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Resistance of Wood Treated with Iron Compounds against Wood-Destroying Decay and Mould Fungi

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Abstract: Treatment of wood with various physical and chemical factors can change the number of wood parameters, which can also lead to changes in resistance to wood-destroying fungi. This study evaluates the effects of hydrothermal treatments (additives Fe₂O₃ or FeCl₃ with and without commercial tannins, also without additives and fresh wood) on decay and mould fungi resistance of modified wood of Scots pine (Pinus sylvestris), Norway spruce (Picea abies), Douglas fir (Pseudotsuga menziesii), walnut (Juglans regia), and Norway maple (Acer platanoides). For wood samples, the resistance against wood decay fungi Trametes versicolor (white rot) and Coniophora puteana (brown rot) and the resistance against mould fungi Aspergillus niger and Penicillium sp. were assessed. The study findings showed that wood modified with iron compounds could cause a higher resistance to wood-destroying fungi. The weight losses of the modified and control wood, caused by T. versicolor and C. puteana, differed for coniferous and deciduous: the average weight loss of treated pine, spruce, and fir wood caused by C. puteana was higher than that caused by T. versicolor, while these differences on maple and walnut wood were not significant. The wood hydrothermal treatment with Fe₂Cl₃ with and without tannins significantly reduced the weight loss caused by *T. versicolor* and C. puteana, and the treatment with Fe₂O₃ slightly improved the decay resistance. For the wood, hydrothermally modified with FeCl₃ and FeCl₃ + tannins, the mould area for both tested Aspergillus niger and Penicillium sp. was smallest for the wood of all tested tree species compared to other treatments. A different response was obtained for coniferous and deciduous tree species wood. The spruce wood, followed by fir wood, treated with FeCl₃ with and without tannins, was the most resistant against the mould fungi. Relatively low resistance against the mould fungi was fixed for the maple wood treated by various iron compounds, except the treatment with Fe₂O₃ + tannins, which gave a very positive response against the *Penicillium* sp.

Keywords: wood modification; iron oxide; iron salt; tannins; decay test; mould test

1. Introduction

Natural wood and wood products with high availability of oxygen, nutrients, and moisture content can be easily degraded by wood-destroying organisms. The microbial-degradable carbohydrates and polar hydroxyls in wood absorb moisture, making wood an attractive material for active biological agents from the environment [1,2]. Over time, this results in decreased wood resistance to various bacteria, moulds, or decaying fungi [3,4], emphasising that fungi are the dominant agents of wood decomposition [5]. Typically, affected wood gradually loses its physical–mechanical characteristics. Despite the fact that it is a very important degradation stage of organic material under natural conditions, it significantly reduces the ability to use the wood for the wood industry, outdoor and indoor constructions, etc. It also significantly shortens the time of wood products use.

Three categories of wood decay, such as brown rot, white rot, and soft rot, are commonly studied [6-8]. Affecting the wood quality, different fungi attack different wood



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). components: cellulose, hemicellulose, and lignin. The most common types of wood decay are brown rot and white rot, caused by basidiomycete fungi [9]. For decay tests, *Trametes versicolor* (L.) Pilat (white rot fungus) and *Coniophora puteana* (Schumacher) P. Karsten (brown rot fungus) are mainly used. These fungi have different mechanisms of wood degradation: the brown rot fungus primarily attacks cellulose and hemicellulose, while the white rot fungus attacks all of the wood components [8,10].

We could argue that biodegradation of wood with moulds, which are usually fixed on the wood surface, is less dangerous compared to the effects of decaying fungi. However, mould attacks can cause a variety of problems for wooden constructions indoors and outdoors, including the loss of aesthetical view due to the discoloration of wood surfaces and detrimental effects on human health. Individual studies have revealed that mould fungi do not affect the strength properties of wood materials [11]. However, it is important to emphasise that these fungi affect the appearance of wood and can also cause allergic reactions [12,13].

The intensity of mould growth depends on the environmental conditions and on the type of wooden material [14–16]. The growth of mould fungi has been found to be affected by different factors, including oxygen, nutrients, humidity, temperature, light, exposure time, and substrate surface quality (substrate roughness and pH), as well as biotic interactions between different cultures [17–21]. It has been shown that mould fungi need an optimal temperature from 22 to 35 °C and a relative humidity of 71%–95% [22]. Other studies observed that moulds can grow at all temperatures from 0 to 50 °C and at the relative air humidity of above 80% [12,23].

