Do biotic and abiotic factors combine to affect the weathering of wood in use class 3 ?

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Les facteurs biotique et abiotique interagissent-ils pendant le vieillissement du bois utilisé en classe d´emploi 3 ?

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Content

Declarations

I would like to thank all those who supported and encouraged me during my time as a PhD student.

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Résumé étendu

Ce résumé présente un projet de recherche portant sur les processus d'altération du bois de Douglas (*Pseudotsuga menziesii*), de chêne (*Quercus petraea*) et partiellement de châtaignier (*Castanea sativa*). Le projet porte sur l'identification des effets synergiques entre des facteurs biotiques tels que les bactéries (Eubacteria) et les champignons (Basidiomycota et Ascomycota) et des facteurs abiotiques tels que la lumière, la température et l'humidité sur le vieillissement de bois utilisé en classe d´emploi 3.

L'utilisation du bois pour des applications extérieures est actuellement assez à la mode en Europe, mais il n'est encore utilisé qu'en petites quantités par rapport, par exemple, au béton et à l'acier. Les utilisateurs recherchent des façades homogènes, aux couleurs vives et nécessitant peu d'entretien. L'aspect visuel d'une façade en bois change au fil du temps et souvent la décoloration est hétérogène. Cependant, non seulement le bois mais aussi le béton, le métal, le verre et le plâtre ont besoin d'être nettoyés, repeints et réparés après plusieurs années de service. Les analyses du cycle de vie ont montré que l'utilisation du bois comme matière première renouvelable est normalement considérée comme neutre en CO_2 et plus écologique que certains des matériaux mentionnés ci-dessus (Salazar and Meil 2009). Selon Börjesson et Gustavsson (2000), qui ont comparé l'analyse du cycle de vie d'une construction en bois à celle d'un bâtiment en béton, l'utilisation de produits issus du bois peut réduire considérablement les émissions de gaz à effet de serre. Si l'on comprend mieux quels facteurs affectent le bois, de quelle manière, et quelles combinaisons de facteurs biotiques et abiotiques interagissent pour dégrader le bois, il pourrait être possible de développer des techniques qui augmentent la durée de vie du bois de manière éco-responsable. Ce travail apporte donc des éléments pour envisager de nouvelles pistes de recherche et innovation conduisant à des façades en bois naturel et stable.

Des recherches approfondies sur la décomposition du bois par des champignons et des bactéries dans le sol et l'eau (classes d´emploi 4 et 5) ont déjà été menées. Beaucoup moins de recherches ont été faites sur le bois en usage extérieur qui n'est pas en contact avec le sol, c'est-à-dire dans la classe d´emploi 3, qui caractérise les conditions d'exposition des façades.

LES MÉTHODES UTILISÉES POUR VIEILLIR LE BOIS

Des expériences d'altération naturelle et artificielle ont été menées afin d'étudier les changements sur les surfaces de bois et en particulier la présence de micro-organismes sur les surfaces de bois altérées.

Une expérience de vieillissement naturel a été menée afin de fournir des données de référence sur le vieillissement du bois lorsqu'il est exposé à un climat atlantique. L'expérience d'altération en extérieur a été menée dans l'ouest de la France, à Nantes, où les données météorologiques ont été enregistrées sur place. Cette expérience réaliste permettra aux chercheurs mais aussi à l'industrie d'avoir un aperçu du processus de dégradation des espèces de bois spécifiques à cet endroit. Comme la variation du mouillage de la surface est très différente d'une essence de bois à l'autre et joue un rôle

important dans le processus de dégradation du bois, la taille des échantillons, leur orientation et l'alignement des échantillons ont été soigneusement choisi. Le bois a été exposé conformément à la norme EN 927-3.

En parallèle, des échantillons ont été artificiellement altérés dans un appareil QUV. Il était prévu d'appliquer trois cycles d'altération différents dérivés de la norme EN 927 avec des cycles de condensation, avec et sans pulvérisation d'eau, à certaines températures et à différentes forces d'irradiation des lumières UV. L'altération artificielle offre la possibilité d'éliminer certains facteurs de dégradation et donc d'observer l'influence de certains paramètres sur le processus de dégradation du bois. L'expérience QUV a démontré que cette technique d'altération artificielle n'est pas stérile comme on pourrait le penser en raison des fortes lumières UV, connue pour être capable de tuer les micro-organismes.

De plus, un appareil de vieillissement artificiel a été développé en interne. On espérait créer des conditions stériles au sein de dispositif, offrant ainsi la possibilité d'étudier seulement l'impact des facteurs abiotiques au cours du vieillissement. Malheureusement, il s'est avéré impossible de maintenir une stérilité complète au cours de la plupart des expériences, car une souche particulière de bactéries, le *Bacillus* sp. a réussi à envahir. Suite à une expérience sur l'utilisation de l'azide de sodium, le maintien de la stérilité resté difficile.

LES MÉTHODES UTILISÉES POUR CARACTÉRISER LA DÉGRADATION DU BOIS

Comme il a été constaté que le mouillage de la surface de différentes essences de bois pendant l'altération augmente avec le temps d'exposition (Oberhofnerová and Pánek 2016), et que la rugosité augmente, des fissures apparaissent et la couleur change rapidement (Oberhofnerová et al. 2017) respectivement, afin de mesurer l'influence de l'altération, des méthodes visuelles, chimiques, mécaniques et microbiologiques ont été appliquées une fois que les échantillons ont été exposés à l'une des installations d'altération.

Des mesures de couleur CIE Lab*, des mesures de rugosité de surface avec un stylet et la spectroscopie IR par réflexion totale atténuée ont été effectuées. Les résultats obtenus confirment fondamentalement les recherches précédentes, mais n'ont pas permis de tirer des conclusions sur l'importance des facteurs de dégradation biotiques.

La microscopie électronique à balayage, la microscopie optique et les scans des surfaces ont été appliqués pour observer la dégradation sur un plan visuel. Ces investigations, plutôt subjectives, combinées se sont avérées être de bons outils pour détecter les dommages de la matrice en bois ainsi que pour obtenir une image de la contamination microbiologique. Dans le cadre de cette thèse, un système de classement a été créé pour grouper les échantillons en classes de dégradation qui pourraient ensuite être utilisées pour intégrer les données obtenues à une analyse statistique.

La spectroscopie de fluorescence des rayons X a été appliquée pour détecter les éléments inorganiques sur les surfaces de bois exposées à l'appareil de vieillissement fabrique où l'on a essayé d'obtenir des conditions stériles à l'aide d'azoture de sodium. De cuivre, de zinc et de calcium ont été trouvées dans des quantités à ne pas sous-estimer.

La quantification des microorganismes sur les surfaces de bois a été réalisée en récupérant les souches sur des boîtes de Pétri contenant de l'agar sélectif. La quantité de colonies formant des unités (CFU) a été élaborées visuellement, ensuite plusieurs souches ont été isolées et préparées pour être identifiées.

La technique d'identification Maldi-TOF devait aider à identifier les micro-organismes, mais les bases de données disponibles ne permettaient guère de classer les micro-organismes trouvés sur les surfaces de bois altérées. Suite une souche bactérienne du genre *Bacillus* et une autre souche fongique du genre *Aspergillus* ont été identifiées par des aspects morphologiques.

Enfin et surtout, les teneurs en humidité ont été évaluées sur des échantillons exposés à l'extérieur. L'installation d'un enregistreur des données a permis de mesurer constamment les échantillons exposés.

LA DÉGRADATION DU BOIS PAR LES BACTÉRIES

Les bactéries sont probablement le facteur, qui influence le processus de dégradation du bois, le moins étudié. Cependant, les recherches sur les communautés bactériennes sur le bois coulé en mer Méditerranée ont révélé que dans un environnement anaérobie, une grande diversité de communautés microbiennes existe (Fagervold et al. 2014). Il est probable que la diversité des communautés microbiennes soit très élevée, non seulement en milieu anaérobie mais aussi en milieu aérobie. Selon de nombreuses études, les bactéries provoquent différents types d'attaques sur le bois (Greaves 1969; Fengel and Wegener 1989; Eriksson et al. 1990). Les bactéries aérobies dégradant le bois ont été étudiées par exemple par Daniel (1994a) et on rapporte qu'elles dégradent les préparations de lignocellulose à l'aide de l'enzyme lignine peroxydases. Certaines bactéries produiraient de l'ammoniac qui augmente la valeur du pH (Schmitz 1919). Ces modifications de l'environnement de croissance causées par les bactéries peuvent influencer la capacité de développement fongique. Ces recherches rendent particulièrement intéressante la formulation de l'hypothèse suivante : Les bactéries, présent dans un environnement aérobie, influencent le processus de dégradation du bois sans contact direct avec le sol ou l'eau.

Il a été constaté que les communautés microbiennes étaient très dépendantes du fait que le bois soit exposé naturellement ou artificiellement. Dans un environnement naturel, où les souches fongiques semblent constituer la majorité des organismes envahisseurs, les propriétés antifongiques de certaines souches bactériennes pourraient être utilisées pour contrecarrer la dégradation fongique du bois.

Abbreviations

ATR.....*Attenuated total reflection*
BPT.....*Black panel temperature*
C……..*Chestnut wood*
Ca.....*Calcium*
CFU..... *Colony forming units*
CO_2..... *Carbon dioxide*
Cu..... *Copper*
D……..... *Douglas fir wood*
FCBA.....*Institut Technologique Foret Cellulose Bois- Construction Ameublement*
FSP.....*Fibre saturation point*
FTIR..... *Fourier transform infrared spectroscopy*
G.lignin.....*Guaiacyl*
H_2OT..... *Water temperature*
Hum..... *Air humidity*
I……..... *6 weeks weathering to NSWE*
II……..... *12 weeks weathering to NSWE*
LFB..... *Laminar flow bench*
LR.....*Linear regression*
MC..... *Moisture content*
ME- agar..... *Malt extract agar*
Mn.....*Manganese*
N……..*Exposure to a natural environment*
Ni……..*Nickel*
NSAWE.....*Non-sterile artificial weathering experiment*
NSWE.....*Non-sterile weathering experiment*
NWE..... *Natural weathering experiment*
O_2..... *Oxygen*
Pb..... *Lead*
PCA.....*Principle component analysis*
PC-agar.....*Plate count agar*
PMMA.....*Poly methyl methacrylate*
Q.....*Oak wood*
QUV.....*Standardized accelerated weathering device*
RR.....*Recovery rate*
SA.....*Staphylococcus aureus*
SAWE..... *Sterile artificial weathering experiment*
Sb……..*Antimony*
SCT.....*Surface contact test*
SD.....*Standard deviation*
Se……..*Selenium*
SR.....*Surface roughness*
Sr……..*Strontium*
UVAC..... *Exposure to adapted UV irradiation force and condensation*
UVC..... *Exposure to UV irradiation and condensation*
UVSC..... *Exposure to UV irradiation, spray and condensation*
Wdirect.....*Wind direction*
Wspd.....*Wind speed*
XRF..... *X-ray fluorescence spectrometry*
Zn..... *Zinc*

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If not indicated otherwise, graphs and photos derive from a personal collection.

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1. Chapter: Introduction

When wood is used in outdoor applications, it is susceptible to environmental degradation, as are all common building materials. In France, maintenance of treated oak and Douglas fir facades is recommended every 2-3 years (Deval 2019), which seems to demand a lot of effort, therefore the use of treated wood is often not considered for facades in residential buildings. Culturally, the use of non-treated and non-coated wood in exterior applications is currently fashionable in France. Such façades do not require maintenance, are recyclable and all pollution associated with the application of finishing products is avoided (Envirobat Grand Est 2014). Wood weathering is caused by weather conditions such as light irradiation and rain, as well as by decay, caused by microbes e.g. fungi (Feist 1989). Investigations of wood weathering require careful planning because it is a complex phenomenon where many parameters impact the appearance and performance of the material. This research tries to improve our understanding of weathering. The main focus of this research is on weathering of solid wood, non-treated and uncoated, that is not in ground or soil contact, e.g. the exposure conditions of facades, and is classified according to EN 335 (2013) as use class 3.

1.1 Case examples

In order to get an insight into the phenomenon of heterogeneous discolorations, some examples of weathered wood facades and their evolution over time are presented below. The changes are shown at a macroscopic level, i.e. by eye, and a microscopic level using optical and electron microscopes.

1.1.1 Macroscopic observation

Figure 1: Façade of the Biotechnical faculty of the University of Ljubljana shortly after construction (Source: https://www.e-architect.com/images/stories/slovenia/biotechnical_ faculty_ljubljana_k260510_m4tb.jpg) (left) and after 8 years of exposure (right)

Figure 1 shows how the wooden façade of the biotechnical faculty of Ljubljana University has changed after 8 years of weathering. A strong difference between areas exposed to weather conditions, and light or rain shaded areas can be observed. Areas completely exposed appear in dark grey, which might be related to leaching of wood chemicals damaged by UV light. Shaded areas however remain yellowish but turned darker which could be linked to moisture accumulation. The transition from shaded to non-shaded areas appear in bright silvery grey. The horizontal construction frame causes water to drip onto the façade and this causes leaching in these areas. Moreover, the weathered façade shows several horizontal bands (very visible in the fourth panelling). It is likely that the bands

coincide with the support battens which attach the façade to the building. The presence of these battens may affect the ventilation behind the façade, and/or its temperature. Both factors would have an impact on the moisture content (MC) of the façade thus creating a micro-climate that may favour the appearance or not of micro-organisms or favour a particular microbe over another. Such micro-climates can lead to very local differences in discoloration.

Figure 2: Wooden facades in Nantes. Exposure towards south-west (left) and north-east (right)

In Figure 2 we can observe a more severe greying on the façade facing the more sun exposed south-west compass direction (left picture) compared to north-east (right). Greying in upper floors appears stronger versus in ground floors. This difference is likely due to higher irradiation, since only top parts of the building are exposed to sun as it rises. Moreover, uppermost parts of the building are exposed to sturdier rain as surrounding buildings form a sort of wind shield at the bottom. Areas underneath the rims of the different floors as well as below the balconies of the façade facing south-west, remain yellowish. Since these areas are protected from direct sunlight and rain and therefore leaching, greying occurs at slower rates. The façade facing north-east however shows darker areas at the rims of the stories. As solar radiation is less pronounced, and moisture dries out more slowly when facing north-east, most likely accumulation of humidity occurs.

Figure 3: Facades with horizontal and vertical wood grain alignment in (left to right) Nantes, Biel and Les Menuires

As in the pictures of Figure 2, the left picture in Figure 3 shows less pronounced leaching along the wood grain in lower parts of the façade. Some areas are still yellowish, the windowsills seem to

protect areas just underneath from weathering through UV irradiation and rain. Possibly other degrading factors such as wind and pollution are reduced as well through a shield effect. The example in Biel shows that leaching along the grain impacts the colour change to a greater extent than across the grain. The pronounced dark colour at the bottom of the wooden boards might be related to humidity and / or growth of microorganisms. The phenomenon of leaching and accumulation of humidity can also be observed in the picture on the right showing a garage gate. Leached areas appear in bright silvery grey. Water runs down till the bottom of the gate where it gathers since it cannot run off, therefore the MC increases. Moreover, splashing water rejected from the pavement towards the lower part of the garage gate might enhancing the humidity as well.

