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The Effect of Surface Treatment on the Antibacterial Properties of Wood and the Possibility to Detect the Antibacteriality with Fluorescence Method

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Abstract: Wood is a widely used indoor material, and thus it is important to consider wood performance in microbial cross contamination via surfaces. In this study, both untreated and surface-treated wood materials were studied by simulating airborne bacterial contamination and evaluating the number of bacterial colonies on the material surfaces. The materials studied were untreated pine heartwood and sapwood, spruce, silver birch, and glass as a reference. The intensity of UV-excited fluorescence was measured to find the most antibacterial Scots pine heartwood. The coatings used were varnish and wax for Scots pine sapwood. The surface roughness of all material types was compared, and it was found to be uniform. All untreated wood surfaces had less viable bacterial colonies at all time points compared to the glass reference, and the surface treated samples did not differ from the glass reference indicating that the antibacterial properties of wood were blocked by both varnish and wax. The fluorescence method was practical to use, but wider testing will be needed to validate it more thoroughly. The results indicate also that silver birch has similar antibacterial properties as pine and spruce and hence should be studied further.

Keywords: antibacterial wood; pine; spruce; birch; wood coating; stilbene; fluorescence

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Citation: Vainio-Kaila, T.; Harju, A.; Rohumaa, A.; Paajanen, O.; Venäläinen, M.; Seppä, J.; Veijalainen, A.-M.; Pasanen, P. The Effect of Surface Treatment on the Antibacterial Properties of Wood and the Possibility to Detect the Antibacteriality with Fluorescence Method. *Forests* **2023**, *14*, 23. https://doi.org/10.3390/f14010023

Academic Editor: Simon Curling

Received: 29 November 2022
Revised: 12 December 2022
Accepted: 20 December 2022
Published: 22 December 2022

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1. Introduction

With growing interest in sustainable building materials, wood is increasingly of interest in the building sector as a structural material. While often hidden inside the structures, there are solutions like cross laminated timber (CLT), or other massive structures that are left visible and touchable in the interior surfaces. Wood is used also in the furniture or interior cladding where the surfaces are commonly coated to prevent them from getting stained. Wooden surfaces are seen as beautiful and relaxing etc. [1,2] and could hence fit well in buildings where people are given care, whether old, very young or sick. These interiors need to have high hygiene requirements in order to limit the spread of the infections, as the people are often more vulnerable, and at the same time, could benefit from the comfortable and restful [1] effect of the built environment.

In coated wooden surfaces the properties, e.g., hygienic, are hidden and the surface resembles other interior materials. There are many ways to coat wooden surfaces, and very common coating types are paint or lacquer, which create a tight barrier on top of the wood surface, and waxes and oils, which are not a very homogenous group of coating methods. Both groups are represented in this study.

In Finland, the main tree species used in building and other industrial processes like furniture or engineered wood products are Scots pine (*Pinus sylvestris* L.), Norway

spruce (*Picea abies* (L.) H. Karst.) and silver birch (*Betula pendula* Roth). The antibacterial properties of Scots pine and Norway spruce [3–6] are better known than the properties of silver birch [7]. The extractives of the Scots pine and Norway spruce have especially strong antibacterial properties [8]; and lignin [9] has also shown antibacterial properties. Pine is generally found to be more antibacterial than spruce, and the content of extractives is higher, especially in the heartwood [10,11]. The resin acids [12] and stilbenes [13,14] are the groups of active components contributing to the antibacterial properties, and the amount of stilbenes also correlates with the decay resistance [15]. However, the antimicrobial properties are not necessarily the result of certain specific components, but rather an optimal combination of the extractive components, as well as previous wood treatment, porosity, age etc. [16]. The chemical properties of silver birch differ from the two softwood species, and hence the microbiological qualities need to be studied further.

A typical fluorescence of Scots pine heartwood under ultraviolet (UV) radiation has been used to separate the heartwood from sapwood [17]. The intensity of fluorescence varies widely among heartwood specimens and has shown a high positive correlation with the stilbene content and a high negative correlation with the mass loss due to the brown rot fungus *Coniophora puteana* (Schumacher ex Fries) Karsten (Strain BAM Ebv. 15) [18]. It has been suggested that the fluorescence method could be utilized in timber grading and forest tree breeding as a tool to improve decay resistance of heartwood material [18].

This article tackles the question of the transmission through surfaces where there is a multitude of bacteria left on the surface that easily transfers to hands. Both tight surface treatment and a more breathable coating are included. The methods for the antibacterial study were chosen to resemble touching the surface.