Several studies indicated that the prevention of mould growth on wooden materials is an important challenge [24–26]. To increase the resistance of wood and wood products to biological agents, various methods have been recently tested, i.e., the wood is treated with compounds that protect against wood-destroying organisms. However, the use of wood preservatives is often limited due to environmental risks [27,28]. Therefore, technological solutions to protect wood using ecological, naturally derived materials are increasingly being tested [29,30]. For example, to protect the wood from fungi or other wood-destroying organisms, essential oils could be used instead of highly toxic traditional wood preservatives [31,32]. Quite widely tested is heat treatment of wood, which has been found to be quite effective against wood decay fungi [33–37]. Data from several studies suggest that thermal modifications change the chemical wood properties and increase wood resistance to decay [38,39].

This study aimed to evaluate the effect of different hydrothermal treatments with additives Fe₂O₃ or FeCl₃ with and without commercial tannins on wood resistance to decay and mould fungi. Searching for a more reliable wood modification technology, we sampled the wood from different tree species—*Pinus sylvestris* L., *Picea abies* (L.) H. Karst., *Pseudotsuga menziesii* (Mirb.) Franco, *Juglans regia* L., and *Acer platanoides* L.—and modified it with iron-containing compounds, which were considered of relatively low toxicity.

2. Materials and Methods

2.1. Wood Treatment

Five tree species Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), Douglas fir (*Pseudotsuga menziesii*), walnut (*Juglans regia*), and Norway maple (*Acer platanoides*) were obtained from a sawmill, and their wood samples were used for the experiment. The sets of 20 wood samples with dimensions of $20 \times 20 \times 30$ mm from each tree species were prepared for the treatment process. The selected wood samples were without knots and free of visible evidence of resins and showed no visual infection by mould, stain, and wood decay fungi.

The prepared wood samples were exposed to the different solvents prepared of 4 L of distilled water and iron oxide (Fe₂O₃), iron salt (FeCl₃), and commercial tannins. In total, there were six treatments: (1) $H_2O + 16$ g FeCl₃; (2) $H_2O + 16$ g FeCl₃ + 50 g commercial tannins; (3) $H_2O + 16$ g Fe₂O₃; (4) $H_2O + 16$ g Fe₂O₃ + 50 g commercial tannins; (5) $H_2O + no$

additives, taken as Control 1; and (6) not treated, fresh wood, taken as Control 2. The wood samples were immersed into the solvents and boiled at a constant temperature of 100 $^{\circ}$ C in a laboratory pot for 75 h (hereafter, hydrothermal treatment).

To provide basic information about the treatments, the ratio between the solvent amount and the wood mass was calculated for each tree species. This ratio was 12 for maple wood, 14–15 for walnut, 20 for spruce and fir wood, and 22 for pine wood, irrespective of the treatment. After each hydrothermal treatment, the percentage of dry mass loss was calculated for all tree species and it was as follows: 14%-21% for the treatment with FeCl₃; 6%-15% for FeCl₃ + tannins; 4%-8% for Fe₂O₃; 1%-6% for Fe₂O₃ + tannins; and 5%-10% for Control 1. For the evaluation of wood resistance, two controls were used in this study.

After the treatment, the wood samples were removed and dried at the room temperature. Then, the samples were prepared for the fungal decay and mould tests. These tests were carried out at the Laboratory of Entomology and Phytopathology in Forest Institute, Lithuanian Research Centre for Agriculture and Forestry.

2.2. Fungal Decay Test

For the fungal decay test, the smaller samples of $20 \times 15 \times 5$ mm were cut from the modified wood samples (hydrothermal treatment with FeCl₃, FeCl₃ + tannins, Fe₂O₃, Fe₂O₃ + tannins) and the controls (Control 1—hydrothermal treatment without additives and Control 2—fresh wood) were taken from pine, spruce, fir, maple, and walnut. In a general case, three smaller samples were cut from each wood sample. For the fungal decay test, 96 wood samples of each tree species, in total 480 samples, were prepared. These samples were dried at 80 ± 2 °C for 10 h until constant weight and weighed with digital scales with 0.01 g precision (the initial weight in grams, *Wi*).

The decay resistance of wood against white rot (*Trametes versicolor*) and brown rot (*Coniophora puteana*) was tested according to the standard EN 113 [40] with some modifications. In our experiment, the wood samples of $20 \times 15 \times 5$ mm instead of $50 \times 25 \times 15$ mm, as given in the standard EN 113, were used; the time of the fungal test was 12 weeks instead of 16 weeks; and Petri dishes of 90 mm diameter instead of 1 L Kolle's flasks were used for the wood exposure.

Fungal cultures were obtained from Westerdijk Fungal Biodiversity Institute (The Netherlands). The culture of *T. versicolor* was grown on 3.7% malt extract agar medium, and the culture of *C. puteana* was grown on 3.7% potato dextrose extract agar medium. The Petri dishes (diameter 90 mm) were filled with 15 mL of the sterile culture medium $(121 \pm 1 \text{ °C} \text{ for 30 min})$ and incubated with a piece of mycelium at a temperature of 24 °C and relative humidity of 75% for one week. In total, 120 Petri dishes with fungal cultures were prepared. After inoculation, sterilised samples (after autoclaving at $105 \pm 2^{\circ}$ C for 30 min) were placed into the Petri dishes. In each Petri dish, four samples were placed. The wood samples were left in contact with the fungus at a temperature of 24 °C and relative humidity of 70% in an incubator chamber for 12 weeks. After this time, the samples were removed from the Petri dish and cleaned of the fungal mycelia. Then, the samples were dried at 80 ± 2 °C for 10 h and weighed again (final weight in grams, *Wf*) to calculate the percentage of weight loss.