Figure 4: Wood exposed and attacked by fungi, visible to the naked eye

The left and middle pictures in Figure 4 show a poplar wood façade in Florence. Water stains and discoloration such as greying are noticeable. A close-up view reveals dark spots, which are an indicator for blue stain. The picture on the right shows a softwood beam exposed in Nantes, invaded by fungi, moss and lichen. It is interesting to observe is the fact that not all faces of the beam are covered with the green film, only those that incline more than 90 ° towards the ground.

1.1.2 Microscopic observation

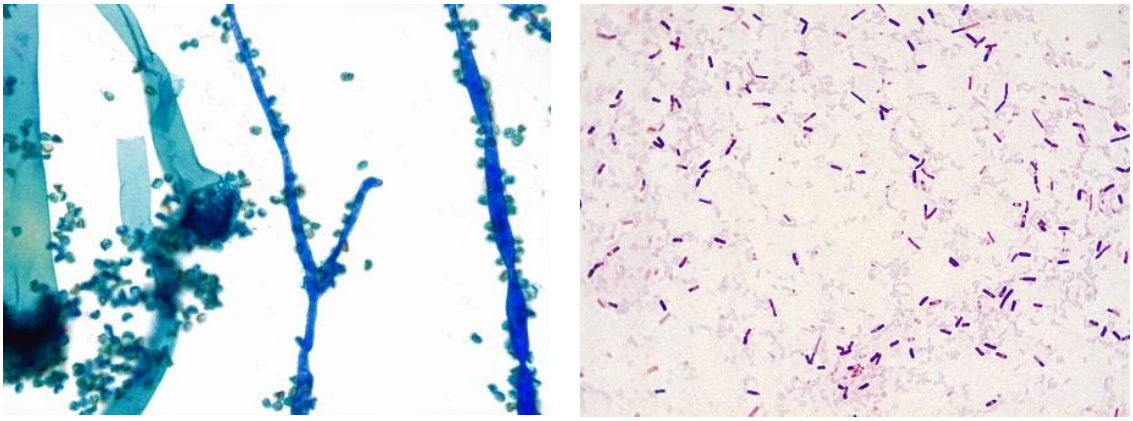


Figure 5: Light- microscopic images of fungi (left) and bacteria (right)

Figure 5 shows microscopic pictures of fungal (left) and bacterial (right) strains isolated from artificially weathered oak wood surfaces. The fungi were coloured with methylene blue and the bacteria was gram stained in order to obtain clear images. These images prove the presence of fungi as well as bacteria on artificially degraded wood.

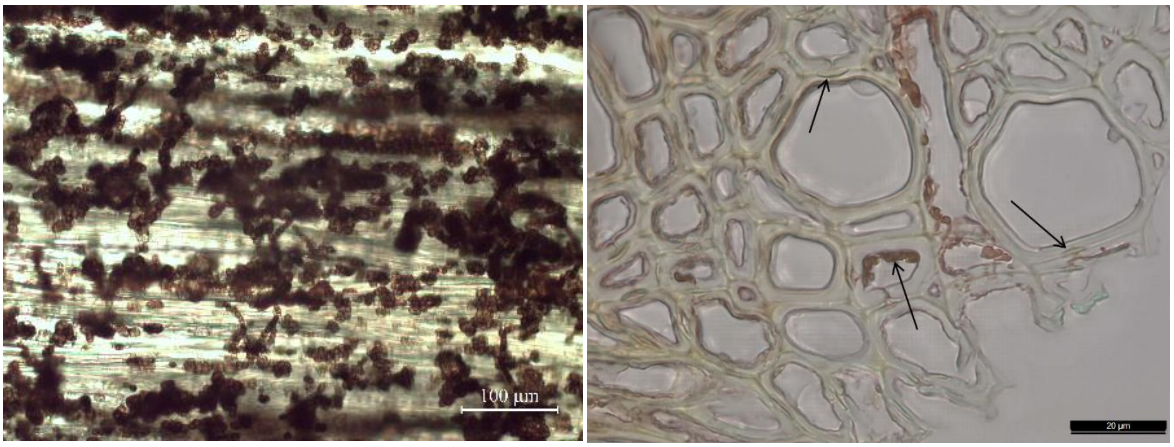


Figure 6: Microscopic observation of degraded oak wood

Weathered oak wood (see Figure 6) demonstrate the presence of fungi and their impact on outdoor weathered wood. The left picture shows oak wood invaded by blue stain fungi. We observed numerous dark spores in the top layer of the wood. The image on the right shows a microtome slice of a weathered oak specimen. Spores, attached to the lumen as well as detachment of the S2 layer of vessels cell walls can be observed.

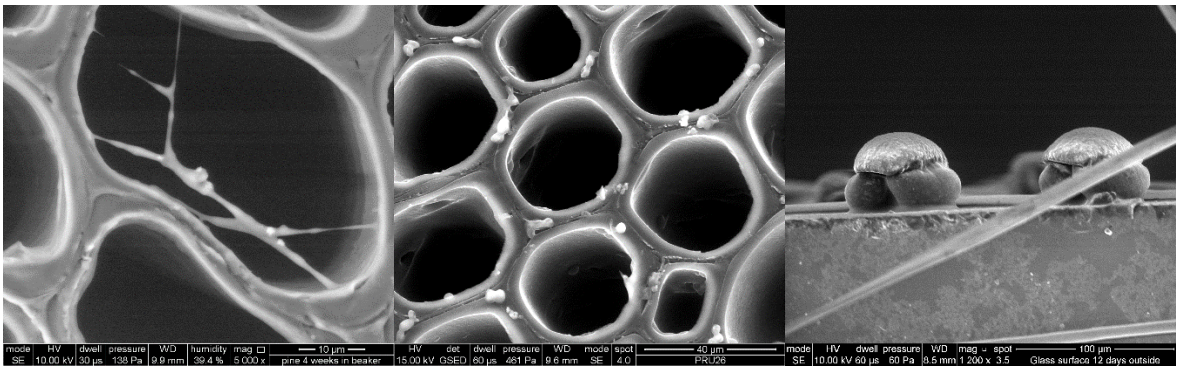


Figure 7: ESEM observation of surfaces exposed to humidity or weathering

Two images of a wood sample and a glass surface, taken with an environmental scanning electron microscope (ESEM) are shown in Figure 7. The left and middle pictures show *Pinus radiata* specimens, previously submerged in water for 4 weeks. Inside the lumen, hyphae were noticeable, a clear evidence of fungal attack. On the tracheid cell walls, small spherical particles can be seen. Even though no clear visible distinction between debris and living cells could have been established with the ESEM, with increasing time to water exposure, particles appeared in different and larger shapes. This led to the assumption that bacteria underwent cell division and that the detected particles were living cells. Pollen, visible in the image on the right, as well as other particles and small debris can easily be observed on outdoor exposed glass surfaces.

1.1.3 Summary

The photos presented above provide a rough insight to the many factors that influence the weathering of untreated wood. Conditions crucial for the pace and homogeneity at which wood degrades, are also mentioned in standard EN 927- 1 (2013).

The following table lists some parameters decisive for the degradation of wood in exterior applications and shows an overview of some exposure conditions and their observed impact on the MC, temperature (Temp) and UV-irradiation (UV). Comparing high density wood to lower density wood, the heavier the wood species is, the slower the MC and Temp will change. Moreover, there is greater penetration of UV light into the surface layers of low density wood. When wood exposed to different compass directions, on boards exposed towards north more elevated MC will be found compared to wood facing south.

Table 1: A summary of how moisture content, temperature and UV- irradiation change in specific exposure conditions. The factors are either higher (↑), lower (↓) or do not show strong differences (=).

Exposure condition	*Specification*	*MC*	*Temp*	*UV*
Wood properties (e.g. species)	High density	↓	↓	↓
	Low density	↑	↑	↑
Exposure direction (compass direction)	South	↓	↑	↑
	North	↑	↓	↓
Grain direction	Horizontal	↑	=	=
	Vertical	↓	=	=
Distance to the ground	Close to the ground	↑	↓	↓
	Upper floors	↓	↑	↑
Design (e.g. shelter or ventilation)	Sheltered	↑	↓	↓
	Non sheltered	↓	↑	↑
Inclination (towards the ground)	Allowing fast drying (90°)	↓	=	=
	Doesn´t allow fast drying (0°)	↑	=	=

However, the above photos reveal as well, that the extent and impact of each single degradation factor on the weathering process is not well understood. Interactions between climatic factors are complex and only assumptions can be made. Shade diminishes the decomposition of chemicals through reduced UV irradiation and the amount of leached products decreases, hence greying may reduce. Nevertheless, shade caused by an overhang should increase the MC which therefore may enhance the growth of microorganisms. On the other hand, the same overhang would reduce exposure to liquid water (rain) and, therefore, lower moisture content. In addition, very local microclimatic variations occur, minor changes of the exposure conditions might have severe impact on abiotic as well as biotic weathering factors.

The previously shown microscopic images manifest the fact that many different kinds of pollution particles, fungi as well as bacteria are ubiquitous and will be present on all real-world exposed wood surfaces. The presence of microorganisms does not necessarily come with a degradation of the wood structure right away but is highly likely to affect the prevailing microclimate. Therefore, the question arises whether biotic and abiotic weathering factors interact during wood degradation.

1.2 Hypothesis

The hypothesis is set that biotic and abiotic factors combine to affect the degradation process of wood in use class 3.

1.3 Aims and objectives

The aim of the project is to identify the relative importance of the effects of biotic factors such as bacteria (*Eubacteria*) and fungi (*Basidiomycota* and *Ascomycota*) and abiotic factors such as light, temperature and moisture on the weathering of wood surfaces in use class 3.

As shown in Figure 8, multiple weathering procedures were applied in order to degrade wood samples in natural conditions, as well as to simulate weathering while eliminating certain degrading factors and therefore observe the influence of specific factors on the degradation process of wood.

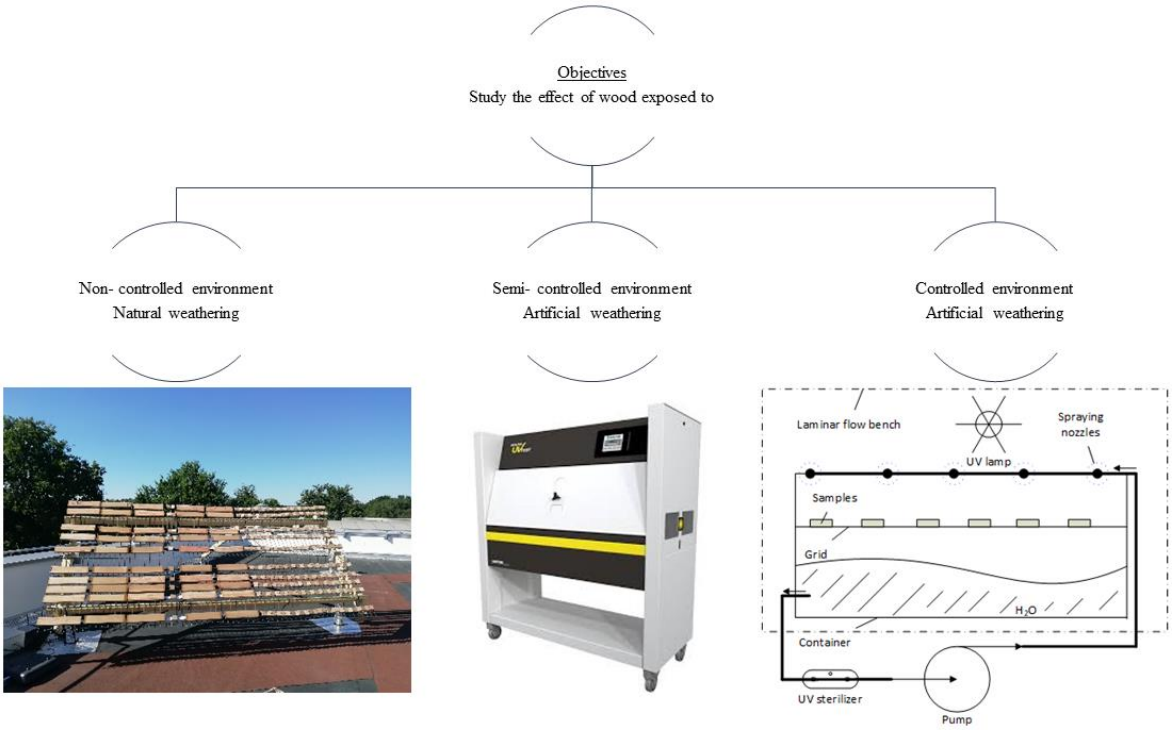


Figure 8: Natural and artificial weathering test scenarios

A natural weathering experiment was carried out to observe the general phenomenon when exposed to an Atlantic climate and served as a benchmark. The experiment also provided the opportunity to collect microbial strains found in this specific region. A real advantage of natural weathering is that it exposes specimens to the complete spectrum of solar radiation, which is rather complicated to be mimic artificially. Although some experiment parameters like orientation, inclination and duration, can be controlled, the actual environmental conditions are “non-controlled”. As a consequence, the above mentioned abiotic and biotic parameters are not set but measured.

Artificial weathering of specimens was conducted using QUV machines. Although such devices are traditionally used to accelerate weathering, the aim of the experiments conducted in this thesis with

the QUV was to observe the differences in weathering observed as abiotic parameters were changed. The aim was to determine the relative importance of abiotic factors, as well as the extent of microbial contamination, through the application of various degradation cycles with different UV-light intensities, at certain temperatures and creating alternative wetting histories by the application or omitting of spraying cycles. The possible presence of microbes in the QUV machine infers that any differences observed cannot be completely attributed to abiotic factors.

Consequently, a third group of experiments were carried out with the aim to observe weathering caused entirely by abiotic factors, which requires weathering in a sterile environment. A lot of time and effort was expended in designing, making and testing a device that would weather wood in a sterile environment. Moreover, the high humidity conditions within the device should be very favourable to microbial growth. Two versions of the device were made: one that was kept sterile and the other non-sterile. Both devices ran in parallel to avoid differences in the climatic and seasonal variations found in the laboratory. So in the sterile version of these experiments, all parameters and conditions are controlled.

In order to measure the influence of the different applied weathering procedures, different visual, chemical, mechanical and microbiological methods were applied and are described in the following chapters.

1.4 Importance of this research- Environmental and social issues

Weathering is one of the reasons for premature replacement of wood used outdoors. Customers seek homogeneous, colourfast facades with low maintenance requirements. That is to say that the choice of the appropriate wood species for each application and a suitable design adapted to its environment can prevent damage, nevertheless, today we are not able to define precise criteria, responsible for wood degradation in different applications and exposure conditions.

There are many different approaches in order to prevent degradation. Modified wood may appear homogeneous for a longer time. Not only a wide range of chemical treatments are on the market, also thermal modification became more and more popular (Jirouš-Rajković and Miklečić 2019). However, it seems that these approaches are not satisfactory. Treatments do not last forever and still degrade with time through, e.g. UV irradiation and erosion, even if more slowly.

Protecting wood by the application of chemicals can lead to environmental problems. The wood will still be broken down into small particles through erosion, allowing chemical residues to enter and potentially persist in ground and water. As the introduction of antibiotics has resulted in antimicrobial resistance, the introduction of pesticides is one of the causes of the decline in insect populations (Braak et al. 2018) and the persistence of microorganism in general. We shall be aware that the use of chemical treatments on wood in exterior applications comprises a certain risk to the environment

as well as to human health. The use of older wood preservatives such as pentachlorophenol or creosote have been banned or restricted (Stockholm Convention on Persistent Organic Pollutants 2017), still, new products and their derivatives are continuously being introduced to the market even though environmental consequences are largely unknown.