The aims of the study were to evaluate the possibility to find the most antibacterial Scots pine heartwood material based on the fluorescence of stilbenes under UV-excitation, to compare the antibacterial properties of pine, spruce, and birch wood in relation to each other and to coated (lacquer and wax) Scots pine sapwood surfaces. Also, a novel contamination method with aerosolization of bacteria by collision nebulizer was used to resemble airborne bacteria landing on the surfaces. The effect of the surface smoothness is also discussed.

2. Materials and Methods

2.1. Wood Material Selection and Specimen Preparation

For this study untreated Scots pine, Norway spruce, and silver birch wood materials were selected. In addition, pine sapwood samples were coated with two different surface coating systems (Table 1). In the selection of wood species, the aim was to take into consideration the materials commonly used in interior surfaces e.g., furniture. The wood materials were obtained from the Siparila Oy plant in Parkano in November 2019 and the material coating was conducted at Tikkurila Oyj.

Table 1. Overview of used materials and test methods.

Wood Species		Surface Treatment of Wood	Testing Methods		
			Surface Roughness	UV-Excited Fluorescence	Bacterial Viability on the Surfaces
Norway spruce	Sapwood	untreated	x		x
Silver birch	Sapwood	untreated	x		x
Scots pine	Sapwood	untreated	x		x
	Heartwood	untreated		x	x
	Sapwood	Wax coating Akviwax Satin	x		x
	Sapwood	Varnish Akvilac FD-J 10	x		x

The untreated planks from all wood species were processed into 5-mm-thick and 50 × 50-mm-wide test specimens (T × R × L) exposing the radial surface for bacterial con-

tamination. Scots pine heartwood specimens were directed for fluorescence measurement to select suitable specimens for bacterial contamination. The material used in this study was freshly planed before the surface roughness and UV-excited fluorescence measurements were taken. However, it took approximately one month until the wood samples were contaminated with bacteria.

Part of the Scots pine sapwood planks were coated in the Tikkurila Oyj product development laboratory under well controlled conditions; after which, the coated planks were similarly cut into the final test specimens as uncoated wood material.

2.2. Wood Specimen Surface Quality Verification by Roughness Measurements

The surface roughness of the specimens was measured from all different test specimen groups. The aim of the roughness measurement was to examine the surface quality after processing. While the process and equipment settings were the same for all materials, the different wood species and surface treatments may affect the surface roughness. The properties of the wood surface could influence the antibacterial research methods and results by creating difference in the surface area and conditions for the bacterial adhering to the surface. The measurements were done using Dektak 6M stylus profiler. The radius of the stylus tip was 2.5 µm. The results were analyzed using software Dektak32 release 8.35 (2006) by Veeco Instruments Inc. Ra values were used in the comparison. The process was made following EN ISO 21920-2:2021.

2.3. Selection of Scots Pine Heartwood Samples Based on Their UV-Excited Fluorescence

UV-excited fluorescence measurements were performed using a custom-built device (3K-factory of Electronics at South-Eastern Finland University of Applied Sciences) as was described in Belt et al. [18]. The UV-LED excitation source (UVCLEAN315FW-5,3-5 mW, TO39 by Sensor Electronic Technology, Inc.) of the device directed UV radiation (315 $\pm$ 5 nm) perpendicularly to the sample surface. The spectrometer recorded the fluorescence emission spectra at a 45° angle from an elliptic detection area approx. 3 mm in length and 4 mm in width. The intensity maximum of UV-excited fluorescence (Imax) was used as the quantitative measure of fluorescence intensity [18].

In the present study, the UV-excited fluorescence was recorded from a radially cut surface of 5-mm-thick and 50 $\times$ 50-mm-wide Scots pine heartwood samples. The wood samples were held on a sample holder that moved step-by-step under the fixed excitation detection assembly by an automated system. The movement was perpendicular to the radially visible annual rings. A single measurement cycle consisted of 8 heartwood samples on the holder at a time. The total number of the oval-shaped measurement areas and thus the number of Imax values per sample was 66 (Figure 1a). Directly after measurement, the samples were photographed under visible light and under UV excitation source (excitation peak at 313 nm) that excited the whole area of the samples (Figure 1a,b).

Fluorescence Imax value was measured from 199 heartwood specimens. Specimens for microbial tests were selected based on the range of Imax among the single measurement areas per sample and the sample-wide average. The grouping to the low fluorescence group of Scots pine heartwood (LF) and to the high fluorescence group of Scots pine heartwood (HF) had an Imax limit value of 12,000 a.u. for single measurement areas of each wood sample. The number of samples with the Imax values less than 12,000 a.u. or more than 12,000 a.u. was 48 and 52, respectively (Table 2). No overlapping of the Imax values between the low and high group was allowed. The group that was not included to the microbial tests, named mixed fluorescence, had Imax values varying around 12,000 a.u. Samples belonging to the low and high fluorescence groups were used to microbial analyses. Part of those samples were used for testing the experimental set-up.