The susceptibility of wood samples to fungi attack was evaluated by the percentage of weight loss (*WL*), calculated according to the Formula (1) [41].

$$WL(\%) = \{(Wi - Wf)/Wi\} \times 100$$
 (1)

where *WL* is the weight loss in percent; *Wi* is the initial weight in grams; and *Wf* is the final weight in grams.

2.3. Mould Test

For the mould test, 48 samples from each tree species (in total, 240 wood samples) of $30 \times 20 \times 7$ mm were prepared. The anti-mould resistance of the wood was tested according to the European Standard EN 15457 [42] with some modifications in the sterilisation

process and the shape of the samples. All wood samples were sterilised (autoclaving at 105 ± 2 °C for 30 min) and dried at a temperature of 80 ± 2 °C for 10 h until constant weight. The sterilised samples were placed into the Petri dishes (diameter 90 mm) with malt extract agar medium and inoculated with spore suspension, prepared as a mixture containing two mould fungi: *Aspergillus niger* Tiegh. and *Penicillium* Link sp. Three samples were placed per one Petri dish. The spore suspension was made using well sporulating cultures on agar dishes. A total of 15 mL of sterile water was added to each agar dish with fungal cultures. A bacteria loop was used to loosen the spores from the mycelium/agar. An equal amount of suspension from two mould fungi was mixed by adding 100 mL of sterile water, totalling 130 mL of suspension. Wood samples were covered by spore suspension (0.4 mL per each sample), containing two mould fungi in a concentration of 10^6 spore mL⁻¹. In total, about 100 mL–130 mL of the spore suspension was applied to all 240 wood samples.

Subsequently, the wood samples were left in contact with the mould fungus at a temperature of 24 ± 2 °C and relative humidity of 85%–90% in an incubator chamber for 28 days. During the exposure period, mould growth on the sample top-side (30×20 mm) was evaluated visually once per week. The development of mould growth on the surface of the wood samples was assessed using the rating scheme described in the Standard EN 15457 [42]. This scheme is based on the percentage of the surface covered with mould fungi. The rating ranged from 0 to 4, and the percentage area for each rating level is presented in Table 1. The individual mould species on the wood surface were identified visually. For later double checking of the registered mould rating, images of the wood samples were taken every week.

Rating Level	Percentage Area of Disfigurements				
0	No growth on the surface of the sample				
1	Up to 10% growth on the surface of the sample				
2	More than 10% up to 30% growth on the surface of the sample				
3	More than 30% up to 50% growth on the surface of the sample				
4	More than 50% up to 100% growth on the surface of the sample				

Table 1. Rating scheme for determination of mould growth (according to the EN 15457 [42]).

2.4. Statistical Analyses

To determine the significant differences of wood weight loss and mould growth area between different wood treatments, ANOVA and Tukey's Studentized Range (HSD) test were used. The different letters next to the mean values show statistically significant differences at p < 0.05 between the treatments. The obtained data were analysed using the statistical package SAS 9.4 (SAS Institute Inc., Wake County, NC, USA).

3. Results

3.1. Weight Loss of Modified Wood Caused by White and Brown Rot Fungi

During the decay test, all wood samples were covered by white rot fungi *Trametes versicolor* and brown rot fungi *Coniophora puteana* mycelia. The weight losses of the modified and control wood, caused by *T. versicolor* and *C. puteana*, differed for coniferous and deciduous wood (Table 2; Figure 1). The average weight loss of treated pine, spruce, and fir wood, caused by *C. puteana*, was higher than that caused by *T. versicolor*. However, the differences between these fungi on maple and walnut wood were not significant. A possible explanation for this might be that fungus *T. versicolor* is more common on deciduous trees than on conifers in natural conditions.

