associated with a pH decrease of degraded wood, though to a lesser degree (Jellison 1992). There is an evidence to support the very important role that oxalic acid plays in the production of acidic environment (Takao 1965; Shimada et al. 1994). Oxalic acid is a small organic acid with two low pK values ($pK_1 = 1.27$, $pK_2 = 4.26$) (Skoog et al. 1992). It is often produced in great quantities by brown-rot fungi (Takao 1965; Green et al. 1991) and may be a very strong candidate for playing a central role in producing the acid environment associated with brown-rot colonization of wood (Jellison et al. 1997). Additionally, incipient brown-rot decay is similar to acid hydrolysis and characterized by acid production (Green et al. 1991). Lastly, fungi produce and secrete many acidic groups containing compounds, such as phenolic acids and other simple organic acids, generated during metabolism (Jellison et al. 1997).

A lot of work has been done in determining pH of undecayed and untreated decayed wood, but to the best knowledge of the authors, reports on pH changes of impregnated wood during decay are very rare in the literature (Göttsche and Borck 1990; Stephan et al. 1996). Thus we were interested in how fungi respond to preserved wood and what happens with the pH-value of infected preserved wood. The focus was on copper-based preservatives, copper being one of the most important ingredients in commercial biocides. The use of these compounds for biocidal purposes has been increased in recent years (Richardson 1997). However, several brown-rot fungi reveal to be copper tolerant and consequently, efficacy of copper-based wood preservatives may not be sufficient. Metal tolerance can be defined as the ability of an organism to survive metal toxicity by means of intrinsic properties and/or environmental modification of toxicity (Gadd 1993). Copper tolerance is especially exhibited by some fungi that are closely aligned to or included in genus

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Antrodia (Sutter et al. 1983; Collett 1992; Tsunoda et al. 1997; Woodward and De Groot 1999). Among *Antrodia vaillantii, A. Sinuisa, A. serialis, A. xantha* and *Oligoporus placenta*, highest copper tolerance was found for *A. vaillantii* followed by *O. placenta* (Schmidt and Moreth 1996). The resistance of some species of decay fungi to copper has been linked to their production of copious amounts of oxalic acid (Murphy and Levy 1983).

A generally used procedure to determine the pH of wood is to suspend wood sawdust in 5 to 10 times as much distilled water by weight, bringing it to a boil, then cooling it or merely stiring it for various lengths of time without heating, followed by determining the pH of the obtained water extract (Stamm 1961). A question arises: do such experiments give the right pH of air-dried material as well as the pH of equilibrium solid-water system? Fortunately, wood shows a large buffering action on the water (Goodell et al. 1997). Therefore, buffer capacity of wood takes a prominent part in conservation of pH (Albert et al. 1999). The extraction method is acceptable for comparative determinations (Stamm 1961; Fengel and Wegener 1989), and it was decided to use the above method in this investigation.

2
Materials and methods

2.1
Sample preparation

Samples were made of Norway spruce [*Picea abies* (L). Karst] sapwood. They were 3.0 (longitudinal direction) × 1.0 × 0.5 cm sized and impregnated with two different copper water-borne preservatives. The concentration of copper was 1.0×10^{-2} mol/l, being equal in both solutions. We chose this concentration, because at this concentration the growth of fungi was slow, but did not stop. We used two aqueous solutions for impregnation, one was acid [copper(II) sulfate, pH = 3.5] and the other one was alkaline [copper(II) octanoate with ethanolamine pH = 11.0]. Additionally, in some cases, we impregnated the wood with solutions of a higher copper concentration ($c_{Cu} = 0.75\%$). For comparison, some samples were impregnated with distilled water.

The samples were vacuum impregnated, according to the EN 113 procedure (ECS 1989). Treatment of small blocks resulted in a solution uptake of about 40.5% of the oven-dry wood mass. The treated wood samples were placed in petri dishes, and were left to dry for three weeks, in the first week in closed, during the second week in half closed and in the third week in opened petri dishes.