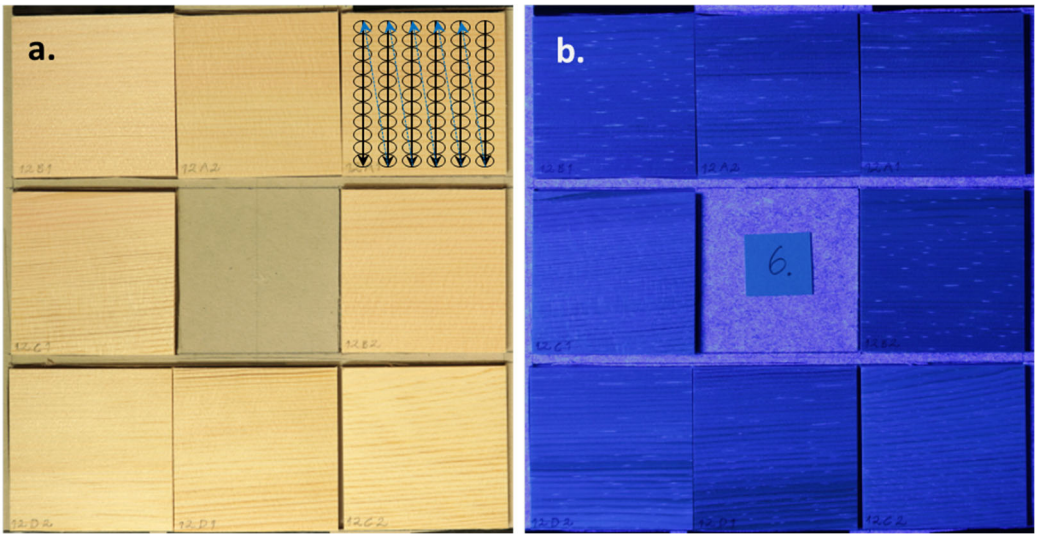


Figure 1. Scots pine heartwood samples on a sample holder (**a**) under visible light, and (**b**) under UV excitation of 313 nm. The drawing in the upper right corner of the image a. shows the positions of the oval-shaped measurement areas and the stepwise progress of the measurement.

Table 2. Grouping of the Scots pine heartwood samples based on their intensity maximum of UV-excited fluorescence (Imax). Presented are the number of samples in each of the three groups, the Imax averages within groups, and coefficient of variation (CV, %).

Fluorescence Group	Number of Samples	Imax	
		Average, a.u. *	CV, %
Low fluorescence	48	10,192	9
Mixed fluorescence	99	13,734	9
High fluorescence	52	17,988	13
All samples	199	13,991	23

* Arbitrary unit.

2.4. Preparation and Spreading of Bacterial Suspension

Wood samples were contaminated with *Staphylococcus epidermidis* aerosols in controlled conditions in a stainless steel chamber (length 1050 mm, width 200 mm, height 150 mm) with a glass lid (Figure 2). *S. epidermidis* is non-motile, facultatively anaerobic, gram-positive coccus. It is ubiquitously present in human skin, and it can be used to simulate the viability and indirect transmission of bacteria from surfaces via touch. *S. epidermidis* is an opportunistic pathogen and belongs to same family with the highly pathogenic *Staphylococcus aureus.* Both bacteria are agents of nosocomial infections [19], *S. epidermidis* mainly in immunocompromised patients or patients with indwelling medical devices [20]. In this viability study, *S. epidermidis* was used as a model organism of *Staphylococcus* species.

The wood specimens of 50 mm × 50 mm were set in a random order in the chamber, shown in Figure 2. Glass pieces with the same dimensions were used as controls. The load of a single test was 48 specimens which allowed testing all five materials and glass control in a single contamination procedure. The contamination was repeated three times for all the specimen types except for the Scots pine heartwood material, which was studied separately in one bacterial contamination.

The fresh *S. epidermidis* culture was prepared before every contamination procedure. In brief, the stock culture was inoculated into 100 mL of Trypticase Soy broth (TSB) and incubated in a shaking incubator (100 rpm, 37 °C) for 24 ± 2 h. The growing culture was refreshed by taking 1 mL of culture into new TSB-broth and repeating the incubation. After that, the cells were washed three times by centrifuging (4500 rpm, 21 °C) for 10 min,

discarding the supernatant and resuspending the cells into 30 mL of sterile water. The concentration of bacterial suspension was checked and adjusted to 10^6 #/mL with Fuchs-Rosenthal chamber.