Tree	T ()	Weight Loss Due to Fungi Effect (%)				
Species	Ireatment	Trametes versicolor		Coniophora puteana		
	FeCl ₃	4.37 (0.47)	b	14.75 (2.76)	d	
_	FeCl ₃ + tannins	3.95 (0.47)	b	25.43 (2.05)	с	
Dina	Fe ₂ O ₃	3.58 (0.66)	b	42.29 (1.15)	ab	
rme –	Fe_2O_3 + tannins	11.84 (0.63)	а	35.94 (1.70)	b	
_	Control 1 *	16.39 (2.11)	а	39.41 (0.87)	ab	
_	Control 2 *	5.70 (1.57)	b	44.68 (2.40)	а	
	FeCl ₃	3.17 (0.52)	cd	15.35 (2.11)	d	
_	FeCl ₃ + tannins	4.17 (0.24)	bc	16.61 (1.74)	cd	
Spruce	Fe ₂ O ₃	3.94 (0.95)	cd	31.29 (2.78)	b	
Spruce =	Fe_2O_3 + tannins	11.32 (0.77)	а	35.55 (3.94)	b	
_	Control 1	7.60 (1.55)	b	29.56 (4.43)	bc	
—	Control 2	0.45 (0.14)	d	57.02 (3.22)	а	
	FeCl ₃	3.59 (0.37)	ab	19.44 (2.93)	bc	
—	FeCl ₃ + tannins	1.46 (0.19)	с	14.68 (0.71)	с	
	Fe ₂ O ₃	2.75 (0.53)	bc	32.54 (3.91)	ab	
FII –	Fe_2O_3 + tannins	1.21 (0.31)	с	21.37 (2.34)	bc	
_	Control 1	4.02 (0.48)	ab	31.74 (1.30)	ab	
_	Control 2	4.78 (0.55)	а	38.70 (5.52)	а	
	FeCl ₃	17.36 (1.74)	cd	15.94 (1.48)	b	
_	FeCl ₃ + tannins	15.49 (0.59)	d	18.64 (1.56)	ab	
	Fe ₂ O ₃	35.13 (5.89)	ab	29.69 (3.34)	а	
wapie –	Fe_2O_3 + tannins	31.04 (2.74)	bc	21.70 (1.82)	ab	
_	Control 1	48.10 (4.94)	а	26.28 (3.21)	ab	
_	Control 2	43.49 (3.20)	ab	26.70 (5.35)	ab	
	FeCl ₃	15.07 (0.54)	cd	18.22 (1.68)	b	
_	FeCl ₃ + tannins	11.61 (0.83)	d	19.19 (1.56)	b	
	Fe ₂ O ₃	25.05 (2.00)	а	30.76 (0.96)	а	
vvainut –	Fe_2O_3 + tannins	17.80 (2.16)	bc	23.97 (4.75)	ab	
_	Control 1	24.14 (0.90)	а	27.77 (2.28)	ab	
-	Control 2	22.16 (0.31)	ab	20.69 (3.11)	ab	

Table 2. Mean (standard error) weight losses of wood samples after exposure (12 weeks) to decay fungi *Trametes versicolor* and *Coniophora puteana*. Different letters given next to the mean value show statistically significant differences for each fungus within each tree species between different treatments at p < 0.05 (n = 8).

* Control 1 is the hydrothermal wood treatment with no additives; Control 2 is fresh wood, without treatment.

The highest weight loss of the coniferous (pine and spruce) wood, caused by the white rot fungus *T. versicolor*, was found for the wood treatment with Fe_2O_3 + tannins and without additives (Control 1) (Table 2). The hydrothermal treatments with Fe_2O_3 , $FeCl_3$ with and without tannins, and the fresh wood (untreated wood; Control 2) showed a similar response to *T. versicolor*: the weight loss of pine wood amounted to 3.6%–4.4% and for spruce wood—3.9%–4.2% (except for Control 2). For fir and maple wood, the highest weight loss caused by *T. versicolor* was obtained for both Control 1 and Control 2, followed by the treatment FeCl₃ for fir wood and Fe_2O_3 with and without tannins for

maple (Table 2). For walnut wood, the weight loss for the treatments Fe_2O_3 , Control 1 and Control 2 ranged between 22.2 and 25.1%, while it was lower for other treatments: Fe_2O_3 + tannins (17.8%), FeCl₃ (15.1%), and FeCl₃ + tannins (11.6%). The data showed that significantly lower values of weight loss were obtained for the wood samples treated with FeCl₃ with and without tannins.



Figure 1. Mean weight loss of wood samples from pine, spruce, fir, maple, and walnut exposed to decay fungi *T. versicolor* and *C. puteana* for 12 weeks. Bars show standard error of the mean.

Similarly, as in the case of *T. versicolor*, the hydrothermal treatments with FeCl₃ with and without tannins significantly decreased the weight loss of the coniferous tree species (pine, spruce, and fir) and deciduous (maple and walnut) wood caused by the brown rot fungus *C. puteana* (Table 2). In this case, the average weight losses of modified wood were 14.8% and 25.4% for pine, respectively, for the treatments with FeCl₃ and FeCl₃ + tannins;

15.4% and 16.6% for spruce; 19.4% and 14.7% for fir; 15.9% and 18.6% for maple; and 18.2% and 19.2% for walnut wood.