2.2
Fungal strains, growth conditions and exposure of the samples

Used were five species or strains of fungi. The white rot species were *Trametes versicolor* (L.: Fr.) Pilát (recently isolated strain) and *Schizophyllum commune* (Fr.: Fr.) ZIM L042. The other three were three isolates of the same species of the brown-rot fungus *Antrodia vaillantii* (DC.: Fr) Ryv. (P163 HUM UH (*A. vaillantii* 1) and P113 DFPG 6911 UH (*A. vaillantii* 2) and ZIM L037 (*A. vaillantii* 3)) (Raspor et al. 1995). *Antrodia vaillantii* 1 and 2 have been kindly provided by O. Schmidt, Hamburg. Cultures were maintained on solid plating media which contained 3.9% potato dextrose agar of pH 5.3 (PDA Difco). Petri dishes with PDA medium were inoculated with small pieces of fungal mycelium. Afterwards, four samples of treated and untreated wood were placed into each inoculated petri dish. The samples were exposed to fungal decay (: dark, 25 °C, RH = 95%) for 2, 4 and 12 weeks.

2.3
Determination of the pH-value

After exposure to fungi, impregnated and unimpregnated spruce samples were isolated. Some samples were used to detect the rate of decay, and the others for pH measurements. The pH of wood was determined by an extraction method. Wood samples were ground into sawdust, which can pass through a 40-mesh screen. Sawdust of 2.0 was then immediately added to 50 ml of boiling de-ionized water (pH = 6.5) and stirred for 5 minutes in an Erlenmeyer flask with reflux. The mixture was standing in the closed (Erlenmeyer) flask for 30 minutes and was then rapidly cooled to room temperature. The extract was then filtered and pH of the solution was measured with a glass electrode. The experiment was performed in three parallel measurements not showing any significant difference between them.

2.4
Determination of the degree of decay

Decay was measured gravimetrically. We determined masses of oven-dry wood samples before and after exposure to wood decay fungi and expressed decay in percent of the initial mass.

3
Results and discussion

3.1
pH-value of preservative solutions and unexposed wood

As can be seen from the Table 1, the pH of spruce wood was measured to be 5.1. The same value was reported in the literature (Fengel and Wegener 1989).

The pH of wood impregnated with a solution of copper(II) sulfate ($c_{Cu} = 1.0 \times 10^{-2}$ mol/l) was 4.7, and therefore a little bit more acid than pH of unimpregnated wood, as the water-based solution of copper(II) sulfate was acid (pH = 3.1). If the wood was impregnated with a

Table 1. pH-values of copper based preservative solutions and of wood treated with these solutions
Tabelle 1. pH werte von Kupfer-haltigen Holzschutzmitteln sowie von Holz nach Behandlung mit diesen Lösungen

Preservative	Cu – Concentration	pH of the solution	pH of impregnated wood
Control (H_2O)		5.7	5.1
Cu sulfate	1.0×10^{-2} mol/l	3.1	4.7
	0.75%	2.7	4.2
Cu octanoate	1.0×10^{-2} mol/l	11.0	6.9
	0.75%	12.3	9.0

solution of higher concentration of copper(II) sulfate and lower pH ($c_{Cu} = 0.75\%$, pH = 2.7), the pH of wood decreased to the value of 4.2 (Table 1).

Wood, protected with copper(II) octanoate with ethanolamine ($c_{EA} = 2\%$) had an almost neutral pH (pH = 6.9), thus this pH is higher than the pH of unimpregnated wood. The pH of the water based solution copper(II) octanoate with ethanolamine solution was 11.0. The pH of wood impregnated with preparation with higher amount of copper(II) octanoate and higher pH ($c_{Cu} = 0.75\%$, $c_{EA} = 20\%$, pH = 12.3) was even higher (9.0).

Therefore, the buffering ability of wood (Goodell et al. 1997; Albert et al. 1999) resulted in higher pH values of treated wood in comparison with the pH of impregnating acidic solutions or lower pH values of impregnated wood than pH of corresponding alkaline solutions (max 9.0, comparing to 12.3 of treatment solution).