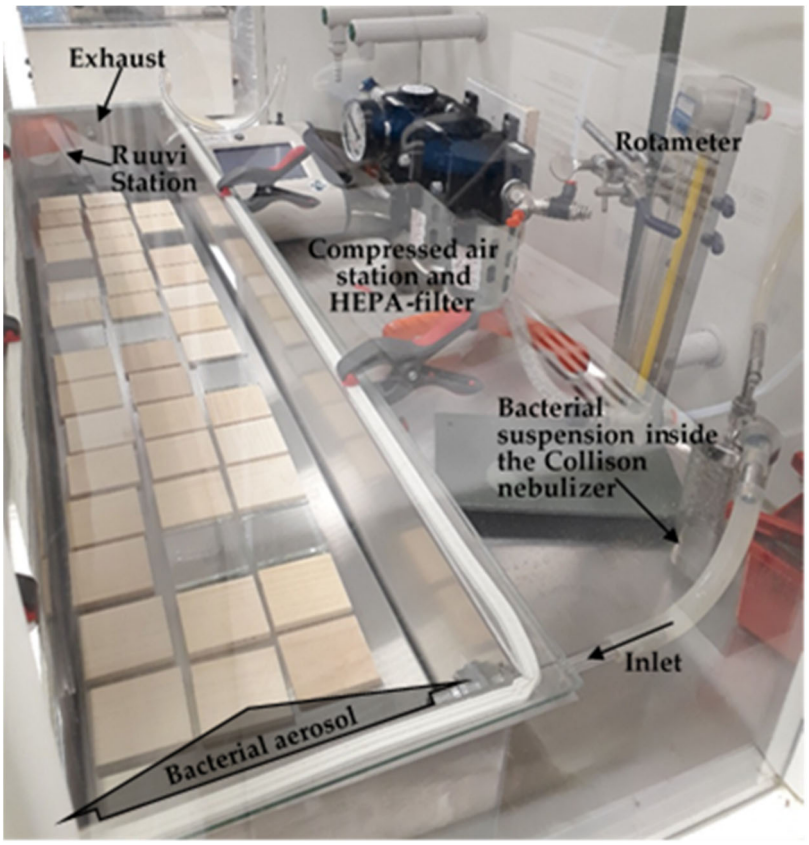


Figure 2. The test set up for the bacterial contamination.

Aerosolization of the *S. epidermidis* was done with a six-hole Collision nebulizer (BGI Inc., Waltham, MA, USA) at an air flow rate of 6 dm^3/min. Compressed HEPA-filtered air was passed through the nebulizer, and the bacterial aerosol was lead through a slit (180 mm × 2 mm) over the samples for an hour. Then, the air flow was stopped and bacterial aerosol was left to deposit on the surfaces for 30 min before the first surface sampling (0 h) was done.

Temperature and relative humidity of the air in the chamber were measured with a Ruuvi Station sensors (Ruuvi Innovations Ltd., Riihimäki, Finland). Temperature during the test ranged between 20–21 °C, which is well within the action limits (18–26 °C) of indoor environments regulated by Finnish regulation 545/2015 "Finnish Decree of the Ministry of Social Affairs and Health on Health-related Conditions of Housing and Other Residential Buildings and Qualification Requirements for Third-party Experts". Compressed air was dried (RH 2%–5%), however, the generation process produced humidity, increasing the average relative humidity during the generations to 50% ± 4%, which decreased during 24-h to 28% ± 4%. This RH range in indoor air is common during heating season in sub arctic regions.

2.5. Estimation of Bacterial Viability on the Surfaces

The number of viable bacterial colonies on the surfaces (cfu/20 cm^2) was analyzed by surface sampling with Petri films (3M^{TM} Petrifilm^{TM} Aerobic count plates, 3M Company, St. Paul, MN, USA) in time points 0 h, 2 h, 4 h and 24 h. Time point 0 h was 1 h 30 min from the beginning of the contamination procedure. The pine heartwood samples were analyzed in time points of 0 h, 2 h, and 24 h. The Petri films were prepared for sampling

according to the manufacturer's instructions by lifting the top film and adding 1 mL of sterile water onto the center of the bottom film, releasing the top film, and gently pressing the wetted film for three seconds with a 3M™ Petrifilm™ spreader to form a circular gel. The prepared films were stored in a refrigerator for 24 h. The films were taken to room temperature one hour before sampling.

Sampling was done by lifting the top film and setting the gel portion of the Petri film on the contaminated wooden or glass surface and pressing the film smoothly with sterile gloves. The film was lifted carefully from the surface, and the top and bottom films were rejoined before incubation at 37 °C for 21 ± 1 h. This was selected for analyses of Staphylococcus epidermidis which has the optimal growth temperature and time of 30–37 °C and 24 h, respectively. The preliminary tests showed that the bacterial growth can be well-observed already after 21 ± 1 h incubation period, and the risk of overgrowth was smaller within this time frame. Then, the incubation the colonies were counted. If the number of colonies was higher than 300, the counting was done from a single square of 10 mm × 10 mm, and the result was multiplied by 20 to get the number of viable bacterial colonies in the total area (20 cm^2) of the film.