If we want to design long-lasting, environmentally friendly wood products for exterior applications, a clear understanding of wood weathering is of great importance. Only if the causes for wood degradation are clearly understood, measures to counteract, in a responsible manner, can be implemented.

2. Chapter: Literature review

This chapter provides information about wood degradation and introduces some microbiological aspects. Only the degradation factors investigated within the experimental part of the thesis are described in detail. The literature review stresses on the microbiological degradation of wood. Standardised artificial as well as natural weathering techniques are introduced in this chapter. Lastly some methods to characterise wood degradation are listed.

2.1 Wood degradation factors

Wood degradation is caused by weathering, so the decomposition of the material through meteorological conditions, i.e. abiotic factors, as well as by decay, i.e. biotic factors.

Moisture, solar radiation, temperature, oxygen and atmospheric pollutants have been described as responsible factors inducing weathering (Feist 1989; Evans 2012). Chemical decomposition through strong acids or bases and mechanical damage such as erosion were additionally mentioned by e.g. Zabel and Morrell (1992). Furthermore, since wood is an organic material, it can be susceptible to biological degradation, i.e. marine borers, termites, beetles, hymenoptera, bacteria and fungi.

Factors that influence the degradation process of wood surfaces, and that are taken into account within this research, are the wood itself; with its specific anatomical and chemical features; the humidity, light irradiation, temperature and microorganisms such as fungi and bacteria (as shown in Figure 9).

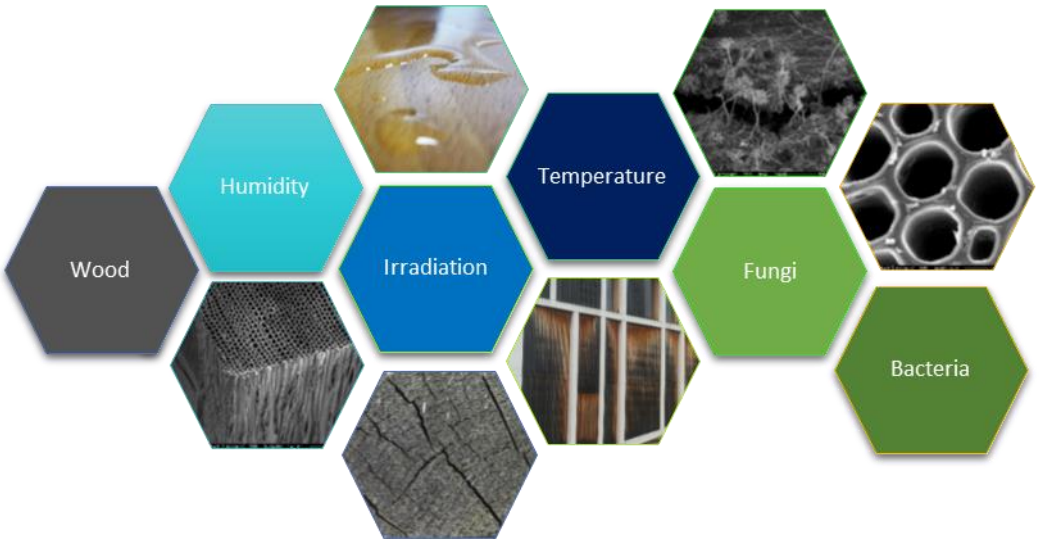


Figure 9: Abiotic and biotic wood degrading factors studied within the research

2.1.1 Wood

The variability of wood, in terms of anatomy and chemistry is very broad (Castera and Morlier 1994; Diaconu et al. 2016). The chemical composition as well as the wood structure, such as cell type and cell wall morphology, vary from one wood species to another and have a significant influence on

wood degradation (Eriksson et al. 1990). The concentration of extractives has been proven to vary among species, between individual trees of the same species as well as within a single tree (Taylor et al. 2006). The variety in-between a single species may be very broad, depending on growth conditions, age and storage. The growth rate plays an important role since it effects the density of the wood which will influence erosion, capillary properties and the absorption and desorption of water (Van den Bulcke et al 2011). It is well-known that there is greater erosion in earlywood than in latewood because of the differences in density and, therefore, mechanical properties (George et al. 2005; Hazneza and Evans 2016). Table 2 presents some characteristics of the wood species used in this research. Douglas fir (*Pseudotsuga menziesii*) was chosen as a representative softwood species and oak (*Quercus robur*) as well as chestnut (*Castanea sativa*) were the tested hardwood species. In **Chapter 3.1** reasons for the choice of the investigated wood species are given.

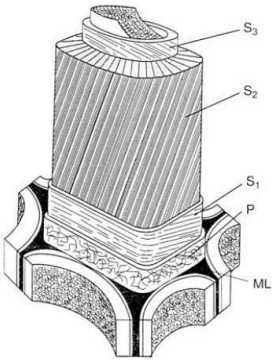
Table 2: Characteristics of the used species. Sources: (Fengel and Wegener 1989)[1], (EN 350 2016)[2], (Cirad 2013)[3],(Geffert et al. 2019)[4],(Kakavas et al. 2018)[5], (Wagenführ 1999)[6]

Wood properties	***Pseudotsuga menziesii***	***Quercus robur***	***Castanea sativa***	**Source**
Wood type	Gymnosperm	Angiosperm	Angiosperm	3
Resin canals	✓	✗	✗	3
Cell content in percent:				6
Vessels	-	8	26	
Fibres tracheids	93	58	56	
Xylem ray parenchyma	7	29	17	
Axial parenchyma	-	5	1	
Application	Veneers, furniture, construction wood (In- and Exterior)	Furniture, carpentry	Furniture (Also exterior)	
Lignin content	27.2%	24.5%	31.8%	1
Cellulose content	50.4%	37.6%	47.3%	1
Holocellulose	67.0%	77.0%		1
Ethanol-benzene extract	4.4%	4.4%	4.7%	1
Hot-water extractive content	5.6%	12.2%		1
Ash	0.2%	0.3%	0.4%	1
pH- value (Heartwood)	3.3	3.46	4.05	4, 5
Density [kg/m3] at 12% MC	470-550	650-760		2
Volumetric shrinkage	0.46%	0.44%	0.42%	3
Total tangential shrinkage	6.9%	9.7%	6.9%	3
Total radial shrinkage	4.7%	4.5%	4.2%	3
Fibre saturation point	27%	31%	30%	3
Natural durability against fungi	Class 3-4 (3-5)	Class 2	Class 2	3
Use class	Class3	Class 3	Class 3	3
Treatability of heartwood	Class 4	Class 4	Class 4	2
Drying rate	rapid to normal	slow	normal to slow	3

Due to the way a tree grows, wood has 3 different faces: radial tangential and transversal. The different orientations of the diverse cell types that make up softwoods or hardwoods influence wood properties. The face of the wood exposed to weathering has an influence on the visual appearance of the surfaces (Hazneza and Evans 2016). For facades, usually radial, tangential or a mixture of these two planes are most often used, the use of transversal cutting directions is very uncommon.

Besides the main component of 40-50 % cellulose ($(C_6H_{10}O_5)n$) and 25-30 % hemicelluloses ($C_5H_{10}O_5$) wood consists of 15-30 % lignin ($C_{81}H_{92}O_{28}$). 0.2- 1 % of wood are organic compounds such as resins, tannins, polyphenols, waxes, fatty acid esters, terpenes, cyclic diisoprenoides and stilbenes. Another 0.2- 1 % of the wood consists of inorganic ashes containing potassium, calcium, magnesium-salts, iron, manganese and boron (George et al. 2005).

Spaces between micro- fibrils are partially filled with hemicelluloses and lignin and are referred to as micro- pores (Hill and Papadopoulos 2001). In the swollen state, the micro- pores; with maximum sizes of 2-4 nm; are open and accessible by entities with diameters smaller than that of the micro-pores (Hill 2006). Figure 10 shows information about the microstructure of the cellular wall.



Cell wall layer	Thickness [µ]	Cellulose (%)	Lignin (%)
Secondary wall (S3)	1	90	10
Secondary wall (S2)	1-10	50	50
Secondary wall (S1)	1		
Primary wall (P)	0.1	10	90
Middle lamella (ML)	1 - 4	10	90

Figure 10: Microstructure of the cellular wall (Booker and Sell 1998) and the thickness and composition of the layers according to Dirol and Deglise (2001)

2.1.1.1 Cellulose

Cellulose is the main cellular component that controls the mechanical, physical and chemical properties of wood and especially its hygroscopicity. Highest cellulose concentrations occur in the secondary wall. It is a long-chain polymer constructed of β-1,4-linked D-glucose units, which can appear as a highly crystalline material (Fan et al. 1982). The water molecules can be absorbed through the hydroxyl groups on the surface (Dirol and Deglise 2001).

In the centre of micro fibrils, crystalline regions with orientated molecules are found. Due to the high crystallinity of micro- fibrils, cellulose is relatively unreactive and thermally stable (Hill 2006). The micro fibrils are surrounded by amorphous regions of non-orientated cellulose molecules as well as hemicelluloses and lignin (Ah Chee 2001).

2.1.1.2 Hemicelluloses

This polymer differs from cellulose in that it has shorter chains. Hemicelluloses are polysaccharides made from sugars other than carbohydrates: xylose, arabinose and uronic acids. Its role is probably structural and consists in binding micro fibrils in a common matrix which prevents the cellulose from being too crystalline (Dirol and Deglise 2001). The middle lamella shows highest concentration of hemicelluloses (Ah Chee 2001).

2.1.1.3 Lignin

Lignin is a highly polymerised aromatic compound with a phenylpropane skeleton. It is a three-dimensional polymer with many different types of linkages between the building blocks, which makes lignin by far the most complex of all natural polymers (Nascimento et al. 2013). Resinous trees contain more lignin than hardwoods. While hardwoods contain syringyl and guaiacyl types of lignin, the primary lignin type in conifers is guaiacyl (Fengel and Wegener 1989). The phenolic structure of lignin acts as a binder between the micro fibrils in the cell walls of wood tracheids (George et al. 2005). As shown in Figure 10 the middle lamella consists to about 90 % of lignin, degradation of lignin is thus mainly taking place in the middle lamella, but the bulk of lignin is in the secondary wall (Ah Chee 2001).

The structural role of lignin is important, but it is also decisive for wood´s durability (Dirol and Deglise 2001). It is the key structure in wood photo-degradation (George et al. 2005). The colour of lignin is naturally dark and strongly affected when exposed to light irradiation (Volkmer et al. 2016), resulting in colour change.

2.1.1.4 Extractives

Extractives are mainly found in resin canals (Softwoods) and in the ray parenchyma tissue (Hardwoods). E.g. terpenes, lignans, stilbenes, flavonoids, tannins, fats, waxes, fatty acids and alcohols are compounds attributed to resin compounds or extractives (Fengel and Wegener 1989). Organic extractives in wood can significantly influence the natural resistance to colonisation by microorganisms (Schönwälder et al. 2002; Windeisen et al. 2002; Taylor et al. 2006; Nascimento et al. 2013). During drying processes or storage, the extractives content may decrease significantly through volatilisation (Zabel and Morrell 1992).

2.1.2 Light irradiation

The sunlight consists of ultraviolet (280-400 nm), visible (400-780 nm) and infrared light (>780 nm) (ISO 9022-9 2016). Ultraviolet (UV) light can be further grouped for example in UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm) spectra (ISO 21348 2007).

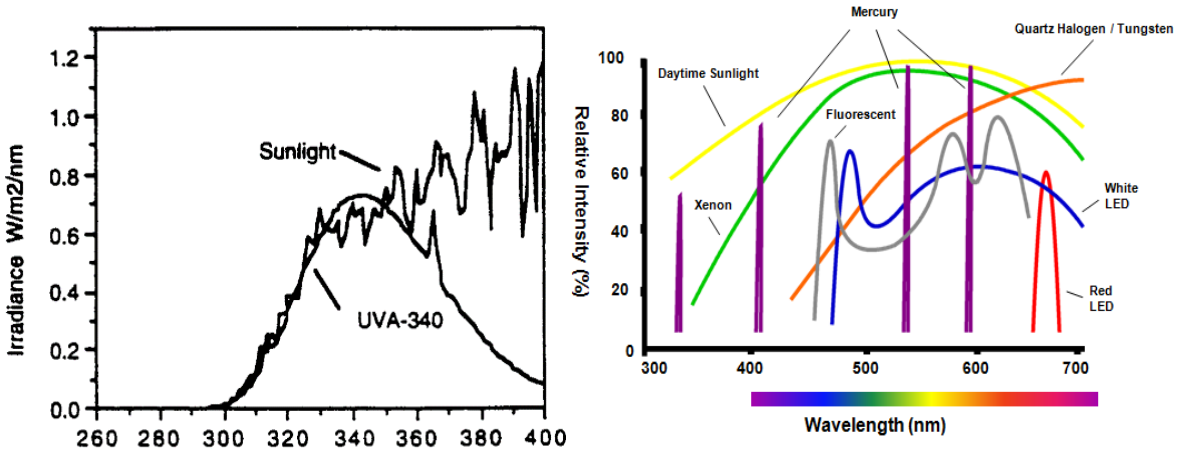


Figure 11: Left: Direct sunlight compared to UVA-340 (Source: ASTM G 154-2012), right: Spectrum from different light sources in comparison (Source: http://www.ni.com/white-paper/6901/en/)

The graph on the left side of Figure 11 shows that from 350 nm onwards the UVA-340 lamp is not imitating sunlight sufficiently. The UV spectrum is fully covered though. Not only UV light but also visible light such as violet and blue light can lead to photodegradation of wood (George et al. 2005; Kataoka et al. 2007). On the right of Figure 11, the spectrum of daylight, xenon lamp, mercury lamp, fluorescent light, different LED lights and a halogen lamp is shown. The xenon lamp mimics sunlight the best from ~450 nm onwards, however the UV region is not well simulated by xenon lamps. Xenon light simulates sunlight only in long-term irradiation experiments and were found to be quite unstable (Tolvaj and Mitsui 2005).

The light excites molecules, and the absorbed energy can be transformed into heat or start chemical reactions (Volkmer et al. 2016) which is capable of cleaving the chemical bonds of wood (Rowell 2013) and leads therefore to surface degradation.

Photodegradation causes colour changes in the short term and noticeable reductions in the mechanical and visco-elastic properties in the long term. It is influenced by moisture content and a higher relative humidity will strongly increase degradation. Moreover, a higher temperature will favour the volatilisation of photolysis degradation products and the degradation rate is increasing with increasing temperature (Volkmer et al. 2016).

UV light causes decomposition of lignin, extractives and partly hemicelluloses (Derbyshire and Miller 1981; Hon and Chang 1984), whilst lignin is the cell wall component most sensitive to UV light (Huang et al. 2012a). Lignin serves as a sort of shield against UV light, so cellulose is protected to be degraded to a certain extent (Volkmer et al. 2016). Free phenoxy radical, phenyl and ketyl are transformed into quinoid structures which leads to a yellowing of wood surfaces (George et al. 2005). Free radicals can react with oxygen and form hydroperoxide or new chromophores. Radicals can recombine to produce neutral molecules and can induce further chain cleavage, creating new ortho- and para-quinone groups. Most end-products of photodegradation are gases: carbon monoxide, dioxide, hydrogen and some organic acids as well as higher molecular substances which are water soluble and can therefore be leached out by rain (Volkmer et al. 2016). It has been found that after

only 72h of UV exposure vescalagin and ellagic acid and after 216 h of irradiation castalagin and gallic acid are destroyed, the main extractives of oak wood (Zahri et al. 2007).