The mean weight losses in the pine wood samples exposed to decay fungi varied in a range from 3.6% (Fe₂O₃) to 16.4% (Control 1) caused by *T. versicolor* and from 14.8% (FeCl₃) to 44.7% (Control 1) caused by *C. puteana* (Figure 1). The mean weight losses in the spruce wood samples exposed to decay fungi varied in a range from 0.5% (Control 2) to 11.3% (Fe₂O₃ + tannins) caused by *T. versicolor* and from 15.4% (FeCl₃) to 57.0% (Control 2) caused by *C. puteana* (Figure 1). As an example, the spruce wood samples exposed to brown rot fungus *C. puteana* for treatments with FeCl₃ and fresh wood (Control 2) are presented in Figure 2.



Figure 2. Examples of spruce wood samples exposed to brown rot fungus *C. puteana* after treatment with FeCl₃ (**A**) and on fresh wood Control 2 (**B**) after 12 weeks (Photos: Aiste Povilaitiene).

For the fir wood exposed to *T. versicolor*, the lowest weight losses were obtained for the treatments with Fe_2O_3 + tannins (1.2%) and $FeCl_3$ + tannins (1.5%). While for the fir wood exposed to *C. puteana*, the lowest weight loss was obtained for the treatment with $FeCl_3$ + tannins (14.7%) (Figure 2).

Maple wood samples treated with Fe_2O_3 with and without tannins were not as resistant to decay fungi as the wood treated with $FeCl_3$ with and without tannins. The mean weight losses in the maple wood exposed to decay fungus *T. versicolor* varied in a range from 15.5% (FeCl₃ + tannins) to 48.1% (Control 1) and from 15.9% (FeCl₃) to 29.7% (Fe₂O₃) when caused by *C. puteana* (Figure 1). As an example, the maple wood exposed to white rot fungus *T.versicolor* for treatments with FeCl₃ and fresh wood (Control 2) is given in Figure 3.



Figure 3. Examples of maple wood samples exposed to white rot fungus *T. versicolor* after treatment with FeCl₃ (**A**) and on fresh wood Control 2 (**B**) after 12 weeks (Photos: Aistė Povilaitienė).

The lowest weight loss of the walnut wood, exposed to *T. versicolor*, was observed in the treatment with $FeCl_3$ + tannins (Figure 1). For fir wood samples, the lowest weight loss was in the treatments with $FeCl_3$ and $FeCl_3$ + tannins (18.2% and 19.2%, respectively) caused by *C. puteana*.

3.2. Wood Response to Mould Test

The two moulds *Aspergillus niger* and *Penicillium* sp., used as a mixture, were tested on wood of different tree species. Growth activity of moulds on pine and maple wood, taking one example from coniferous and one example from deciduous species, are given in Figure 4. During the mould test, two fungal species were obtained on wood. In most cases, the *Aspergillus niger* was the dominating species, possibly because this species is able to adhere more strongly to the wood surface than *Penicillium* sp. [43].



Figure 4. Growth activity of moulds *Aspergillus niger* and *Penicillium* sp. on pine and maple wood samples from the 7th to 28th day (n = 8). Note: the wood samples were exposed to the suspension made from a mixture containing the two fungi.

For the wood, hydrothermally modified with FeCl₃ and FeCl₃ + tannins, the mould area for both *Aspergillus niger* and *Penicillium* sp. was smallest for all tested tree species wood (Table 3). The mould *Aspergillus niger* area on wood treated with FeCl₃ was 2.2–2.3 times (maple wood), 5.5–8.8-fold (pine wood), and 14.8–16.7-fold (walnut wood) to 24.7–30.8-fold (fir wood) smaller than on wood treated without additives (Control 1) or on fresh wood (Control 2). No active growth of the *Aspergillus niger* was fixed on spruce wood modified with FeCl₃.

The growth area of the mould *Aspergillus niger* on wood treated with Fe_2O_3 with and without tannins was slightly smaller than that of the controls (Table 3). For the treatment with Fe_2O_3 + tannins, the growth area of the *Aspergillus niger* was 1.5–2.0 times smaller than Control 1 for all species. While the growth area of the *Aspergillus niger* for the Fe_2O_3 treatment did not differ from Control 1 for all wood samples, except for fir wood (the difference was 2.6 times). In comparison with the fresh wood (Control 2), the growth area of *Aspergillus niger* was smaller for wood treated with Fe_2O_3 + tannins but no effect was fixed for the wood treated with pure Fe_2O_3 .

The *Penicillium* sp. did not grow on spruce and fir wood treated with $FeCl_3$ (Table 3). For wood treated with $FeCl_3$ + tannins, the smallest area of the *Penicillium* sp. was fixed on fir wood, which differed by almost 26 times when compared to that of Control 1 and Control 2. After the exposure period of 28 days, the *Penicillium* sp. growth area on pine,

Treatment

maple, and spruce wood treated with $FeCl_3$ + tannins was 2.8–6.7 times smaller than the controls.