2.6. Statistical Analyses

Because there was only one replication of the Scots pine heartwood exposure, the actual number of counted colonies was used to compare the low and high fluorescence samples. The hypothesis of high fluorescence samples having a lower number of bacterial colonies was tested using independent samples t-test (IBM SPSS statistics). Equal variances were not assumed. The testing of hypothesis was based on one-tailed significance deduction.

For other materials, three separate replications of bacterial exposure were performed, and the results are presented in relation to number of bacterial colonies on the glass surface at the timepoint of 0 h. The average of two glass pieces was used as the reference and was set to 100%.

3. Results

3.1. Surface Roughness

The results of the statistical analysis of the roughness data showed that the roughness values of the planed surfaces of all wood species were similar (Table 3). It was also confirmed that the mechanical processing of the surfaces did not have a major effect on the surface roughness. This indicates that the topography of the surface should not have a large impact on the results from antibacterial tests. The numerical results are included in the Table 3.

Table 3. Roughness description of wood materials and coating systems.

Wood Species	Surface Treatment	Image of Surface	Roughness, µm R_a (Sdev.) *
Norway spruce (*Picea abies* (L.) H. Karst.)	untreated		9.5 (2.03)
Silver birch (*Betula pendula* Roth)	untreated		11.8 (3.83)

Table 3. *Cont.*

Wood Species	Surface Treatment	Image of Surface	Roughness, µm R_a (Sdev.) *
Scots pine (*Pinus sylvestris* L.)	untreated		13.7 (4.72)
Scots pine (*Pinus sylvestris* L.)	Wax coating Akviwax Satin, application rate 65–70 g/m^2		11.6 (2.78)
Scots pine (*Pinus sylvestris* L.)	Varnish Akvilac FD-J 10), gloss 10, application rate 2 × 100 g/m^2		13.0 (4.92)

* R_a—Arithmetic mean height (EN ISO 21920-2:2021).

3.2. Growth of Bacterial Colonies on Low- and High-Fluorescence Scots Pine Heartwood Samples

Regarding the maximum intensity of UV excited fluorescence, Imax, the two groups of Scots pine heartwood having low or high fluorescence were clearly separated from each other (Figure 3a). At the beginning of the bacterial aerosol experiment (0 h), the two groups did not differ from each other in the number of bacterial colonies. After two hours, the number of colonies was higher on the high fluorescence samples, but their order was reversed at 24 h. Thus, only at the timepoint of 24 h, the expectation of a lower number of bacterial colonies on high fluorescence heartwood samples was realized. Generally, the variation in the number of bacterial colonies was high between individual samples and the lowest for all glass references and for the HF at 24 h. (Table 4 and Figure 4).

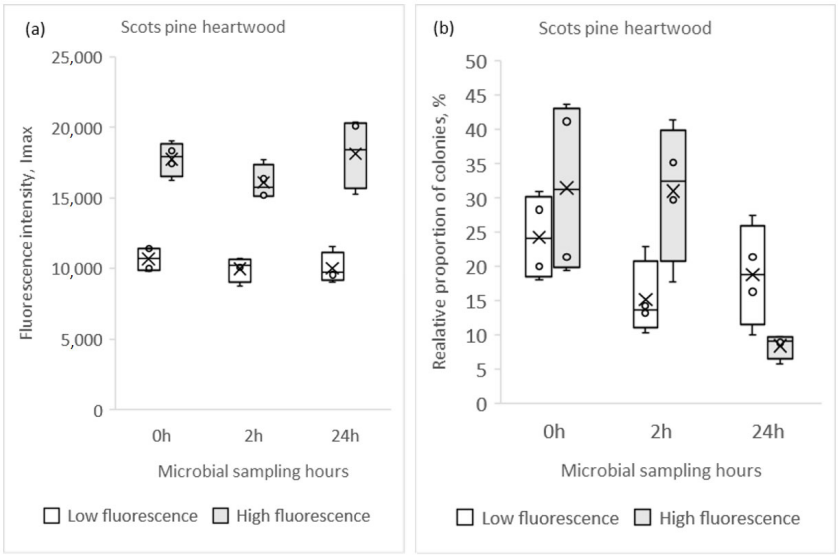


Figure 3. (**a**) Imax of the Scots pine heartwood samples used in *S. epidermidis* aerosol generation, (**b**) relative proportion of *S. epidermidis* colonies in Petrifilm. Wood measurements were presented in relation to glass average at 0 h (two measurements). $n = 4$ in each group. 0 h was defined to half an hour after the end of the contamination process.