Photo-degradation is essentially a surface phenomenon (Derbyshire and Miller 1981). Erosion rate was suggested to be at approximately 6 mm per century in European softwood species (Williams 2005). Experiments on the degradation of wood have shown photodegradation in depths up to 80 µm (Hon and Chang 1984). More recent research could detect penetration depths of up to 540 µm (Kataoka et al. 2007). Reinprecht found that colour changes can occur even to depths up to 2.5 mm (2018). Since UV degradation products will be leached from the wood surface and underlying cell layers are exposed and further degraded (Reinprecht 2008), defining the degradation depth depends on exposure length and extent of leaching. Moreover, the radiation penetration depth is strongly depending on wood density and thus is lower in latewood compared to earlywood (Volkmer et al. 2016). Another mayor factor decisive for how deep light can penetrate into the wood, is the cutting direction. Tangential wood surfaces were found to erode to a higher extent during natural weathering compared to end-grain surfaces, due to the presence of lens-shaped voids (Hazneza and Evans 2016).

2.1.3 Humidity

Wood is a hygroscopic material which means that the wood moisture content (MC) corresponds to surrounding environmental conditions such as air temperature and relative air humidity (Hum). MC is the mass of water in wood expressed as a proportion of its oven-dry mass (EN 1995-1-1 2004). A lot of research concerning wood and MC has been carried out (Brischke and Rapp 2008; Niklewski et al. 2018; Baar et al. 2019; Sjökvist et al. 2019), stressing the importance of the factor. The MC in wood in use class 3 can often exceed 30 % and thus the material is susceptible to microbiological attacks (Dirol and Deglise 2001). A MC higher than 18 % will already strongly increase the degradation process of wood, depending on the wood species (Volkmer et al. 2016). Moisture dynamics are strongly influencing wood performances and therefore the water exclusion efficacy is an important factor related to the performance of wood in above-ground applications (Žlahtič and Humar 2016). Wood starts drying out on the surface while it can still be moist in the inside (Niemz et al. 2007). The variation of the surface wetting is very different from one wood species to another but also the specimen size, orientation (meaning the compass direction), inclination (meaning the angle to the ground) as well as the alignment of the samples (wood grain horizontal or vertical) strongly influence the MC.

The contact angle represents the wettability of a material and thus the expected behaviour of water after the contact with wood, whilst a lower contact angle indicates greater wettability. Studying the surface wetting during natural weathering, Oberhofnerová and Pánek found in general a significant decrease of the contact angle between 6 and 12 months of weathering. For English oak wood the contact angle changed drastically and was related to the large opened vessels and thus a faster soaking of water to the wood. Douglas fir wood however did not follow the trend and showed an increase of

contact angle in the initial stage of surface degradation and a decrease only after 6 months. A better performance of the contact angle was attributed to a higher density and amount of extractives in their heartwood zones (Oberhofnerová and Pánek 2016).

Micro-checks appearing in weathered wood, primarily in earlywood fibres, often follow the microfibril angle and occur on pits (Derbyshire and Miller 1981; Evans et al. 2015). These micro-cracks allow water to enter the surface more easily and thus the wettability increases (Huang et al. 2012b). The bigger the checks the quicker the MC increases in a specimen (Sjökvist et al. 2019).

2.1.4 Temperature

As already mentioned, the depth of light penetration into the wood matrix is, beside other factors, known to rise with increasing temperature. With increasing temperature, the air relative humidity often decreases (Niemz et al. 2007) and influences therefore the MC of wood. The temperature is moreover a decisive parameter whether and in what time microorganisms develop.

Exposing wood to temperatures above 120 °C induces chemical changes which can on the one hand reduce strength properties as a result of thermal treatment (Pfriem et al. 2010), on the other hand, applying temperatures exceeding 200 °C, has shown to improve dimensional stability, reduce hygroscopy and improve the resistance against degradation by microorganisms (Hakkou et al. 2006; Li et al. 2011; Huang et al. 2012b). Heat treatment is known to modify the chemical composition of wood (Shafizadeh 1984; Vainio-Kaila et al. 2013), degradation of hemicelluloses is suggested to be the most important chemical effect due to heat treatment (Pfriem et al. 2010). During outdoor exposure wood can reach surface temperatures of up to 90 °C, depending on its colour (Tolvaj and Molnar 2008), thus temperatures during natural weathering have rather negative effects on the wood.

2.1.5 Fungi

A lot of research has been conducted on the degradation of wood due to fungi (Ritschkoff 1996; Worrall et al. 1997; Hukka and Viitanen 1999; Viitanen 2001; Schwarze 2007; Schmidt 2010; Gupta et al. 2011; Arantes et al. 2012; Fackler and Schwanninger 2012; Arantes and Goodell 2014; Karami et al. 2014; Morris et al. 2016; Teacă et al. 2019). Wood decaying fungi break down structural components of wood and reduce its physical and mechanical properties, whilst wood staining fungi have minor impact on mechanical properties of wood but change the colour and permeability of wood (Reinprecht 2016).

Wood invading, heterotrophic fungi are eukaryotic organisms that do not photosynthesize. Fungal cell walls consist of chitin which is the reason for placing fungi in a different kingdom to plants or bacteria. Wood as a food source is limited to certain strains which are able to utilize the components released during break down (Srivastava et al. 2013). Mainly fungi belonging to the phylum Basidio-

mycota (white- and brown-rot fungi) and Ascomycota (Staining- mould- and soft-rot fungi) are responsible for degradation in dead wooden cells (Schmidt 2010; Reinprecht 2016). Brown-rot fungi are perhaps the most important agents involved in the biodegradation in dead wood (Arantes and Goodell 2014). Soft rot fungi need elevated moisture conditions; exceeding the fibre saturation point (FSP); to provoke a strong wood degradation (Niemz et al. 2007) and is therefore for this research of less interest. Figure 12 shows a classification of wood decay fungi according to Teaca et al. (2019).

The asexual reproduction of fungi occurs through the release of spores or through mycelial fragmentation. Those spores can be spread by wind, rain and snow. The mycelium is the filamentous lining up of hyphae which form branched hyphae when older. In the light microscope, the mycelium, the actual fungus with wood decay ability, can be visualized (Schmidt 2010). One can see how hyphae is progressively invading wood cell walls and lumen. Hyphae's diameters reach from 0.1 µm (Liese and Schmid 1966) to 60 µm and length from 5 up to several µm (Schmidt 2010). Research conducted by Schwarze (2007) shows a good overview of the different fungal decay types on a macroscopic level. On a chemical level, most fungal strains produce enzymes, contributing significantly to the decay of lignocellulosic residues (Dashtban et al. 2010), which allow fungi to benefit from wood as nutrition source (Eriksson et al. 1990). Enzymes are biocatalysts that accelerate and control biochemical reactions (Reinprecht 2016).

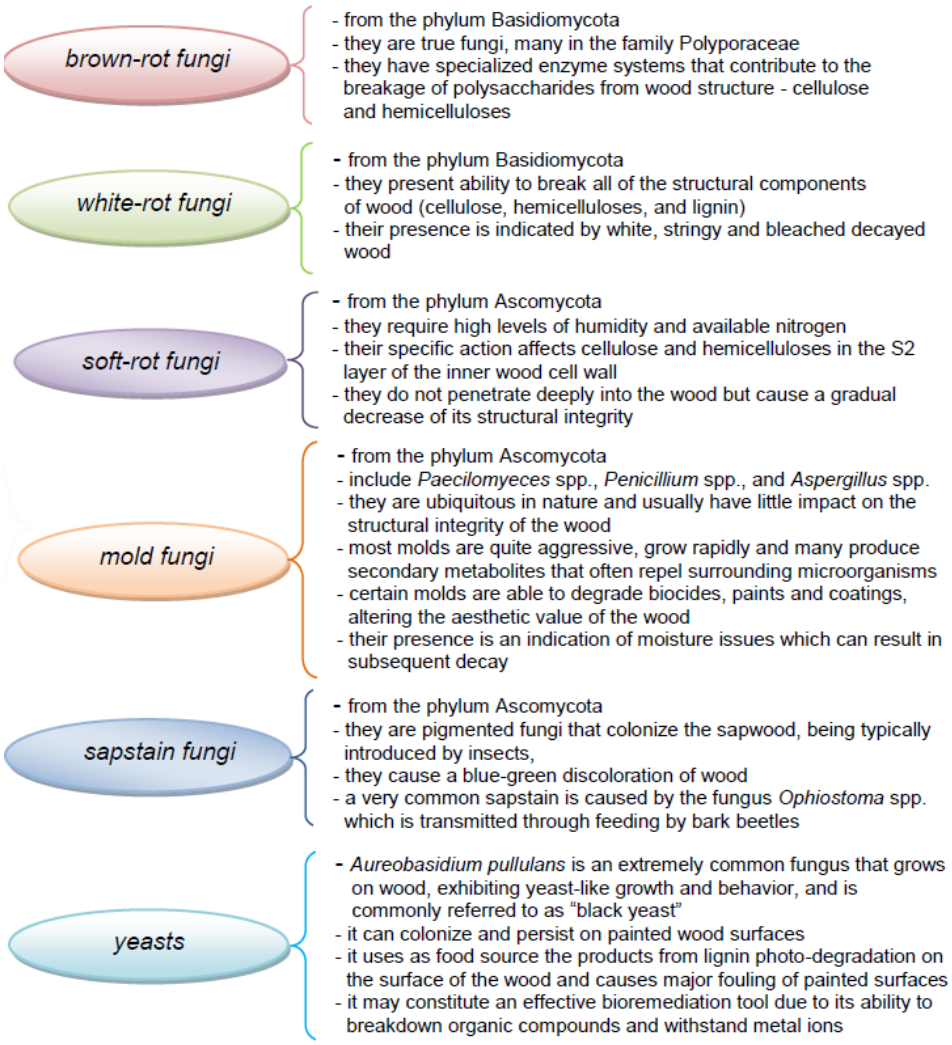


Figure 12: Wood decay fungi classification depending on their involvement in wood degradation (Teaca et al. 2019)

2.1.5.1 Growth conditions

Colonisation, growth and decay ability of basidiomycete fungi are dependent on ecological factors, even though wood decay fungi have fairly basic requirements and can tolerate a wide range of conditions (Xie et al. 1997). Fungi can develop rapidly in desirable conditions and the mechanical properties of wood attacked by fungi can fall quickly (Fengel and Wegener 1989). The major factors are nutrients, water, air, temperature, pH-value, light, force of gravity as well as biological interactions such as antagonism, synergism or symbiosis with other microorganisms (Schmidt 2010).

Fungi consists of about 90 % water and 10 % dry matter (Schmidt 2010). Conditions where the MC is below the FSP make water less available to most fungi and affect its growth (Whiting and Rizzo 1999; Volkmer et al. 2016). The optimum wood MC requirements for brown rot fungi is between 20 % and 60 %, white rot fungi prefers slightly higher moisture contents of between 30 %-70 % (Niemz et al. 2007). Soft rot fungi belonging to the phylum Ascomycetes damage wood if MC is quite elevated (Schmidt 2010).

It has been reported that the access of oxygen (O_2) for different types of fungi can affect the growth (Scheffer 1986). Reinprecht (2016) mentioned that wood decaying fungi need at least 1 - 4 % O_2. Moreover, nitrogen is required for biosynthesis of proteins and enzymes, necessary for wood decomposition. Mineral substances such as phosphorus, sulphur, calcium, magnesium, potassium, iron and copper; inorganic extractives also present in wood; are required, especially for several brown rot fungi that use non-enzymatic decomposition of cellulose (Reinprecht 2016).

Optimum temperature requirements vary from one fungal strain to another. In experimental studies different fungal strains, nutrition agars, environmental conditions, etc. are used, which makes a comparison complex. The temperature interferes with other factors such as MC, nutrition and presence of anti-freezing agents, etc. In general, reaching the freezing point, where no liquid water is available, metabolism may not take place. Fungal growth temperature can span from 0 – 45 °C for some species, however optimum temperature for most wood degrading fungal species is between 22 and 32 °C (Schmidt 2010). For most fungi 40-50 °C is often the maximum tolerated temperature for mycelial growth before proteins are affected. Kempe (2009) mentioned that only extended exposure to temperatures between 50 and 75 °C may lead to death. Optimum temperatures for most fungal enzymes are reported between 50-60 °C (Schmidt 2010). For the majority of fungal species temperatures of 20 °C might be the optimum for mycelium growth (Dirol and Deglise 2001). However, Wälchli (1977) reported that the optimum temperature range for mycelial growth may be broader than for wood degradation. Enzyme activity, and therefore wood degradation processes, are highly affected by the temperature (Volkmer et al. 2016).

In general, slightly acid environments, pH between 4.5 and 5.5, are favourable for fungal growth (Dirol and Deglise 2001). It has been reported that the temperature in which fungal strains, such as *Aspergillus sp.* were produced, influences the optimum pH-value for enzyme activity. Maximum stability was reached at pH-values between 4.3 -6.5 for enzymes produced at 37 °C and 45 °C (Mendicuti Castro et al. 2006).

Prolonged exposure to UV light is known to have lethal effects on mycelium, on the other hand it stimulates the fructification of certain strains. Gamma radiation is mortal at doses of 2.5 megarads (Dirol and Deglise 2001).

Last but not least, it needs to be mentioned that fungi are affected by the presence and activity of other organisms such as fungi and bacteria. Antagonistic or synergetic effects may occur (Eriksson et al. 1990; Schmidt 2010; Reinprecht 2016). Please see the section 2.1.7 on page 25 for more information.

2.1.5.2 Wood invasion by fungi and enzymatic degradation

The wood fungi, saprobes (feeding on dead and decaying wood), use carbon from organic material as a nutrition source. In order to benefit from the wood matrix as a nutrition source, wood decaying

fungi transform polysaccharides, lignin and extractives to water and CO_2. They synthetize organic substances with the help of enzymes, chitin, mycotoxins and compounds of cytoplasm (Reinprecht 2016).

White rot fungi degrade mainly lignin but also celluloses and hemicelluloses. Basidiomycetes and ascomycetes are known white rot fungi that decay mainly the S_2 layer. The wood colour turns bright till white but also dark boundary lines may emerge (Niemz et al. 2007). Polysaccharides are decomposed via hydrolase enzymes, oxidation and oxidation-reduction enzymes. Crystalline cellulose is degraded by means of synergic system of endo-exo-glucanases. Lignin degradation of white rot fungi is driven by different enzymatic systems such as lignin-peroxidases, manganese peroxidases, methanol-oxidases and others (Reinprecht 2016).

Brown rot fungi on the contrary mainly degrade cellulose and hemicellulose, lignin residues have been shown to be modified (Arantes and Goodell 2014). Brown rot degradation starts in the lumen and continues toward the middle lamella. The S2 layer, which is rich in polysaccharides, was found to be degraded rapidly (Eriksson et al. 1990). Shakes parallel and longitudinal to the fibre occur and leaves a cubical rotten pattern due to shrinkage (Niemz et al. 2007). Brown rot fungi produce hydrolase enzymes mainly to degrade amorphous polysaccharides of wood. The crystalline cellulose is degraded by the activity of low molecular weight Fenton oxidation system (Reinprecht 2016).