3.2
Influence of wood-decay fungi on the pH-value of untreated wood

The pH of wood exposed to *A. vaillantii* was higher than that of unexposed samples, and it was increasing with the time of exposure to this fungus. After two weeks of exposure to *A. vaillantii*, a significant reduction of pH was observed (Table 2). The reason for this drop may be oxalic acid produced by brown-rot fungi (Takao 1965). Oxalic acid has an important role in first, pre-visual, non-enzymatic stages of decay (Shimada et al. 1994; Goodell et al. 1997). If we compare pH-values (Table 2) with the rate of decay (Table 2), it can be seen that significant changes of pH were observed before mass loss was exhibited. For instance, the pH- value of wood, exposed to *A. vaillantii* 1. decreased from 5.1 to 4.1 after 2 weeks. However, negligible decay was observed after this period. A decrease of pH was even more prominent when decay proceeded (Table 2), so the decrease of the pH of wood may be an early indicator of a decay process. Similar changes have been observed at a related brown-rot fungus *Oligoporus placenta* (Jellison 1992; Shortle 1990).

White-rot fungi behaved differently. It is known that white-rot fungi do not cause such significant reductions of pH of wood as brown-rot fungi do (Shortle 1990). For instance, fungi related with genus *Antrodia* produce almost three-times more oxalic acid than *T. versicolor* (Takao 1965). Generally, this fact correlates well with our results (Table 2). Two and four weeks of exposure resulted in almost the same pH (5.2), but after the 12th week, the first drop was noticed to the value of (3.9). Nor was the first mass loss noticed before the 12th week. Thus, pH observations are less reliable for detecting the presence of *T. versicolor* in wood before the occurrence of the mass loss.

Schizophyllum commune caused specific changes of the pH of infested wood. This fungus did not decrease pH of exposed wood, but increased it (Table 2). This increase was less significant than the decrease at *A. vaillantii*. A comprehensive investigation using 48 different isolates of *S. commune* revealed that this species prefers a pH-value of about 5 and changes the initial pH to values between 4.2 and 7.5 (Schmidt and Liese 1978). The exposure of wood to *S. commune,* so far for unknown reasons here, did not result in any mass loss of the samples. A slight increase of mass was observed instead. Non-ability of *S. commune* to degrade wood was not surprising since its very low decomposing ability is well known (Eaton and Hale 1993; Vesel-Tratnik 1994). Also the 48 isolates investigated by Schmidt and Liese (1980) only exhibited up to 2.9% mass loss on softwood samples within seven months of exposure.

3.3
pH of wood impregnated with copper sulfate and exposed to wood-decay fungi

Copper(II) sulfate did not prevent decay of protected wood, but it was slowed down at all fungi, with only one slight exception at *A.v.*1 (Table 2). *Antrodia vaillantii* degraded wood to a higher extent than *Trametes versicolor*. *Schizophyllum commune,* as with unimpregnated wood, did not cause any mass loss.

Wood rotting fungi caused similar pH changes of wood protected with copper(II) sulfate, to those of unimpregnated wood. After 12 weeks of exposure to all strains of *A. vaillantii,* could be detected no significant difference in pH between exposed impregnated and exposed unimpregnated wood. As in the case of untreated wood, pH of treated wood also markedly decreased after a two-week exposure period. Again, mass loss was not observed during the first two weeks of decay, thus pH decrease is an important pre-visual sign, which indicates the presence of *A. vaillantii* in wood.

During the first two weeks of exposure to the white-rot fungus *T. versicolor,* no changes of pH were observed. After 4 weeks of inoculation, this fungus increased pH of wood to the value of 5.4 and after 12 weeks of inoculation to 5.3. (Table 2). At this time the first mass loss (1%) was noticed. A question is, what happens with pH when the decay proceeds? If the same pH drop occurred, as at unprotected samples, this method cannot be used as a reliable pre-visual detection of white-rot fungi in wood.

The pH of wood attacked by *S. commune* was only slightly increasing all the time during exposure, from initial 4.7 to 5.2 after two and to 5.6 after 12 weeks of exposure. This shift might be a consequence of exposure of wood to *S. commune*. On the other hand, this fungus did not cause any mass loss.

3.4
Wood impregnated with copper octanoate and ethanolamine

Fungi exhibited problems in degrading wood impregnated with copper(II) octanoate (Table 2). During 12 weeks, wood exposed to different strains of *A. vaillantii* lost max. 3% of mass. Exposure to *T. versicolor* resulted in the same mass loss. As anticipated, *S. commune* did not induce any mass changes. One of the reasons might be the pH-value of impregnated wood (6.9). Some fungi are more resistant to copper in acid media. On the other hand, copper is strongly toxic or inhibitory near neutrality, as reported for soft-rot fungus *Scytalidium* sp. (Starkey 1973).