Table 4. Average number of bacterial colonies growing on glass reference, on low-fluorescence and high-fluorescence Scots pine heartwood samples (LF and HF, respectively). Coefficient of variation (CV%) is based on two samples per sampling hour for glass and four for wood samples. 0 h was defined to half an hour after the end of the contamination process.

Sampling Hour	Average Number of Bacterial Colonies						
	Glass	CV%	LF	CV%	HF	CV%	P [a]
0 h	350	12	85	26	110	41	0.183
2 h	260	11	53	36	109	33	0.021
24 h	198	2	66	40	30	22	0.032

[a] Significance from one tailed *t*-test testing number of bacterial colonies: HF < LF.

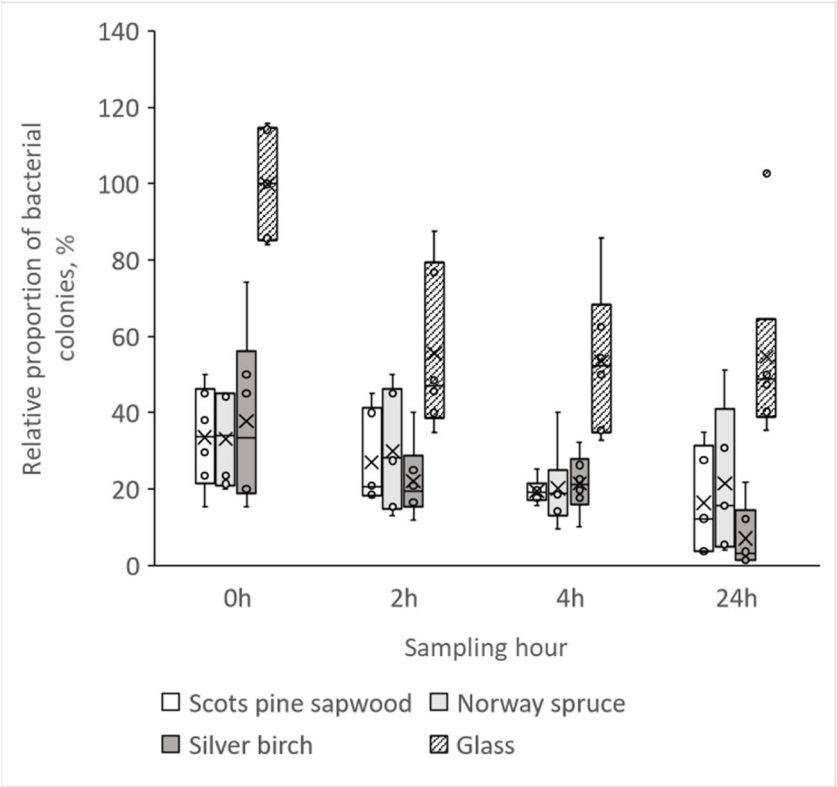


Figure 4. Comparison of untreated Scots pine sapwood, Norway spruce wood, and silver birch wood. Results are a combination from all three bacterial generations. (Average of two glass measurements of each generation at 0 h was used as a reference and was set to be 100%). Wood measurements were presented in relation to glass average at 0 h of each generation. Each group is presented by six observations except for Scots pine and Norway spruce at 24 h, which had five observations. 0 h was defined to half an hour after the end of the one-hour contamination process.

3.3. Growth of Bacterial Colonies on Untreated Wood Materials

At all timepoints, the number of viable bacteria on the untreated samples was lower compared to the glass samples. Untreated Scots pine sapwood, Norway spruce and silver birch wood behaved similarly in this experiment. The variation was large in these experiments most likely due to the contamination procedure. During the experiment, the number of viable bacteria also decreased on glass surfaces (Figure 4).

3.4. Growth of Bacterial Colonies on the Untreated and the Surface Treated Scots Pine Heartwood Specimen

In the beginning, at 0 h, there was more viable bacteria on the wax-and-varnish-treated surface than on the glass or untreated Scots pine surface. The number of viable

bacteria was also lower after 2 and 4 h on the pine sapwood surface compared to glass or treated surfaces. Finally, after 24 h the materials did not differ from each other, and the number of viable bacteria was slightly higher on glass surfaces (Figure 5).