Table 3. The mean mould growth area (%) on wood samples and mean mould rating after 28 days exposure (n = 8). Note: the wood samples were exposed to the suspension made from a mixture containing the two fungi.

Tree	Treatment	Aspergillus niger		Penicillium sp.		Mean Mould
Species		Mean	Range (SE) **	Mean	Range (SE)	Rating ***
Pine -	FeCl ₃	10.6	0-30(4.1)	33.8	0-80(11.0)	2.5
	FeCl ₃ + tannins	38.1	5–90(11.7)	16.8	3–50(5.8)	2.9
	Fe ₂ O ₃	86.3	40–100(7.3)	4.5	0-20(2.3)	3.9
	Fe_2O_3 + tannins	63.1	40-95(7.9)	8.8	0-20(2.3)	4.0
	Control 1 *	93.8	85–100(2.1)	16.3	5-30(3.2)	4.0
	Control 2 *	58.8	40-70(3.5)	46.3	10–70(6.8)	4.0
- Spruce -	FeCl ₃	0.0	0	0.0	0	0.0
	FeCl ₃ + tannins	6.3	0–15(1.8)	4.6	0-10(1.3)	1.5
	Fe ₂ O ₃	75.0	20-100(8.7)	11.9	0-20(2.8)	3.8
	Fe_2O_3 + tannins	60.6	30-100(9.3)	10.8	1-20(2.3)	3.5
	Control 1	91.9	80-100(2.5)	22.5	5-40(4.4)	4.0
-	Control 2	75.0	50-90(5.4)	31.3	10-60(5.8)	4.0
	FeCl ₃	2.9	0-5(0.9)	0.0	0.00	0.6
-	FeCl ₃ + tannins	5.9	1–15(1.6)	0.4	0-3(0.4)	1.1
E:-	Fe ₂ O ₃	34.0	15-70(7.2)	3.6	0–10(1.5)	2.9
FII	Fe_2O_3 + tannins	47.5	20-80(8.4)	8.1	5–10(0.9)	3.3
	Control 1	88.8	60–100(4.7)	6.9	0-20(2.8)	4.0
-	Control 2	71.3	30-90(7.4)	9.8	3–20(2.2)	3.9
	FeCl ₃	40.6	5-70(8.9)	21.3	0-80(9.0)	3.1
	FeCl ₃ + tannins	36.9	5–90(11.5)	7.5	0–25(3.5)	2.6
- Maple -	Fe ₂ O ₃	96.3	80-100(2.6)	13.1	5-30(3.8)	4.0
	Fe_2O_3 + tannins	58.8	30-90(7.2)	0.0	0.00	3.4
	Control 1	91.9	80–100(3.3)	21.9	5-50(5.0)	4.0
	Control 2	90.6	80-100(2.0)	27.5	5-50(5.2)	4.0
Walnut -	FeCl ₃	5.9	0-20(2.5)	11.3	0-20(2.8)	1.6
	FeCl ₃ + tannins	26.9	5-100(10.9)	6.9	0-20(3.0)	2.4
	Fe ₂ O ₃	72.5	60-90(4.5)	18.8	0-50(6.6)	4.0
	Fe_2O_3 + tannins	43.8	5-100(12.3)	2.3	0–10(1.3)	2.6
	Control 1	86.9	70–100(3.3)	10.0	0-25(2.8)	4.0
	Control 2	98.1	90–100(1.3)	3.9	0-10(1.1)	4.0

* Control 1 is the hydrothermal wood treatment with no additives; Control 2 is fresh wood, without treatment. ** range (SE), where range is the values from the minimum to maximum; SE is standard error of the mean. *** mean mould rating was calculated averaging the rating levels of 8 wood samples for different tree species per each treatment.

The growth area of *Penicillium* sp. was 3.6 times smaller on pine wood treated with Fe_2O_3 than that of Control 1 and 1.7–1.9 times smaller on spruce, fir, and maple wood (Table 3). For the treatment with Fe_2O_3 + tannins, the growth area of *Penicillium* sp. on pine and spruce wood was 1.9–2.1 times and on walnut wood 4.4 times smaller than that of

Control 1. The growth area of *Penicillium* sp. was 10.3 times smaller on pine wood treated with Fe_2O_3 than that of Control 2 and 2.1–2.7 times smaller on spruce, fir, and maple wood. For the treatment with Fe_2O_3 + tannins, the growth area of *Penicillium* sp. was from 1.2 (fir wood) to 5.3 (pine wood) times smaller than that of Control 2. The *Penicillium* sp. did not grow on the maple wood treated with Fe_2O_3 + tannins.