Soft rot fungi degrade, due to hydrolase enzymes and oxidation-reduction lignonolytic enzymes, celluloses, hemicelluloses and lignin. Soft rot typically attacks wood in wet conditions and is quite resistant against fungicides (Reinprecht 2016).

Nowadays there are more than 3000 enzymes known (Schmidt 2010). Terri and Hendriksson (2009) give a good overview about the enzymes involved in wood degradation. Cellulases are one type of enzyme responsible for fibre digestion, the decomposition of cellulose and some polysaccharides (Creative Enzymes 2020). Cellobiose is the major product from hydrolysis of cellulose. Cellulolytic enzymes appear to recognize the cellobiose unit of cellulose. Crystalline cellulose is quite durable towards microbial and enzymatic degradation, amorphous cellulose however is hydrolysed much faster. Cellulolytic enzymes are produced by fungi and certain bacteria. Depending on the microorganism, hydrolytic, oxidative or phosphorylases enzymes may participate in cellulose degradation. (Eriksson et al. 1990). Table 3 shows an overview of the enzymes ensuring the biochemical decomposition of wood. For a total biodegradation of wood multiple types of enzymes are needed (Reinprecht 2016). The xylanolytic complex of enzymes is present in several wood decaying fungi but is also present in some bacteria and is responsible for xylans, hemicelluloses; to be affected (Eriksson et al. 1990). Mannans, mainly present in softwood, are affected by a similar enzyme system consisting of other types of enzymes such as endo-1,4-β-mannanase (Reinprecht 2016). The ligninolytic complex of enzymes produced by white- and soft rot fungi as well as some bacteria responsible for

the lignin decomposition process in wood entails decomposition of polysaccharides. Brown rot fungi attack lignin by specific enzymes or by Fenton-type reagents (Daniel 2014).

Table 3: Enzymes identified to be responsible for the biochemical decomposition of substrates in wood (Reinprecht 2016)

Enzyme name	Substrate attacked	Catalysed reaction
endo-1,4-β-Glucanase	Cellulose	Statistically random hydrolysis of glucoside bonds in positions 1,4 in amorphous and crystalline cellulose
exo-1,4-β-Glucanase	Cellulose	Intended hydrolysis of glucoside bonds in positions 1,4 at the non-reducing ends of cellulose, leading to the splitting-off of cellobiose
1,4-β-Glucosidase	Cellobiose, cellodextrines	Hydrolysis of cellobiose and water-soluble cellodextrines to glucose
Cellobiose:quinone oxidoreductase (CBQ)	Cellobiose + quinones	Oxidation of cellobiose and other cellodextrines to lactones and acids + reduction of quinones and phenoxyl radicals (from lignin) to phenols
endo-1,4-β-Xylanase	Xylose	Statistically random hydrolysis of glycoside bonds in positions 1,4 in the frame of xylanes, while oligosaccharides, xylobiose and xylose are produced
1,4-β-Xylosidase	Xylobiose	Hydrolysis of xylobiose, and xylane oligosaccharides, to xylose
Acetyl(xylane)esterase	Xylanes	Hydrolysis splitting-off of acetic acid from xylanes (i.e. from C-2, C-3 acetylated carbohydrates)
endo-1,4-β-Mannanase	Mannanes	Statistically random hydrolysis of glycoside bonds in positions 1,4 in the frame of mannanes, while oligosaccharides, mannobiose and mannose are produced
1,4-β-Mannosidase	Mannobiose	Hydrolysis of mannobiose, and mannane oligosaccharides, to mannose
Lignin peroxidase	Lignin	Oxidation splitting of C–O–C and C–C bonds, splitting of aromatic ring while lactones are produced, oxidation of benzyl-alcohols, polymerization of phenols
Mn^{2+} peroxidase	Lignin	Oxidation splitting of C–O–C and C–C bonds, oxidation of phenols to phenoxyl radicals (and quinones)
Laccase	Lignin	Oxidation splitting of C–O–C and C–C bonds, oxidation of phenols to phenoxyl radicals (and quinones)
Aryl-alcohol oxidase	Aryl alcohols	Oxidation of aromatic C_α alcohols and production of H_2O_2, needed for peroxidases
Glucose-oxidase	Glucose	Oxidation of glucose to gluconolactone and production of H_2O_2
Methanol-oxidase	Glucose	Oxidation of methanol to formaldehyde and production of H_2O_2
Dioxygenase	Aryl alcohols	Splitting-off of aromatic ring in position 3,4 or 4,5, during which both atoms of the oxygen molecule are built into the substrate

2.1.6 Bacteria

In all natural resources, microorganisms occur more or less in large numbers. $6x10^8$ bacterial cells per g of soil, were reported to be present (Kilian et al. 2000). Bacteria play a key role in the metabolic cycle since they are continuously running mineralization processes. These primitive entities are mostly unicellular and have a ball-, stick- or screw-like shape. Depending on the species, they can occur in characteristic groups or chains. The length of individual bacteria varies according to type between 0.5 and 5 µm. Bacteria are composed of: Ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and proteins. Their cell nucleus is not surrounded by a membrane which distinguishes bacterial cell structures from vegetative and animal cells. The so called Eubacteria, meaning bacteria, are one main domain of life since they are prokaryotic (Alexander 1988).

The reproduction of bacteria is normally done by division which, in optimal living conditions, can take place within 20 to 30 min for many species. Some bacteria are freely moving with the so called flagella. Others have shorter filamentous attachments, the fimbriae, which allow them to attach to surfaces. Another morphological characteristic is the spore formation. Under certain unfavourable conditions endospores arise inside the bacterial cell. Many bacteria secrete extracellular material in form of capsules or a slime layer which can enclose bacteria into a biofilm (Alexander 1988).

2.1.6.1 Growth conditions

It has been stated that some bacterial strains can tolerate very harsh conditions such as desiccation (Alpert 2006), temperatures up to 80 °C (Alexander 1988) and may create resistance towards chemicals such as chlorine (Obe et al. 2018). Bacteria may be classified in aerobic and anaerobic, whilst latter do not need oxygen to survive. The abundance of bacterial phyla is depending rather on the combination of wood and chemical properties than on a single parameter such as pH alone (Hoppe et al. 2015). Nevertheless, some environmental settings decisive for bacterial growth and activity are mentioned below.

Bacterial wood degradation is mainly active when a water flux through the wood is present (Klaassen et al. 2008). Fengel and Wegener (1989) state that bacteria degradation is limited because they cannot move, except when there is water. Highly diverse bacterial communities colonizing wood sunken in the Mediterranean Sea were reported (Bessette et al. 2014). In conditions where the wood is submerged in water or conserved in a very humid environment, bacteria is able to destroy punctuations in the cell wall and increase wood permeability (Dirol and Deglise 2001).

The ambient temperature plays a major role in the reproduction of bacteria (Alexander 1988). Optimum temperature conditions for bacterial growth can vary from one species to another between 15 °C- 40 °C, at 37 °C mesophilic organisms are the most active. Another group, the psychrophilic bacteria are already active in a temperature range of below zero up to 20 °C. They are likely to be present before mesophilic bacteria invade, since they develop in lower temperature ranges (World of

Microbiology and Immunology 2020). Optimum temperatures for nitrogen fixation were reported around 30 °C (Hicks et al. 2003).

Most bacteria grow at pH values ranging from 5.5 to 9.0, and maintain a cytoplasmic pH that lies within the range of pH 7.4 to 7.8 (Booth 1985). Hence, bacteria are able to acidify or alkalinize the cytoplasm relative to the external milieu and are in general able to survive and grow in alkaline environments (Padan et al. 2005).

2.1.6.2 Wood degrading bacteria

Bacterial taxonomy is complex, classification for bacterial phyla is still ongoing via genome sequencing. So far, communities of bacteria from the phyla Proteobacteria such as alpha-, beta- and gamma-proteobacteria (Hervé et al. 2014) and gamma- and epsilon- proteobacteria in maritime conditions (Bessette et al. 2014), as well as Acidobacteria and Firmicutes such as Bacilli (Folman et al. 2008) were identified to invade decomposing wood. Figure 13 shows a classification tree with major functional traits, whilst the above mentioned phyla are highlighted in red.

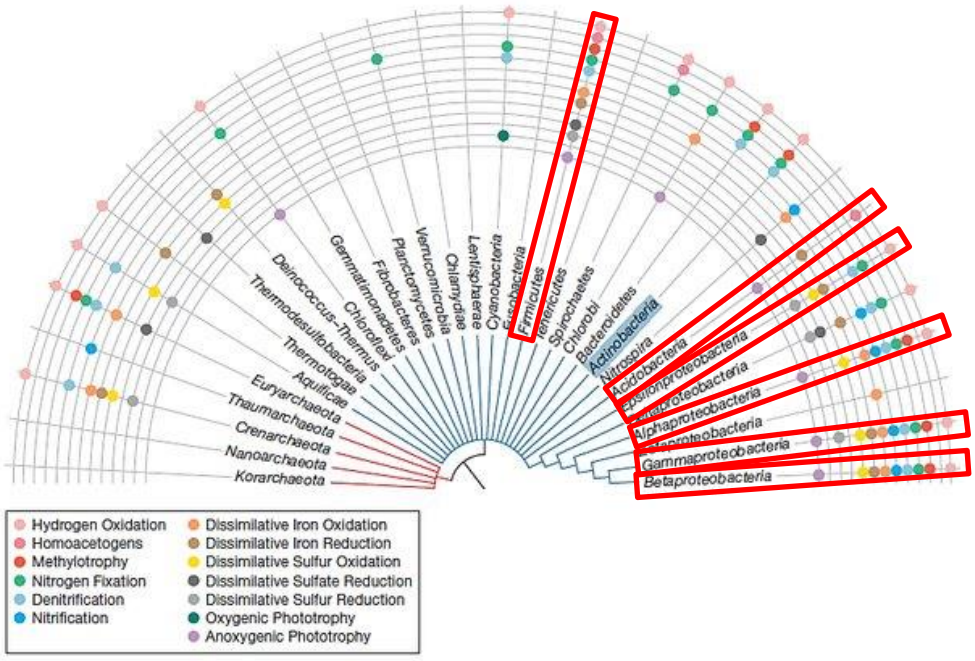


Figure 13: Functional traits mapped across major phyla of bacteria and archaea (Jayashantha 2015)

Firmicutes have a bifunctional enzyme and moreover one (*Enterococcus faecalis*) or two (*Bacillus subtilis*) proteins containing only the synthesis domain. Bacteria from the phylum *Firmicutes* produce endospores and are therefore resistant to desiccation (Cashel and Potrykus 2013).

The heterotrophic phylum of *Acidobacteria* have the ability to use nitrite as nitrogen source and glucose and xylose as carbon source, respond to soil macro-, micro nutrients and soil acidity and produce exopolysaccharide (high-molecular-weight polymers). Enzymatic activity has been observed, however is not fully understood yet. Cellulose degradation by most *Acidobacteria* has not

yet been demonstrated, however, glucosidases are known to be involved in the degradation of polysaccharides, mainly starch but also cellulose (Kielak et al. 2016a).

Proteobacteria comprise one of the largest divisions within prokaryotes (Gupta 2000). They are possibly the most diverse phylogenetic lineage showing extreme metabolic diversity. A lot of *Proteobacteria* are motile by means of polar or peritrichous flagella. They are likely to establish symbiosis with leguminous plants or animals (Kersters et al. 2006).

2.1.6.3 Wood invasion by bacteria

The provenance of bacteria in wood is largely unknown, they have limited motility and probably enter by means of soil, rain, air or with the help of other organisms such as fungi into the wood (Johnston et al. 2016). There is very little research available about the effect of bacteria on wood. Bacteria are said to be less destructive to wood compared to fungi, still they can provoke localized micro-cuts or cavities in wood cell walls (Blanchette et al. 1990; Dirol and Deglise 2001). Moreover, studies indicate that interactions between fungi and bacteria may play an important role during the decay process (De Boer and Van der Wal 2007). Wood colonizing bacteria were first classified by Greaves (1971) into four groups:

1. Bacteria using cell content of rays → Affecting permeability but not wood strength
2. Bacteria attacking cell walls directly → May affect wood strength properties
3. Bacteria associated with other microorganisms → Contribute to the breakdown of wood
4. Antagonistic bacteria → Interact with other microorganisms colonizing wood

According to multiple studies there are three different kind of attacks on pit borders caused by bacteria: Tunnelling, erosion and cavitation (Greaves 1969; Fengel and Wegener 1989; Eriksson et al. 1990). The study by Singh et al. (2016) describes the tunnelling and erosion bacteria in detail.

Bacteria appear primarily with parenchymatous tissues; thus, they often accumulate in rays and resin ducts. Moreover, an affinity towards the S3 layer in softwood tracheids and hardwood fibres and vessels has been observed (Wilcox 1970). Some bacteria are able to degrade pectin, hemicelluloses and cellulose. Schmidt and Liese (1994) mention, that only few bacteria show activity towards lignin. But actinomycetes have shown to produce lignin peroxidase-type enzymes allowing them to degrade lignocellulose preparations (Daniel 1994b; Hatakka 2005). Eubacteria can only degrade low-molecular weight parts and degradation products of lignin (Hatakka 2005). An overview of enzymes responsible for decomposition of wood is shown on page 21.

The depth of microorganism penetrating in wood surfaces is suggested to be deeper on cross- compared to the longitudinal sections. Maximum penetration on cross section was shown to be 4 mm (Prechter et al. 2002), whilst density and anatomical features, such as vessel size and orientation of tracheids, of the wood species need to be considered.

2.1.6.4 Biofilms

Many bacteria form aqueous slimy layers on surfaces (Alexander 1988). The initial adhesion step, crucial for the formation of biofilms, is driven by van der Waals, electrostatic and acid-base interactions (Soumya et al. 2013). Biofilms grown for longer periods are more resistant than biofilms developing in 2 days only, it was assumed that this is not due to higher cell density but starvation effects or coalescence of the biofilm that are responsible for higher cell resistance (LeChevallier et al. 1988). LeChevallier et al. showed that bacteria is able to survive disinfection through chlorine since they attach to surfaces (1988).

2.1.7 Microbial communities

Bacteria are said to degrade wood to a limited extent (Fengel and Wegener 1989). However, wood can naturally inhibit microbial, fungal as well as bacterial, population; depending on its MC, decay status and storage (Dutkiewicz et al. 1992). Both, synergistic as well as antagonistic effects between bacteria and fungi have been reported (Käärik 1975; Eriksson et al. 1990; Dutkiewicz et al. 1992; Clausen 1996; De Boer and Van der Wal 2007; O'Callahan et al. 2012). These interactions between the microorganisms may catalyse or slow down wood decomposition. Schmitz (1919) reported first that a mix of bacteria and fungi populations caused a greater deterioration in oak wood compared to decay by the basidiomycetes alone. Richness of bacterial communities was suggested to potentially increase as decay of the wood substrate progresses and may be linked to the equally increasing richness of fungal species (Hoppe et al. 2015).