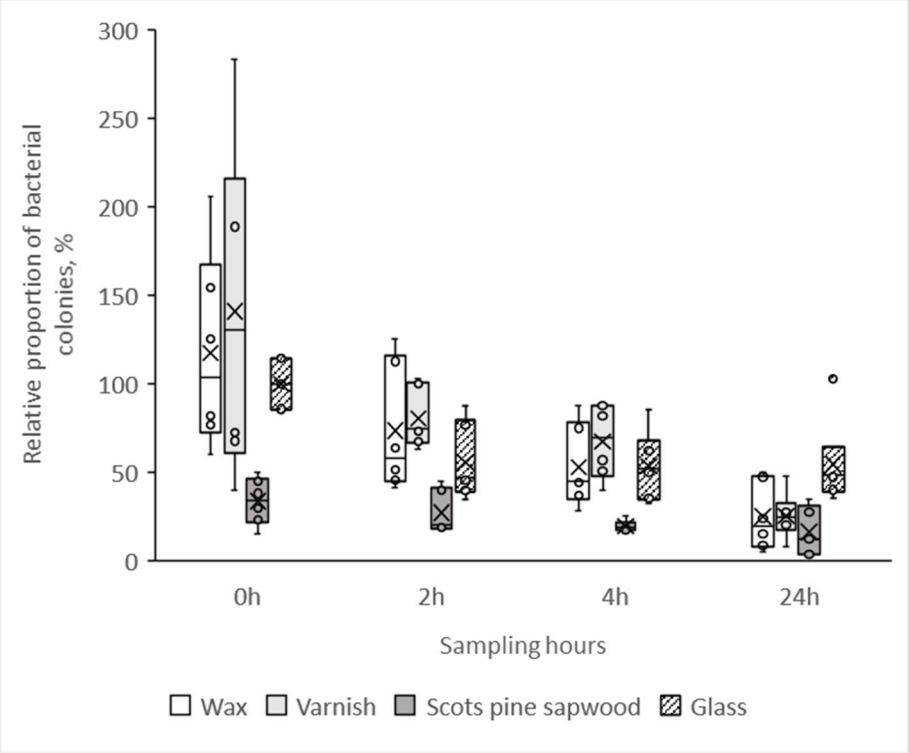


Figure 5. Relative proportion of bacterial colonies on wax or varnish treated Scots pine sapwood, untreated Scots pine sapwood, and glass reference. Results are a combination from all three bacterial generations. (Average of two glass measurements of each generation at 0 h was used as a reference and was set to be 100%). Measurements were presented in relation to glass average at 0 h of each generation. 0 h was defined to half an hour after the end of the contamination process.

4. Discussion

In the present study, the microbial testing was designed to simulate the real-life conditions, where airborne contamination lands on a vertical surface. The viability of *S. epidermidis* was studied on untreated Scots pine (sapwood and heartwood), Norway spruce and silver birch wood materials and on the coated wood materials of Scots pine sapwood.

On all the surfaces, the relative proportion of the viable bacteria declined in time during the 24-h experiment (Figures 3b, 4 and 5). At all of the time points, the highest proportion of viable bacteria was observed on the glass reference when comparing to the untreated wood surfaces. The coated surfaces had a similar number of viable bacteria as the glass reference. The differences between the materials decreased towards the 24 h time point.

The three tree species in this study differ from each other both anatomically and chemically [21]. When considering the untreated wood surfaces, the relative proportion of viable bacteria at 0 h time point was ca. 30%–40% lower on all the untreated wood specimens than on the glass surface. The difference to the glass reference was smaller at 2 h and 4 h time points—the wood specimen still all having lower average bacterial counts. The relative proportion of viable bacteria at the 24 h time point was the lowest on the high fluorescence Scots pine heartwood (Figure 3b) and on silver birch (Figure 4) samples, being less than 10% of the average of the glass reference at 0 h. The relative proportion of viable bacteria at 24 h time on the glass surface was about 50%. In general, the viability of bacteria has shown to decrease on dry and clean indoor surfaces [22]. In this study, the relative humidity of the air increased during the generation up to 54%, and it decreased to 28%

during the 24 h test period. These conditions had only minor effect on the moisture content of the wood samples. In a previous study, relative humidity was found to be less significant than the material of the surface to the viability of *S. aureus* in the range of RH 42%–65% [23].

Scots pine heartwood is rich in extractives compared to other wood materials used in this study [21]. The wide antibacterial spectrum of pine wood extracts [8] and pure stilbenes (PS, PSM) against various bacteria has been found, e.g., in foodstuffs [13,24,25], and in paper mills [14]. In solid Scots pine heartwood material, PS and PSM are the main stilbenes, and they are known to delay decay due to cellar fungus *C. puteana* both in natural heartwood [18], and in the stilbene-impregnated sapwood [26]. The degree of delay was dependent on the stilbene content [18,26]. In the present study, due to the limited number of heartwood samples, only one *S. epidermidis* aerosol generation was possible. Although the scale of the experiment is dependent on the available resources, the future studies would benefit from a larger number of heartwood samples originating from a wider distribution of Imax.