Mould growth was defined by five mould growth categories, and the mean of the evaluations after 28 days of exposure are given in Table 3. The highest mould rating (3.8–4.0) was obtained for the treatment with Fe_2O_3 (except fir wood) and the control wood samples (treated without additives—Control 1 and fresh wood—Control 2) of all studied tree species. Fir wood had generally the lowest mould rating for the treatment with Fe_2O_3 with and without tannins. Overall, the lowest mould rating was recorded for the pine, spruce, fir, maple, and walnut wood treated with $FeCl_3$, and a lower mould rating was recorded for the wood treated with $FeCl_3$ together with tannins.

The species wood resistance against *Aspergillus niger* in accordance with different treatments can be shown in the following order, from the highest to lowest growth area: (1) for the FeCl₃ with and without tannins—spruce (the highest resistant) < fir < walnut < pine < maple (the lowest resistance); (2) for the Fe₂O₃—fir < walnut = spruce < pine < maple; and (3) no significant differences in wood resistance among tree species for the Fe₂O₃ + tannins and both controls were found. The order of species wood resistance against *Penicillium* sp. in accordance with different treatments was different: (1) for both FeCl₃ treatments—spruce = fir < walnut < maple < pine; (2) for the Fe₂O₃—pine = fir < spruce = maple < walnut; and (3) for the Fe₂O₃ + tannins—maple < walnut < pine = fir = spruce.

Overall, after four weeks, the surfaces of the treated wood samples were fully covered by mycelia of both mould species (Figure 5A, pine wood chosen as an example). Only spruce wood samples treated with FeCl₃ remained the most resistant to both mould species (Figure 5B). The maple wood samples treated with Fe_2O_3 + tannins were highly resistant to the *Penicillium* sp. but were highly covered by *Apergillus niger* (Figure 5C).



Figure 5. Examples of mould growth on pine (**A**), spruce (**B**), and maple (**C**) wood samples after the treatments with Fe_2O_3 , $FeCl_3$, and Fe_2O_3 + tannins, respectively, and Control 1 (**A**,**B**) and Control 2 (**C**) exposed in each Petri dish for 28 days after inoculation. Note: the wood samples were exposed to the suspension made from a mixture containing the two fungi.

4. Discussion

The present study was designed to determine the effect of different hydrothermal treatments (Fe_2O_3 , Fe_2O_3 + tannins, $FeCl_3$, $FeCl_3$ + tannins, including Control 1 or the treatment without additives and Control 2 or the fresh wood) on wood resistance to decay (white rot fungus *Trametes versicolor*; brown rot fungus *Coniophora puteana*) and mould fungi (*Aspergillus niger* and *Penicillium* sp.). The response of modified wood samples from five different tree species—pine, spruce, fir, maple, and walnut—was tested. Previous studies show that wood modification affects the basic wood properties, such as dimensional

stability, hardness, durability, and UV stability [44]. Several studies also indicated the higher resistance of modified wood against the wood-destroying fungi [45,46].

The current study found that the hydrothermal treatment with various Fe compounds caused the increased anti-decay resistance of the modified wood samples. Overall, the white rot fungus (*T. versicolor*) caused lower weight loss than the brown rot fungus (*C. puteana*), except for the maple wood. The stronger positive effect was obtained for brown rot C. puteana than white rot T. versicolor. Previous studies showed weak or no improvement of the resistance against *T. versicolor* when the wood of pine (*Pinus radiata; Pinus sylvestris*), spruce, fir, and ash trees were thermally treated [35,47,48]. For example, the study by Sivrikaya et al. [48] showed that the weight loss for thermally treated spruce and ash wood at a 210 °C temperature was higher than 5% due to the white rot *T. versicolor*, but the weight loss was lower than 2% due to brown rot C. puteana. The higher resistance against brown rot *C. puteana* than that of white rot *T. versicolor* for the thermally treated wood was obtained by Tjeerdsma et al. [35]. Interesting findings were concluded by Leithoff and Peek [49], who described the optimal thermal regimes that are necessary for obtaining the significant resistance against the white rot T. versicolor and brown rot C. puteana, which were, respectively, the wood exposure at a 220°C temperature for 120 min and at 200 °C for 60 min. Ayata et al. [50] defined that the heat treatment can be used effectively against fungal attack for Scots pine, oak, and beech wood, identifying lower weight losses at a higher intensity of the heat treatment. Yalcin and Sahin [51] also concluded that thermally treated wood was more resistant to both brown and white rot fungi.

In our study, we used the basic decay test with the evaluation of weight loss as the measure of decay. This is one of the most common methods to measure wood degradation in laboratory studies because the wood cell wall components are converted to CO_2 by the fungus [52]. Furthermore, the wood resistance against wood-destroying fungi could be evaluated additionally by including the strength loss indicator and biochemical changes in the wood [53]. Otherwise, the mentioned study found a direct relation between wood strength and weight losses. Therefore, it is highly possible that the use of additional methods could only confirm the results obtained in our study.