There is evidence of potential mutualistic interactions between fungi and nitrogen-fixing bacteria that consume by-products of enzymatic lignin degradation such as methanol (Hoppe et al. 2015). Antagonistic effects were reported as well: Bacteria in the presence of decay fungi must have sufficient anti-oxidative activity to protect them from being attacked by free radicals (De Boer and Van der Wal 2007). Changes of the pH value, caused by some bacteria through the production of ammonia (Schmitz 1919), may have an influence on the ability of fungal strains to development. O´Callahan et al. identified bacteria having antifungal properties (2012). Hervé et al. (2016) found, that fungal growth was significantly lower when soil bacteria, i.e. diverse bacterial communities, were present. On the other hand, bacteria were found to be able to dissolve toxic components and therefore favour degradation by soft rot fungi (Blanchette et al. 1989).

The mentioned studies do not allow to draw a conclusive statement to which extent interactions take place and whether bacterial or fungal communities may encounter disadvantages or profit from the environment. Furthermore, not only microbial interactions but also abiotic and biotic factors were found to act synergistically during the degradation process (Žlahtič and Humar 2016).

2.2 Wood weathering techniques

The weathering of wood can be observed with both natural and artificial weathering techniques. One or more surface characteristic is measured before weathering and then again after various durations of weathering. Different natural and artificial standardized weathering techniques are suggested, which propose more or less specific exposure settings. There is continuing debate about the correlation between natural and various artificial, or accelerated, weathering techniques.

2.2.1 Natural weathering

Standards such as EN 927, allow the evaluation of the durability of paints and varnishes applied to wood surfaces that are then exposed to an exterior environment. According to the standard, samples shall not be sheltered by a roof and shall be located where walls will not block the wind, rain or sun, in order to test in severe exposure conditions (see Table 4). A total score of 9 represents most severe settings (EN 927-3 2006). However, there are other factors which contribute to the degradation process of wood and are not considered by the standard. So does for example the site of exposure (location) strongly affect the surface degradation (Sandak et al. 2015). Since a natural environment is non-controlled, during natural weathering experiments, meteorological data should be collected. Meteorological conditions are unpredictable, vary significantly between sites, period of the year, as well as in between years. These variations make a comparison of wood exposed to different field test conditions rather complicated.

Table 4: Determination of exposure conditions from some climatic factors (EN 927-1 2013)

Factor[a]	Score			Total score (sum)	Relative exposure condition
	1	2	3		
Orientation	North west to north east (moderate)	North east to south east and west north west to north west (hard)	South east to north west (extreme)	3	Mild
Degree of shelter	Sheltered	Partly sheltered	Unsheltered	4 to 6	Medium
Inclination	Vertical	≈ 45°	Horizontal	7 to 9	Severe
[a] For explanation, see Annex A.					

2.2.2 Artificial weathering

The benefit of an artificial weathering apparatus is the fact that abiotic influencing factors can be set and controlled. Possible guidelines such as ASTM G15, EN 927-6, ISO 11341, ISO 4892-3 and ISO 16474-3 propose accelerated weathering scenarios in specific machines, where the parameters light source and irradiation, water spray, condensation and temperature can be set. In this thesis, those machines are referred to as QUV devices. Another weathering apparatus is the wheel, a photo-ageing instrument in accordance with standards such as EN 16472 and ISO 4892-1. This weathering setup

does not allow to control neither the ambient temperature nor humidity, thus the use of the wheel was neglected for this research.

The probably most critical factor during artificial weathering is the choice of light source due to the fact that photodegradation is the fastest and strongest degradation pathway of wood products (Cogulet et al. 2016). Energy in UV fraction is significantly higher than in visible light (Volkmer et al. 2016). According to Tolvaj and Mitsui, Xenon light simulates sunlight only in long-term irradiation experiments. In their work they demonstrated that the yellowing caused by xenon light was more pronounced after short-time exposure. Moreover, they mentioned, that xenon lamps do not emit UVB light, which is becoming, due to thinning of the ozone layer, more and more important. Mercury light however, as a strong UV light and especially UVB light emitter, proved to be inconvenient to mimic sunlight (Tolvaj and Mitsui 2005). Applying artificial weathering cycles, it has to be considered that electric lamps are not able to mimic sunlight perfectly.

For example, in a study by Žlahtič and Humar (2016) the accelerated weathering experiment was conducted as following: Cycles consisted of 18 min water spray and 102 min xenon-arc radiation (1000 h in total), at 38 °C and a relative humidity of 68 %, a UV radiation intensity of 0.35 W/m^2 was set. Cogulet et al. (2016) conducted a similar study with the same length of wet and dry periods, but with an irradiation intensity of 0.89 W/m^2 using a UVA-340 lamp, for a 2000 h exposure. A comparison of different studies applying artificial weathering cycles seems rather unrealistic as firstly, many factors are considered and secondly, they often differ greatly.

2.2.3 Correlation between artificial and natural weathering

Both, natural and artificial exposure of wood, provoke longitudinal macro-cracks, thinning of cell walls, greening and blueing, significant decrease of lignin; faster decrease of guaiacyl than syringyl lignin; decrease of carbonyl groups and cellulose crystallinity (Reinprecht et al. 2018). C=C lignin bonds in *Picea* wood were found to disappear after 2 months of outdoor weathering in Biel, Switzerland (Volkmer et al. 2016) whilst according to Cogulet et al. during artificial weathering, lignin is completely degraded after only 7 days (2016). Naturally exposed *Pinus* samples in Canberra, Australia, showed almost complete surface delignification after 6 days only (Evans et al. 1996). Some research suggests that outdoor weathering show more severe aging, especially on a chemical level, compared to artificial weathering (Žlahtič and Humar 2016), contrary, artificial light sources are said to cause faster changes in lightness of wood surfaces than sunlight during the first 30 h of exposure (Tolvaj and Mitsui 2005).

Studies, trying to correlate outdoor exposure to artificial ones, showed results of samples exposed in different ways, where climatic conditions differ. 1000 h of artificial weathering were stated to be comparable to 6 months outdoor weathering in Ljubljana, Slovenia (Žlahtič and Humar 2016), and 540 h of artificial cycles were said to correspond to 1 year outdoor exposure in Poland, (Jankowska

and Kozakiewicz 2014). As outdoor conditions in Nantes, France are not the same, again a different duration of artificial weathering is assumed to be correlative with outdoor weathering to this Atlantic climate. Moreover, great differences between the examined wood species were observed whilst compression strength of European oak wood decreased faster compared to scots pine (Jankowska and Kozakiewicz 2014). The wide range of different densities and anatomical structures of various wood species, differences of exposure sites and in artificial weathering cycles are all factors which make the evaluation of the relation between artificial and natural weathering even more difficult.

Evaluating outdoor exposure conditions for specific regions may help correlate field tests with artificial weathering. Indexes serve to estimate the decay hazard of wood depending on its exposure regions and the service life of structures can be calculated (Brischke 2017). First attempts to estimate the hazard potential of different climates was done by correlation of climate data and empirically determined decay from field tests in the early 70´s. This index focuses on the parameters temperature and rainfall (Scheffer 1971). More recent modelling, also including the weight loss of wood, was done by Viitanen et al. (2010). With a focus on decay of bio-based building materials, Niklewski et al. developed a fungal decay hazard map for Europe (2016), as shown in Figure 14: Relative decay potential for Europe indicated as relative dose according to an empirical exposure model and a logistic model for fungal decay of wood for European sites (black dots) based on data from Meteonorm (Niklewski et al. 2016).

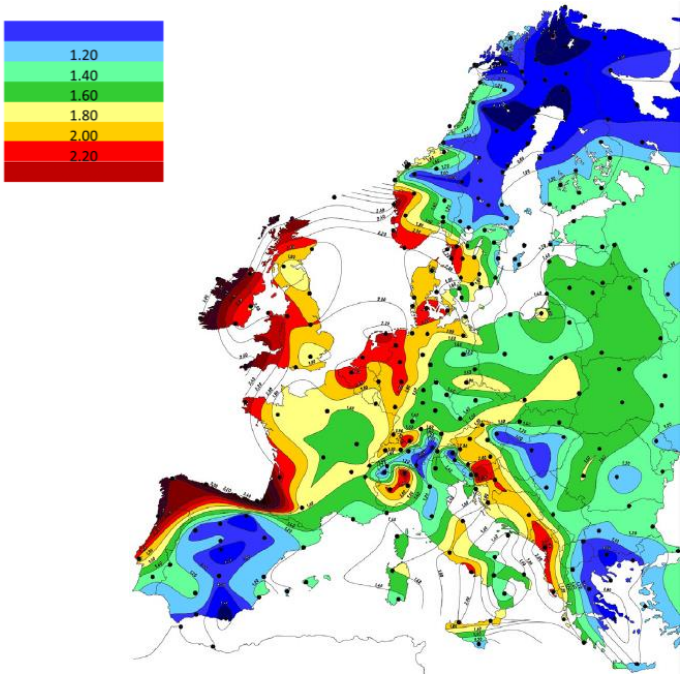


Figure 14: Relative decay potential for Europe indicated as relative dose according to an empirical exposure model and a logistic model for fungal decay of wood for European sites (black dots) based on data from Meteonorm (Niklewski et al. 2016)

2.3 Methods to characterise wood degradation

This part of chapter 2 gives background information about methods that seem to be appropriate for characterising the level of wood degradation caused in a given situation. Those methods are often dependent on the MC of wood, thus various techniques to evaluate the MC are equally listed.

2.3.1 MC

There are different ways to evaluate the moisture content (MC) of wood samples. The most precise and standardized method is the gravimetric method where samples are weighed before and after oven drying (ISO 13061-1 2014). This method is, however, time consuming and, in a sense, destructive so it is not suitable for an ongoing experiment, where samples are re-exposed to weathering after MC determination, nor applicable to sterile samples. Ideally, MC evaluation should take place continuously since changes may occur very locally (Viitanen et al. 2010) and quite rapidly. Other ways to evaluate the MC is the use of resistance type or capacitance meters, while the latter is more affected by elevated surface MC, density and temperature (Forsén and Tarvainen 2000).

2.3.2 Colorimetric evaluation

An important aspect is the appearance of wood over time of exposure. Colour change can be used as an indicator for the change of chemical decomposition of weathered wood specimens and is used in many studies (Pandey 2005; Kržišnik, et al. 2016; Žlahtič and Humar 2016; Cogulet et al. 2016; Oberhofnerová et al. 2017; Reinprecht et al. 2018; Kubovský et al. 2018; Baar et al. 2019). In order to investigate and compare colour changes caused by the different weathering techniques, colorimetric evaluation was carried out within this thesis. The standard ISO 7724-3 describes methods for determining trichromatic coordinates and colour differences with the help of specific instruments (ISO 7724-3 1984).

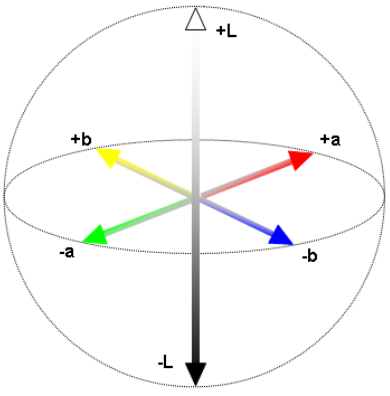


*Figure 15: CIE L*a*b* colour space. (Source:http://vision.gel.ulaval.ca/~jflalonde/cours/4105/h18/tps/results/projet/111080041/index.html)*

The CIE L*a*b* colour space is a three-dimensional colour space produced by plotting in rectangular coordinates (EN ISO 11664-4 2019). As shown in Figure 15, L* represents the luminosity where 100

indicates white colour and 0 indicates black. The coordinate a* signifies green (-120) and red (+120) whilst the b* value reaches from blue (-120) to yellow (+120) (Mouw 2018).

Evidence of wood decomposition through weathering can be gathered by measuring changes in its visual appearance (Derbyshire and Miller 1981). This change can become obvious even after only short exposure lengths (Tolvaj and Faix 1995). A significant colour change was noticed after less than one month of natural weathering (Schnabel et al. 2009). The yellowing of wood, so the change of the b* coordinate, is caused by photochemical breakdown of lignin (Sandoval-Torres et al. 2010; Volkmer et al. 2016). The grey colour occurs in later stages of weathering, when extractives and photodegraded lignin parts are washed out (Feist 1989; Huang et al. 2012a). The red hue, so the a* coordinate, in wood correlates with the extractive content (Yazaki et al. 1994). Mainly darkening in the yellow-red region of the CIE Lab colour system was observed in artificially exposed samples (Tolvaj and Faix 1995). Even though most naturally weathered specimens were found to turn darker with increasing exposure length, some artificially degraded samples showed contrary results (Žlahtič and Humar 2016).

The colour is dependent on the MC of surfaces to be measured (Baar et al. 2019). It is generally observed, and easily tested, that a wood surface darkens when liquid water is applied. The water changes the refraction and absorption of light. Consequently, it can be complicated to follow colour change during weathering experiments and especially natural weathering experiments when it might rain just before a measurement is due.

2.3.3 Surface scans

Colour measurements alone do not show local discoloration patterns, e.g. dark spots or blue mould on the surfaces. It is suggested that colour evaluations should be supplemented with visual assessments in order to gain a better understanding of microbial growth (Lie et al. 2019). The visual images help to provide a timeline of very local changes.

2.3.4 Microscopy

Wood weathering triggers deterioration of pits, cracking of the S2 layer and the middle lamella, which in turn cause micro-cracks and checks to appear (Evans and Banks 1988; Feist 1989; Christy et al. 2005; Schultz 2008). Ultimately, cells become separated from the main wood surface (Feist 1989; Volkmer et al. 2016). The fact that cells are seen to detach from the surface implies that lignin, which serves as a binder between wood fibres (Fengel and Wegener 1989), is preferentially degraded by weathering. These anatomical consequences can be observed with various microscopes. Examining samples on the perpendicular face towards the exposed surface reveals the intensity and depth of degradation caused by weathering.

2.3.4.1 Light microscope- Reflection and transition mode

Basic light microscopy (LM) is sufficient to determine the depth of weathering effect below the surface. LM might also allow to gain insight into the microbiological contamination of the samples through the detection of spores and hyphae. This technique cannot, however, differentiate between live and dead bacterial or fungal cells, whereas confocal or fluorescence microscopy can. These last two methods, however, require more expensive equipment and are more time consuming (Ak et al. 1994; Johnson and Criss 2013), thus LM was the preferred method.

For the use of a LM in transition mode, a common sample preparation is the use of a Microtome, where samples are sliced up to 60 µm thickness. Applying dyes such as safranin and light green facilitate the observation of lignin degradation (Mulisch and Welsch 2015). A distinguishing between gram-positive and gram-negative bacteria can be made by using gram staining, as well as a better visibility of fungi through the application of e.g. methylene-blue dies is achieved. Disadvantages of using the light microscope in transmission mode is the fact that sample preparation is time consuming and big numbers of replicates are needed and interpretation through image analysis is limited.

Another possibility, less time consuming, is working in the reflection mode, where there is no need to prepare slices, still changes in anatomy and colour can be observed. The weakness of this method is that small samples can be observed only superficially. Moreover, the visualisation of fungi and bacteria is not assured.

2.3.4.2 ESEM

LM in combination with environmental scanning electron microscopy (ESEM) can provide complimentary findings (Singh et al. 2010). ESEM is a good supplementary tool besides biochemical and chemical approaches (Daniel 1994b).The ESEM can visualise wood degradation anatomically (Evans et al. 2015) and is said to detect bacteria (Singh et al. 2019) without any sample preparations such as dehydration, fixation or application of coatings. Contrary to LM, the pictures obtained with the ESEM are grey scale images, therefore colour changes are not visible. A benefit of the ESEM however is the possibility of high magnifications as well as the fact that 3D images can be obtained.