In addition to stilbenes, Scots pine heartwood also contains resin acids, the content of which was not analysed in the present study. The total resin acid content is higher than the stilbene content [10,27–29]. The resin acids are also the main components of pine sapwood and spruce extracts [10,11]. Of resin acids, especially abietic acid [12,30], dehydroabietic acid [12] and isopimaric acid [31] have shown antibacterial properties. In addition to the wood material, the cones [32], bark and needles [33,34] of spruce also have components with antibacterial properties. There are many factors explaining the decrease of the bacterial count on the pine and spruce surfaces, with the chemical combination of several antibacterial compounds and the porosity of the wood surface absorbing the free water [35]. The results for birch are in line with earlier results [7], but so far this topic has not been studied extensively. In silver birch, low content of a total number of 23 phenolic compounds extracted from wood material has been reported [36]. Several phenolics in plants [24,37] and extracts of *Betula* species [38] have antibacterial properties.

High phenotypic (0.6–0.8), [15,39] and additive genetic correlations (around 0.7), [40] between the content of stilbenes and the sum of resin acids in Scots pine heartwood has been reported. Thus, it is expected that the intensity of fluorescence under UV excitation reflects the content of resin acids, which also have antibacterial effects [12,31,41]. One of the goals of this study was to evaluate the fluorescence method for predicting the antibacterial properties of Scots pine heartwood. The fluorescence method was easy to use in practice and therefore a promising method for analyzing wooden surfaces. If the high content of stilbenes would predict high content of the resin acids, the fluorescence method could well be suitable for detecting particularly antibacterial properties of Scots pine heartwood.

The coatings studied were wax and varnish. Both had a similar amount of bacteria at each time point as the glass reference, which means that the antibacterial properties of Scots pine sapwood had no effect through these coatings. Varnish forms a very tight layer on wood surfaces, which prevents all components from wood from interacting with the bacterial cells. Similarly, the wax used in this study is water repellent and prevented the contact between the antibacterial components in spruce and the bacteria.

There were no great differences in surface roughness between the tested wood materials, so it is probable that the Petri film method was equally successful in catching bacteria from the surfaces.

The contamination procedure used in this experiment was designed so that the bacteria cells settled as aerosol containing single or few cells in a settled particle imitating the natural surface contamination process in indoor environments. The method has been successfully applied in enclosed habitats with well described ventilation and air streams [42].

For future studies, coatings which do not form a solid layer should be included to study the possibility of having a naturally antibacterial surface which is easy to clean and stays nice looking. Microbiological properties of birch and its extracts should also be studied further, especially as it is commonly used in furniture and hence is often touched by hands.

5. Conclusions

The main findings of this study were:

- Both surface coatings, wax and varnish, had a similar number of bacteria at each time point as the glass reference, which clearly shows that these coatings inhibit the antibacterial properties of wood.
- The relative proportion of viable bacteria was lower on all untreated wood specimens, also on birch, compared to the number of bacteria on the glass surface, showing that all studied wood species have antibacterial properties.
- The relative proportion of viable bacteria at the 24 h time point was the lowest on the high fluorescence Scots pine heartwood.
- This study showed that the fluorescence method for predicting the antibacterial properties of Scots pine heartwood was successfully applied.

Author Contributions: Conceptualization, T.V.-K., A.H., A.R., O.P., M.V. and P.P.; methodology, T.V.-K., A.H., A.R., O.P, M.V., A.-M.V. and P.P.; validation, T.V.-K., A.H., M.V. and A.-M.V. and P.P.; formal analysis, T.V.-K. and A.H.; investigation, A.H., A.R., O.P., M.V., J.S., A.-M.V. and P.P.; resources, A.H., M.V., O.P., A.-M.V. and P.P.; data curation, A.H., A.R., M.V., J.S., A.-M.V. and P.P.; writing—original draft preparation, T.V.-K., A.H., A.R. and O.P; writing—review and editing, T.V.-K., A.H., A.R., O.P., M.V., A.-M.V. and P.P.; visualization, A.H.; project administration, T.V.-K., A.H., O.P. and P.P.; funding acquisition, T.V.-K., A.H., A.R., O.P, M.V. and P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of the Environment, Wood building programme VN/2878/2019.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We acknowledge Siparila Oy for providing the materials and Tikkurila Oyj for the coatings. We are grateful to Matti Kilpiäinen and Antti Janhonen for help in the preparation of the wood specimens and Maarit Myllymäki for the funding administration in Luke. We acknowledge Sirpa Martikainen for assistance in microbial analysis.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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