The pH of treated wood exposed to brown-rot fungi rapidly decreased from 6.9 to 4.9–5.6 after two and to 3.3–4.3 after 12 weeks of exposure. We again believe that this drop of pH is an early sign of decay, although mass loss

Table 2. Decay, moisture content and pH-value of impregnated and unimpregnated wood ($c_{Cu} = 1 \times 10^{-2}$ mol/l) after 2, 4 and 12 weeks of exposure to wood-destroying fungi (for isolates see Methods)
Tabelle 2. Abbaugrad, Feuchte und pH-Wert von imprägniertem und nicht imprägnierten Holz ($c_{Cu} = 1 \times 10^{-2}$ mol/l) nach 2-, 4- und 12-wöchigem Abbau durch holzzerstörende Pilze

Preservative	Fungi		Weeks of exposure			
			0	2	4	12
Control	*Antrodia vaillantii 1*	decay		0%	1%	7%
		MC	10%	22%	77%	57%
		pH	5.1	4.1	3.7	3.5
	Antrodia vaillantii 2	decay		0%	3%	10%
		MC	10%	18%	25%	42%
		pH	5.1	4.2	4.0	3.5
	Antrodia vaillantii 3	decay		0%	6%	14%
		MC	10%	47%	30%	40%
		pH	5.1	4.0	3.7	3.2
	Trametes versicolor	decay		0%	0%	8%
		MC	10%	19%	28%	57%
		pH	5.1	5.2	5.1	3.9
	Schizophyllum commune	decay		0%	−1%	−5%
		MC	10%	24%	36%	62%
		pH	5.1	5.4	6.2	5.5
Copper sulfate	*Antrodia vaillantii 1*	decay		0%	1%	8%
		MC	11%	33%	33%	86%
		pH	4.7	3.7	3.6	3.5
	Antrodia vaillantii 2	decay		0%	1%	9%
		MC	11%	19%	35%	67%
		pH	4.7	4.1	3.8	3.5
	Antrodia vaillantii 3	decay		0%	2%	11%
		MC	11%	31%	28%	39%
		pH	4.7	3.7	3.8	3.3
	Trametes versicolor	decay		0%	0%	1%
		MC	11%	19%	26%	56%
		pH	4.7	4.7	5.4	5.3
	Schizophyllum commune	decay		0%	0%	−4%
		MC	11%	22%	33%	47%
		pH	4.7	5.2	5.5	5.6
Copper octanoate and ethanolamine	*Antrodia vaillantii 1*	decay		0%	1%	0%
		MC	19%	53%	33%	142%
		pH	6.9	5.3	4.6	4.3
	Antrodia vaillantii 2	decay		0%	0%	1%
		MC	19%	40%	61%	109%
		pH	6.9	5.6	4.8	3.9
	Antrodia vaillantii 3	decay		0%	0%	3%
		MC	19%	64%	68%	116%
		pH	6.9	4.9	5.0	3.3
	Trametes versicolor	decay		0%	0%	3%
		MC	19%	27%	58%	76%
		pH	6.9	7.7	7.7	4.8
	Schizophyllum commune	decay		0%	0%	0%
		MC	19%	37%	63%	95%
		pH	6.9	7.4	6.8	6.0

after two weeks was still not noticeable. When these fungi overgrow samples impregnated with copper(II) octanoate, the color of wood samples is changed from greenish to brownish. Some authors (Murphy and Leavy 1983; Collet 1992) reported it to be copper oxalate.