2.3.5 FTIR- ATR

Fourier transform infrared (FTIR) spectroscopy was found to be a useful tool to characterize chemical degradation (Anderson et al. 1991a; Pandey 2005; Evans 2012; Cogulet et al. 2016; Kubovský et al. 2018). Within this work the aim was to investigate the differences in chemical decomposition when wood exposed to various abiotic and/or biotic degradation factors and thus gain an insight into the relative importance of various parameters.

Table 5 compiles assignments of various infrared absorption bands according to Tolvaj and Faix (1995), Temiz et al. (2007), Faix (1991) , Pandey and Pitman (2003), Schwanninger et al. (2004), Tamburini et al. (2017), Naumann et al. (2005), Hergert (1971) and Gupta (2015).

Table 5: Assignment of infrared absorption bands in wood

Wavenumber cm^{-1}	*Assignment / (Interpretation)*	*Source*
1740-1720	CO stretch, in unconjugated ketone, acetyl, carboxyl groups	Tolvaj and Faix 1995, Temiz et al. 2007
1730-1736	C=O stretch in unconjugated ketones, carbonyls and ester groups (Hemicellulose)	Schwanninger et al. 2004, Faix 1991, Pandey & Pitman 2003, Tamburini et al. 2017
1645	O-H and C-O	Faix 1991, Pandey & Pitman 2003
1660-1645	C=O in para- OH substituted aryl ketones, quinines	Temiz 2007
1647	Absorbed O-H and conjugated C=O	Faix 1991, Pandey & Pitman 2003
1646-1633	Amide I protein	Naumann et al.2005
1635	Absorbed water	Schwanninger et al. 2004
1600-1593	C=C,Aromatic skeletal vibrations (Lignin)	Schwanninger et al. 2004, Temiz et al. 2007, Hergert 1971, Faix 1991, Pandey & Pitman 2003
1578	C-O vibration in metal chelates or salts of conjugated diketones	Tamburini et al. 2017
1552	Amide II protein	Naumann et al. 2005
1510	C=C, Aromatic skeletal vibrations (Lignin)	Schwanninger et al. 2004, Temiz et al. 2007
1504	Aromatic skeletal vibrations (Lignin)	Faix 1991, Pandey & Pitman 2003, Tamburini et al. 2017
1455	C-H deformation in lignin and carbohydrates	Faix 1991, Pandey & Pitman 2003, Tamburini et al. 2017, Schwanninger et al. 2004
1430	Aromatic skeletal vibrations combined with C-H in plane deformation (Lignin), CH2 scissoring (Cellulose)	Schwanninger et al. 2004
1421-1414	C-H bending	Naumann et al. 2005
1425-1423	C-H deformation in lignin and carbohydrates	Faix 1991, Pandey & Pitman 2003, Gupta 2015
1400-1415	C-O vibration in carboylates coupled	Tamburini et al. 2017
1374-1367	Amide III protein	Naumann et al. 2005
1369	C-H deformation in cellulose and hemicellulose	Faix 1991, Pandey & Pitman 2003
1370	C-H symmetric deformation in carbohydrates	Tamburini et al. 2017
1365	C-H deformatin (Hemicellulose, Cellulose)	Hergert 1971
1330-1325	C-O in Syringyl ring	Hergert 1971, Tamburini et al. 2017, Faix 1991, Pandey & Pitman 2003
1317-1315	CH2 rocking vibration	Schwanninger et al. 2004
1319	C-H vibration in cellulose and C-O vibration in syringyl derivates	Faix 1991, Pandey & Pitman 2003
1326-1315	Amide III protein	Naumann et al. 2005
1320	Syringyl Lignin	Faix 1991, Pandey & Pitman 2003
1319	CH2 wagging, C-H deformation	Tolvaj and Faix1994
1317	CH2 wagging in cellulose	Tamburini et al. 2017
1270-1275	Guaiacyl ring breathing (lignin)	Hergert 1971, Tolvaj and Faix 1995
1265-1268	CO in lignin and hemicellulose, Guaiacyl ring breating with CO stretching	Tamburini et al. 2017, Schwanninger et al. 2004, Faix 1991
1235	Syringyl ring and C-O stretch in lignin and xylan	Faix 1991, Pandey & Pitman 2003
1230	C-C plus C-O plus C=O stretch (Acetyl in xylan/ Hemicelluloses)	Schwanninger et al. 2004, Tamburini et al. 2017
1217	C-O bond of guaiacyl ring	Tamburini et al. 2017

1205-1200	OH plane deformation (Cellulose)	Schwanninger et al. 2004
1203	CH + OH in plane (D-Glucose)	Ibrahim et al 2006
1170	C-O-C stretching	Tolvaj and Faix 1995
1162	C-O-C in cellulose	Temiz et al. 2007
1155	C-O-C vibration in cellulose and hemicellulose	Faix 1991, Pandey & Pitman 2003, Gupta 2015
1120	C-O stretching in lignin	Tamburini et al. 2017
1110-1107	Ring asymmetric valence vibration	Schwanninger et al. 2004
1030-1025	C-O-C deformations in polysaccharides (cellulose and hemicellulose)	Gupta 2015, Tamburini et al. 2017, Faix 1991, Pandey & Pitman 2003
896-898	C-H deformation of cellulose	Tolvaj and Faix 1995, Gupta 2015, Faix 1991, Pandey & Pitman 2003, Tamburini et al. 2017
800	Pyran ring vibration	Tolvaj and Faix 1995, Schwanninger et al. 2004

Pre-processing of the data is required in order to eliminate effects of unwanted signals such as detector noise, calibration error etc. as well as to amplify fine differences between samples (Srinivasan 2010). Normalisation is of big importance since spectra from same materials could have different intensity levels. Through normalisation one makes sure that the intensity of a given band is as similar as possible across the spectra (Gautam et al. 2015). The min-max normalisation is the most common procedure applied for FTIR spectra obtained from wood and wood-products and vary between research from 1650- 1546 cm^{-1} (Gupta et al. 2015), 1404- 1352 cm^{-1} (Tolvaj and Faix 1995), at ~ 1016 cm^{-1} (Cogulet et al. 2016), 1800- 700 cm^{-1} (Tamburini et al. 2017) or 1800- 1500 cm^{-1} (Capano et al. 2015).

Latewood regions of softwood showed limited lignin degradation in deeper layers of the wood matrix compared to early wood (Volkmer et al. 2016). In softwoods, complete degradation of many absorption bands after 300-600 h artificial weathering were observed (Anderson et al. 1991a). Exposure to UV light only, can produce reaction by-products that remain in the wood matrix since they are not leached out. Since natural weathering includes intermittent wetting and, therefore, leaching of the reaction products, it seems necessary that accelerated weathering methods also include leaching. According to Anderson et al. white oak showed to be the most resistant compared to the other observed hardwood species aspen, poplar and maple (1991b). In softwoods, carbonyl absorption bands initially increase and then decrease, whilst in hardwoods, the peak decreases in intensity immediately. However, for degradation with light only the carbonyl absorption increases for both, soft and hardwoods. Hardwood's composition is lower in lignin and higher in di-methoxy phenolic groups, which might affect the stabilisation of free radicals during initial photochemical process (Anderson et al. 1991a, b).

Artificial weathering with UVA irradiation up to 24 h was investigated by Timar et al. (2016). Correlations of the colour coordinate b*, expressing yellow colour, and FTIR analysis were found, showing that yellowing of samples was mostly related to lignin photodegradation. Investigation of the a* colour coordinate, expressing red tones, could not be correlated with FTIR measurements, since extractives are usually not detectable by FTIR analysis.

Lignin and hemicelluloses are found to be affected first and the most by natural weathering. After one year exposure in Trento, Italy, dramatic degradation was noticed. Crystalline cellulose infrared bands did not show much of a change, showing the resistance of this chemical structure of wood to natural weathering (Sandak et al. 2015).

Žlahtič and Humar found in their study that outdoor exposed samples (up to 18 months) showed more severe chemical changes compared to artificially weathered samples including spraying cycles (1000 h) (2016).

2.3.6 XRF

Inorganic elements such as copper are known to have antimicrobial and antifungal properties. Copper as a biocide has become indispensable for many different applications. But also zinc and nickel are showing antifungal properties (Borkow 2012). Regardless of its efficacy, chemically treated wood will eventually be disposed of, and it might not be recognised as being treated and be placed with other recovered wood causing potential contamination of recycled wood products (Rasem Hasan et al. 2011). To counter this problem, regulations and thresholds for inorganic substances are in place in most countries. X-ray fluorescence (XRF) spectroscopy is one method widely used to detect trace metal elements. Chemical elements such as copper, calcium, manganese, iron, nickel, zinc and strontium can be detected by XRF spectroscopy (Morgan et al. 2015). This method was applied to samples in sterile and non-sterile exposure scenarios in order to verify if antimicrobial or antifungal properties of inorganic elements may play a role. The analysis can be performed directly, with limited prior sample preparation.

2.3.7 Surface roughness

The characterization of wood surface quality is often analysed by surface roughness (SR) measurements. SR strongly depends on the cutting tools, sample preparation, wood species and anatomy (Aydin and Colakoglu 2005; Sinn et al. 2009). Still, the SR is an indicator for erosion (Nzokou et al. 2011). The detachment of fibres is heterogeneous, some remain on the top of the wood surface and therefore the roughness increases (Tolvaj et al. 2014; Volkmer et al. 2016) and the surfaces appear fuzzy (Futó 1976). Especially photodegradation and the leaching of lignin are said to change the surface roughness values (Ozgenc et al. 2013). In **Annex II** some of the important SR parameters are listed. The SR on samples before and after exposure to various weathering conditions was established. The hypothesis was set, that cracks may provide very local conditions that might enable microorganisms to develop and survive. Thus, SR may be not only be an indicator for abiotic but also for biotic degradation.

Monitoring the SR can be done either using contact or non-contact measuring instruments (Gurau et al. 2005; Aydin and Colakoglu 2005) such as light sectioning lasers or by perthometers using a stylus in direct contact with the surface. Phase shifting interferometric microscopy, confocal microscopy

and white light interferometric microscopy were compared to the stylus contact method whilst non-contact methods show larger distortions (Vorburger et al. 2007). Even though optical, non-contact measuring methods do not damage the surfaces and are much faster than contact methods, they are more sensitive to surface quality, surface height, slopes, fine surface features and deep valleys, therefore scattering may occur and consequently the accuracy of the method is affected (Vorburger et al. 2007). These findings make it clear that contact methods are better adapted to measure SR on porous materials such as wood, where vessels and tracheids show as big valleys and peaks.

2.3.8 Microbiological analysis

Today, we are aware that microorganisms, and especially bacteria, are ubiquitous. Even though wood does not seem to be the ideal nutrition for these microorganisms, they are able to colonise and feed on wooden surfaces. Visual methods such as LM are neither suitable to quantify the total amount of microorganisms present on a specimen (van Nieuwenhuijzen et al. 2015), nor can the species of fungi or bacteria be established. Moreover, LM cannot differentiate between live and dead microorganisms. Since within this thesis investigation of microorganisms on wood surfaces is envisaged, methods that allow to quantify microbiological contamination as well as to identify strains are needed.

2.3.8.1 Recovery of microorganisms from wood surfaces

The recovery of microorganisms towards agar plates may on the one hand be used as an indicator for the degree of microbiological contamination and on the other hand serve so that stains can be identified following. Much of the existing work in this field derives from the food processing industry and the regulations that come with hygienic aspects. The standard ISO 18593 presents multiple methods to recover microorganisms from surfaces in the food chain environment (2018), however up to now there is no standardized method in order to recover microorganisms from porous surfaces such as wood (Ismaïl et al. 2013). The mentioned standard describes the recovery of microorganisms towards nutritious medium. The amount of colony forming units (CFU) growing on selective or non-selective agar plates are, after incubation, enumerated. The transfer can take place in different manners, as e.g.:

The conventional printing procedure, which uses agar plates firmly pressed onto the solid surface to be examined. Benefits of this method is the fact that the whole surface area is tested, no sample preparation is needed, and it is a non-destructive technique.

Scraping and grinding methods are destructive procedures, which demand careful sample preparation. The samples need to be ground and following the material can be mixed with a buffer for subsequent analysis. This destructive method is not appropriate for hard materials. If grinding wood, the samples need to be relative thin in order to be ground which is a big disadvantage. It is suggested that through grinding heat is developed and might have an influence on the vitality of certain microorganisms (Ismaïl et al. 2013, 2015).

A similar destructive method, more suitable for wood, is the recovery via scratching, so planing a specific area of the wood specimen with a razor blade, producing shavings which are homogenized in a solution and consequently plated onto agar plates. This scratching technique was first described by Zangerl et al. (2010).

Different destructive recovery methods from porous surfaces have shown higher recovery rates of microorganisms compared to non-destructive techniques (Hedge 2015; Ismaïl et al. 2015). Non-destructive methods such as printing have still shown efficiency, even though recovery rates are lower (Prechter et al. 2002; Moore et al. 2003; Buttner et al. 2007; Coughenour et al. 2011; Vainio-Kaila et al. 2013; Ismaïl et al. 2015).

2.3.8.2 Determining the CFU

The determination of the total number of colonies growing on nutrition agar allows to draw conclusions about possible contamination of materials (Pichhardt 1998). Defining the number of colony forming units may have limited accuracy due to variables such as choice of nutrition, inoculation temperature, humidity and time. Moreover, microbial cells may exist in cryptobiotic, dormant, moribund or latent states in which no colonies will be formed but cells may have other measurable activities and are therefore “alive”. (Davey 2011). All microbiological methods have limitations (Sutton 2012), still, in microbiology repeated division of a cell on agar surface is usually considered as viability as they produce visible colonies. No growth is not necessary an indicator for no viable cells in the sample (Davey 2011), nevertheless the CFU can be attributed to a certain indicative function (Pichhardt 1998).

Counting the CFU manually is still a widespread approach. Several reports describe alternative technologies where colony counting is automated (Marotz et al. 2001; Dahle et al. 2004; Putman et al. 2005). These technologies however demand access to specific equipment. The acceptable number of countable CFU on a plate are set to a range of 25-300 (Sutton 2012), below 25 CFU cross contamination cannot be disregarded, above 300 CFU counting the colonies manually is rather intolerable.

2.3.8.3 Nutrition agar

A lot of different agar types are available on the market. Selective agars are achieved by adding e.g. antibiotics or bile salts, specific proteins, hydrolysates and carbohydrates. The use of chromogens and fluorogens are indicators for specific enzymes. Non- selective agars are used for general purposes, observing either bacterial or fungal strains, and do not hinder or support the growth of specific strains (Zimbro 2009).

As the choice of agar is a crucial parameter to determine the CFU, to verify the presence of microorganisms on wood surfaces, agars with a wide range of nutrients are needed. On plate count agar

(PC- agar) more colonies were shown to grow compared to nutrient agar plates (Jeršek 2017), probably because this medium is specifically used for the evaluation of the total bacterial growth (Pichhardt 1998). Malt extract agar (ME- agar) is used by various researchers in order to observe fungal growth (Börjesson et al. 1990; Hakkou et al. 2006; Soumya et al. 2011, 2013; Žlahtič and Humar 2016; De Ligne et al. 2020). It is a medium that allows to isolate and enumerate fungi and to develop specifically moulds and yeasts (Sigmaaldrich 2018).