In this study, the response to the tests of wood-destroying and mould fungi showed the complex effect of heat and iron compounds (Fe_2O_3 , $FeCl_3$) with or without commercial tannins, but the effect of a single treatment factor could not be determined. As noted by Ohno et al. [54], the treatment of wood with metals was only partially effective in combating soft rot and brown rot fungus, such as Fibroporia radiculosa (Peck) Parmasto, detoxifying copper compounds to form oxalates. A similar fungi response to metal-treated wood was found by Gadd [55]. The content of compounds such as tannins in wood was also related to the susceptibility to mould of different species [56–61]. This was best illustrated by the differences obtained in heartwood to sapwood, i.e., if the heartwood contained high levels of extractives and reduced nutrient levels, the susceptibility to biodegradation was directly affected [56,62,63]. In a broader perspective, heat treatment induces significant degradation of amorphous polysaccharides, which occurs at the cell membrane, and the hygroscopicity of the wood decreases [37]. Therefore, the weight loss correlates with the changes in chemical composition of the thermally treated wood. If the content of available nutrients decreased, the resistance against the fungi increased [37]. As Hill [7] showed, the brown rot fungi are related to the removal of the polysaccharide components and white rot fungi degrade both lignin and polysaccharides.

The most important finding of our study was the increase in wood resistance of all studied tree species wood due to the impact of hydrothermal treatment with FeCl₃ compounds. The treatments with FeCl₃ with and without tannins significantly decreased the weight loss of the coniferous and deciduous wood caused by the white rot *T. versicolor* and brown rot *C. puteana*. Similar results were obtained after the mould test, i.e., in comparison to the treated (without additives) and untreated (fresh wood) control, the mould area for *Aspergillus niger* and *Penicillium* sp., tested as a mixture containing the two fungi, was smallest on wood treated with FeCl₃ and FeCl₃ + tannins for all tree species.

A similar finding was also reported by Råberg et al. [1], who indicated that the toxic or inhibiting substances affect fungal survival and spread in the wood; therefore, the changing nutrient status of the wood during the successive stages of decay should be estimated.

As the natural durability of the wood varies between wood species and is explained mainly by the composition and amount of wood extractives, the better result in our study was obtained for the treated wood when additional commercial tannins were added. This, to some extent, accords with the finding that trees with high content of extractives, mainly tannins and flavonoids, are more resistant against fungi [64].

As mentioned earlier in the text, hydrothermal modification changes the chemical wood structure, making the modified wood more resistant to decay. In order to have a more accurate response, it would be appropriate to study the chemical composition of the wood (cellulose, hemicellulose, lignin, etc.) after hydrothermal treatment with various iron compounds, together with the tests of the wood resistance caused by wood-destroying fungi. Similar tests can be performed with other tree species or by modifying the wood using other metals, such as those with antimicrobial activity.

5. Conclusions

This study was set out to evaluate the effect of different hydrothermal treatments (Fe₂O₃ or FeCl₃ with and without commercial tannins) on Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), Douglas fir (*Pseudotsuga menziesii*), walnut (*Juglans regia*), and Norway maple (*Acer platanoides*) wood resistance to white-rot fungus *Trametes versicolor* and brown rot fungus *Coniophora puteana* and two moulds *Aspergillus niger* and *Penicillium* sp., used as a mixture of two mould fungi. The results showed a positive response of the treated wood to different tests of wood-destroying fungi. The best obtained treatment technology included more aggressive FeCl₃ compounds, and, while less effectively, yet still significantly, the wood resistance against these fungi was increased when the wood was treated with Fe₂O₃ together with tannins.

The wood modified with iron compounds caused a higher resistance to wood-destroying fungi. The weight losses of the modified and control wood, caused by *T. versicolor* and *C. puteana*, differed for coniferous and deciduous: the average weight loss of treated pine, spruce, and fir wood caused by *C. puteana* was higher than that by *T. versicolor*, while these differences on maple and walnut wood were insignificant. The wood hydrothermal treatment with Fe₂Cl₃ with and without tannins significantly reduced the weight loss caused by *T. versicolor* and *C. puteana*, and the treatment with Fe₂O₃ slightly improved the decay resistance.

For the wood, hydrothermally modified with the pure $FeCl_3$ and with $FeCl_3$ applied together with tannins, the mould area for both *Aspergillus niger* and *Penicillium* sp. was the smallest for all tested tree species compared to other treatments. The spruce wood, followed by fir wood, treated with $FeCl_3$ with and without tannins, was the most resistant against the mould fungi. Relatively low resistance against the mould fungi was fixed for the maple wood treated by various iron compounds, except the treatment with Fe_2O_3 together with tannins, which gave a very positive response against the *Penicillium* sp.

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