As already mentioned, exposure of wood treated with Cu(II) octanoate/ethanolamine to *A. vaillantii* resulted in max 3% mass loss. This value is low compared to 11% in wood treated with Cu(II) sulfate. Obviously, Cu(II) octanoate/ethanolamine is at least for *A. vaillantii* a more efficient preservative than Cu(II) sulfate. Anyway, decomposition of Cu(II) octanoate/ethanolamine treated wood was not prevented. In spite of a relatively high initial pH of impregnated wood (6.9), *A. vaillantii* caused its fast and substantial decrease (*A. vaillantii* 3 after two weeks to 4.9 and after 12 weeks to 3.3). The final value of pH of wood, exposed to *A. vaillantii* (3.3), is nearly the same as the one measured on exposed untreated wood samples and exposed samples treated with copper(II) sulfate. This means that although we noticed the first signs of brown-rot after 12 weeks, the degradation process started long before any mass loss. As it could be expected from previous experiments, the

white-rot fungi *T. versicolor* and *S. commune* behaved differently. Firstly, they increased pH of copper(II) octanoate/ethanolamine treated wood (7.7 and 7.4), and decreased it after 12 weeks to 4.8 and 6.0, respectively. This decrease of pH occurred at the same time as the mass loss, so it cannot be a pre-visual indicator for the infection of wood with white-rot fungi.

3.5
Moisture content of wood exposed to fungi

The humidity of the exposed samples was checked (Table 2). Moisture content of untreated wood was increasing all the time during the exposure to fungi, from initially 10% to in one case 62%, after 12 weeks of exposure, which is above the fiber saturation point (FSP) (Table 2) at about 30% for Norway spruce. As in the present investigations, moisture content above FSP has been also reported by some other authors (e.g. Shortle 1982). Wetting of protected wood was more prominent. Moisture content of wood impregnated with copper(II) sulfate after 12 weeks of exposure to brown-rot fungi reached 39–86%. Wetting of wood impregnated with copper(II) octanoate with ethanolamine was even more drastic (116–142%) as Cu(II) octanoate is poorly soluble in acid medium, thus fungi have to humidify wood better. Fungi try to make copper soluble and than transport it, or transform it into another, less toxic form. This presumption was suggested based on our recent Electron Paramagnetic Resonance (EPR) studies (Humar et al. 1999).

White-rot fungi proved to be less effective in the wetting of wood (Table 2). This may be due to the fact that white-rot fungi are more extensive in situations where abiotic wetting (e.g. rain, etc.) is more easily performed (Carlile and Watkinson 1994).

3.6
Differences between different strains of copper tolerant fungi *A. vaillantii*

We did not find any important differences between strains of *A. vaillantii* except for their activity. The third strain was the most active one. Mass losses of untreated wood and of wood impregnated with both preservatives were the highest in the case of the *A. vaillantii* strain No. 3 (Table 2). Changes of pH values of untreated and treated wood, exposed to three strains of *A. vaillantii* were indicative, too. For instance, decay by the most active strain of *A. vaillantii* (No. 3) caused a decrease of pH of wood, impregnated with Cu(II) octanoate/ ethanolamine to the final value of 3.3. In contrast, the least active strain, *A. vaillantii* (No. 1), induced a change of pH from 6.9 to 4.3 only. The same, but somewhat less considerable phenomenon, was observed on wood, impregnated with Cu(II) sulfate and untreated wood. On the other hand, we should be careful with the interpretation of these results in terms of copper tolerance. Lower activity of a certain strain does not necessarily mean lower copper tolerance. As reported by Woodward and De Groot (1999), activity and tolerance are not closely correlated. Among the ten isolates of *A. vaillantii* investigated for copper tolerance isolate DFPG 6911 (*A.v.* 2) belonged to the most tolerant strains (Schmidt and Moreth 1996).

4
Conclusions

Both preservatives, copper(II) sulfate and copper(II) octanoate with ethanolamine were effective against the tested wood-decay fungi *Antrodia vaillantii*, *Trametes versicolor* and *Schizophyllum commune*. At the same copper concentration ($cCu = 1.0 \times 10^{-2}$ mol/l) copper(II) octanoate with ethanolamine revealed a somewhat higher biological activity against brown-rot fungi. Strains of *A. vaillantii* differed in their activity. However, there is no correlation between activity and copper tolerance.

The extraction method is applicable for the determination of the pH of wood for untreated wood as well as for the impregnated wood.

The pH is one of the most important pre-visual signs of brown-rot decay, and it is useful for an early detection of brown-rot fungi in wood. Significant decrease of pH-value was observed at least two weeks before the first mass loss occurred. This decrease was observed at unimpregnated and at impregnated wood. White-rot fungi did not cause such significant pH changes. Thus, this method is less or even not at all appropriate for a pre-visual detection of white-rot fungi in wood.

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