2.3.8.4 Identification of microorganisms

As described earlier in the section 2.1.7, current research does not answer the question to which extent interactions between microorganisms, present on wood, take place. Identifying these microorganisms may on the one hand facilitate the understanding of e.g. enzymatic interactions between fungal and bacterial communities and on the other hand enable to study the effect of abiotic factors. Following some identification methods are mentioned.

Traditionally, genetic diversity is assessed by measuring morphological and physiological variation. Those factors are however highly influenced by the environmental conditions the strains are developing in (Rodriguez et al. 2004). Isolated cultures are necessary to assess a microorganism's qualitative properties and characteristic features. Pure strains can be obtained by isolation or re-cultivation methods (Pichhardt 1998). Metabolic approaches through biochemical tests are the most commonly used methods, they are said to be easy to apply, fast and not very cost intensive (Zhou and Li 2015). For example, analytical profile index (API) kits detect the enzymatic activity or fermentation of sugars (Tankeshwar 2015). Pre- identification, using e.g. gram stains, is necessary in order to choose the appropriate API test strip. Moreover, there is no guarantee that API kits will lead to an identification of non-pathogenic microorganisms.

It was suggested that biomolecular methods provide more exact and credible identification results (Erem et al. 2009). An identification method, which is capable of identifying viruses, bacterial and fungal strains, is for example the matrix-assisted laser desorption ionization – time of flight mass spectrometry (Maldi-TOF) method (Schmidt 2010). The method even allows to detect antibiotic resistances, however for identification of new isolates, databases containing peptide mass fingerprints are required, in order to determine the microorganisms strain (Singhal et al. 2015).

Ribosomal DNA (rDNA) investigation allows phylogenetic analysis. Information of hundreds of nucleotides of the target DNA is used which makes the analysis precise and suitable to present in and compare with data collections. Nuclear rDNA units of Eukaryotes (e.g. Fungi) consists of the conserved coding domains 18s and 28S rDNA (Schmidt 2010). The probably most accurate identification of bacteria (Prokaryotes) and Eukaryotes nowadays can be achieved by using 16S-rDNA and 18S rDNA sequencing (Woo et al. 2008; Singhal et al. 2015). It is used for the identification of isolates by amplification and sequencing of the species-specific variable V2-V4 and V6-V9 regions

of 16S-rDNA (Arnemann 2019). In combination with next-generation-sequencing (NGS) and connection to bioinformatics approaches, even identification of bacterial and fungal communities can be done (Macrogen Inc. 2017), meaning that purification steps are not necessary. Even though identification through sequencing is very precise, a disadvantage of these services are high prices.

3. Chapter: Material and methods applied to characterise wood degradation

This chapter starts off by introducing the wood species chosen to be observed for investigations. Following, the development and application of methods used within the thesis are described in this chapter. The next chapters 4 to 8, describing experimental studies, refer to the applied methods described in this section. The implementation of methods for the different experiments; the outdoor weathering experiment (OWE), the non-sterile weathering experiment (NSWE) and the artificial weathering experiment in a purpose-built device in sterile (SAWE) and non-sterile (NSAWE) conditions; within this work are shown. An overview of the applied characterisation methods for the different weathering experiments is given in Table 6.

Table 6: Methods used to characterise surface weathering after the application of a weathering technique

Characterisation method	Experiment (Weathering technique)		
	OWE	**NSWE**	**SAWE/ NSAWE**
Colour measurements	x	x	x
Surface scans	x	x	x
Moisture content evaluation	x	x	
Light microscopy		x	x
Environmental scanning electron microscopy (ESEM)		x	x
Attenuated total reflection spectroscopy (ATR)		x	
Surface roughness		x	
Surface contact test (CFU)	x		
Scratching technique (CFU)		x	
Solution plating technique (CFU)			x
XRF			x

3.1 Choice of wood species

As this thesis focuses on the degradation of wood in use class 3, only wood species which are said to cope with such exposure conditions are considered. One of the weathering experiments performed within this work, the outdoor weathering experiment, was carried out in Nantes, France, thus it was of importance that rather local wood species, of commercial interest were chosen.

The high commercial importance and the availability of *Quercus sp.* and especially *Pseudotsuga menziesii* in Europe and specifically in France (Lochu 2012) are primary reasons to study the degradation of these two wood species in more detail. For claddings in France, 57 % of softwoods are

imported. Douglas fir is with 16,8 % (46 000 m^3) the essential wood species and oak wood is the most important hardwood species for the use of facades in France (Lochu 2012).

According to Fioravanti et al., *Castanea sativa* forests in France and Italy cover an area of 14000 km^2. The strong growth of chestnut of up to 24 m^3/ha/year, the highest after poplar wood, allows it to store more CO_2 than numerous indigenous wood species, e.g. oak wood, which make the cultivation of the wood species quite interesting. Unfortunately, missing silviculture, susceptibility to diseases and the lack of innovation and development makes the wood species less popular, even though chestnut has good durability properties (Fioravanti et al. 2010).

Another argument for the choice of theses wood species, is the different wood anatomy. *Quercus sp.* is a ring-porous hardwood species with the presence of large vessels and multiseriate rays. There is the hypothesis that degradation may often start around parenchymatic tissue, thus studying a wood species with numerous ray parenchyma can be an advantage to detect degradation in the first stages of weathering. *Castanea sativa* shows very similar anatomical properties compared to oak wood, except that the ray parenchymatic cells are much thinner. Even though the chosen hardwood species differ only slightly on an anatomical point of view, on the chemical level the two hardwood species distinguish considerably though, by e.g. lignin-, cellulose-, ash content and pH-value. In tracheids of the resinous *Pseudotsuga menziesii*, helical thickenings occur and are very typical for this softwood species.

The choice of these three wood species allows a comparison between softwoods and hardwoods, and between hardwoods of similar anatomical structure. Having said that, it was decided, after the research programme had started, to focus on the differences between hardwood and softwood, i.e. oak compared to Douglas fir, because the inclusion of chestnut would have increased the workload by 50% and only provided interesting, rather than essential, data. The data collected from chestnut has been included as it had been collected.

Douglas fir wood usually has a larger proportion of sapwood compared to oak and chestnut wood. As for all wood species, sapwood is considered not to be durable, thus the sapwood was avoided when preparing the specimens. The choice was made by using the distinct colour differences between the heart and sapwood of the wood species. Careful sample taking was carried out, meaning that mainly heartwood with normal growth characteristics; such as growth speed, proportion of early- and latewood, absence of knots or normal distribution of resin ducts; was chosen.

3.1.1 Origin of wood specimens

Oak wood from 6 trees harvested from 3 different forests have been obtained and 90 Douglas fir boards from 4 different areas were provided by the FCBA (Institut Technologique Foret Cellulose Bois- Construction Ameublement). The chestnut wood observed within this thesis derived from one

single board where the origin is unknown. Figure 16 shows a scheme of the origin of the wood samples.

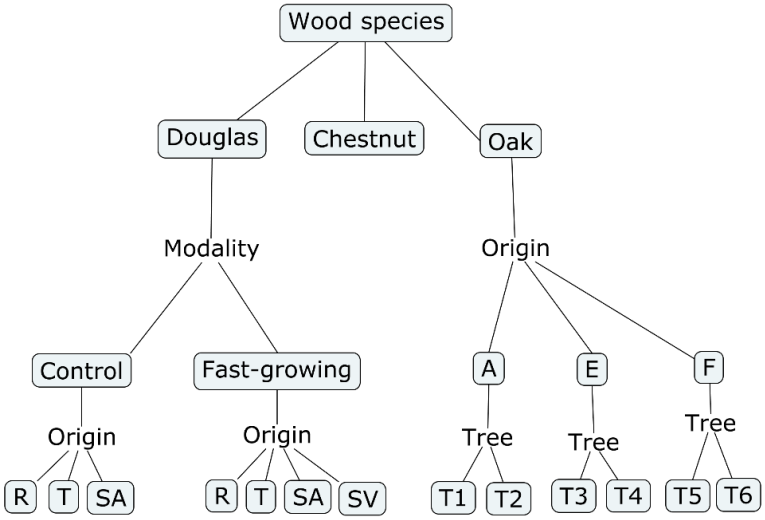


Figure 16: Scheme of wood sampling

3.1.1.1 Pseudotsuga menziesii

The Douglas wood was provided from FCBA, who had prepared and used the wood for their own experiments. The wood boards derived from 4 different areas in France, namely: Larochemillay (R), Le Transet (T), Saint- Amand (SA) and Saint- Victor (SV), all forests in the centre of France. Half of the boards derived from “fast-grown” trees, the other half were “control” trees.

3.1.1.2 Quercus petraea

Oak wood was purchased from the company Boisloco. The trees harvested from three different forests, 4 trees from two different forests close by Parcé-sur-Sarthe, and 2 trees from a forest close by Précigné, France. All three forests have different soil properties and wood populations.

3.1.1.3 Castanea sativa

The origin of chestnut wood, which was used only for one experiment, the NSWE, is unknown.

3.1.2 Drying of wood specimens

Douglas fir and chestnut wood had a MC of ~ 20 % when delivered. The oak wood, however, was dried in-house with a vacuum dryer (W.D.E Maspell SRL). The drying cycles were adapted from proposed cycles by the supplier of the dryer. Oak wood, if dried too fast tends to crack, thus a slow drying cycle, as shown in Table 7, was chosen to reach a MC of ~20%. After which the oak was stored in a conditioning room set to 20 °C and 65% relative humidity.

Table 7: Drying cycles of oak wood

Drying cycles								
Phase	0	1	2	3	4	5	6	Conditioning
MC %		50	40	30	25	20	17	
P mbar		150	150	150	150	150	150	
t °C	20/ h	48	50	52	54	56	58	

3.2 MC measurements

The MC of **OWE** specimens was measured with a resistance moisture meter from Scanntronik (Mugrauer GmbH). A Gigamodule with 8 MC sensors was connected to a 'Thermofox Universal' Datalogger. The data was analysed via the SoftFox software. The resistance measuring curves [10* log(R)] were converted into wood MC [%] based on laboratory experiments by Scanntronik on the specific wood species. A pair of specimens of each Douglas and oak wood with the size of 375*78*20 mm^3 (Longitudinal*tangential*radial) were measured continuously throughout the experiment. The transverse faces of the specimens were sealed according to EN 927-3 with a hydrochrome type paint furnished from the company MILESI. Standard stainless-steel screws (~⌀ 2.5 mm) were used as electrodes. The installation of the electrodes, temperature sensor and screws are shown in Figure 17. Small holes were pre-drilled to prevent cracking and short screws were screwed to a depth of approximately 5 mm into the samples so that the MC is measured on the top layer of the surface only. Following, screws were sealed with a marine grease to minimise the risk of water accumulation. A temperature sensor was fixed on the front side of an exposed wood specimen as well as an integrated temperature sensor measured the ambient temperature. The electrodes as well as the temperature sensor were connected to the Gigamodule and data were collected at intervals of 1 h.

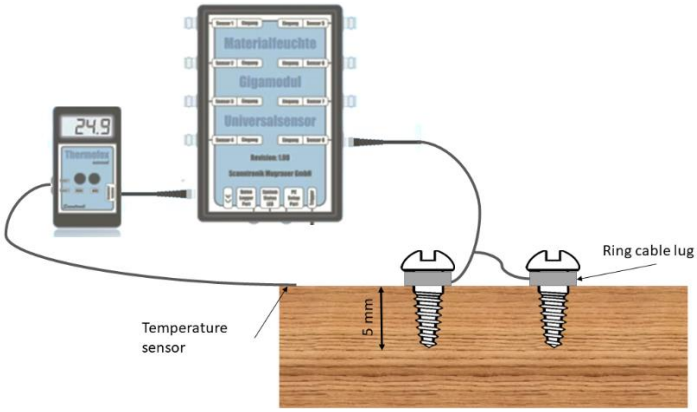


Figure 17: Installation of the Gigamodule device to measure MC and temperature of exposed wood samples continuously

MC measurements of **NSWE** samples were carried out with the Logica LG6NG capacitance moisture meter. Measurements were taken immediately after removing the specimens from the weathering device or after removing them from the outdoor exposure site. Only one measurement was taken on each specimen (n=3) since the microbiological analysis needed to be tested in the laminar flow bench (LFB) and the specimens dried out quite fast with the air circulation in the LFB.

3.3 Colorimetric evaluation

A Spectrophotometer Ci64 from X-rite was used for colour evaluation. The device was set to the CIE standard illumination A (ISO 11664-2 2011) and SPIN values were recorded. The number and position of colour measurements varied between the different experiments:

- **OWE:** the colour was measured in 10 predefined areas using a template for each of the 12 replicates (for each wood species)
- **NSWE:** the colour was measured in 4 predefined areas using a template for each of the 3 replicates (for each wood species)
- **SAWE/NSAWE:** the colour was measured in 4 predefined areas using a template for each of the 5 replicates (for each wood species)

Paperboard templates, as shown in Figure 18 helped ensure that measurements were taken in the same area each time. The data collected allowed the calculation of delta values between before and after exposure for each colour coordinate.

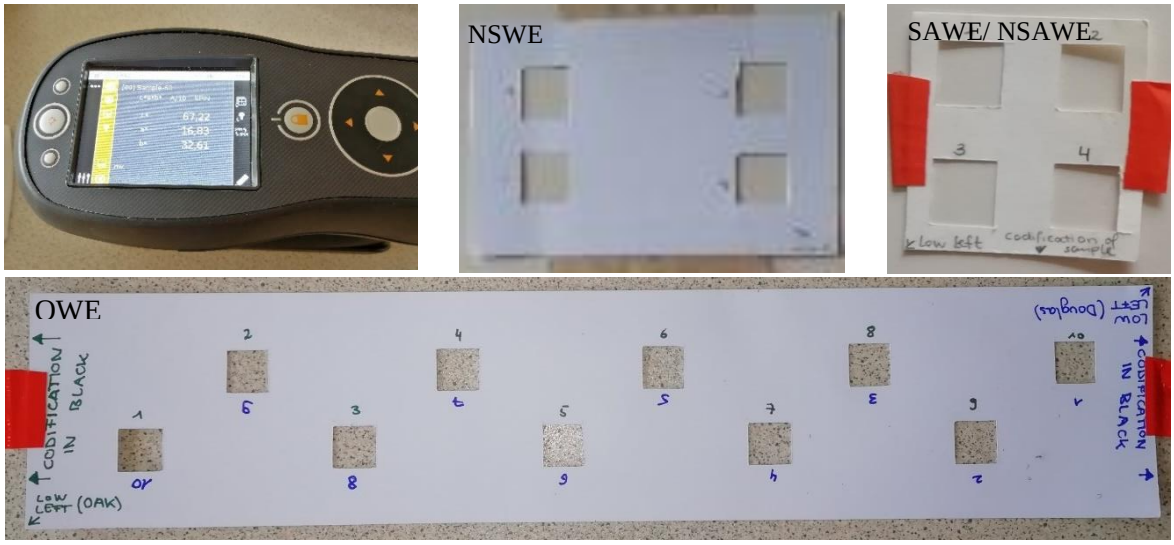


Figure 18: X-rite device and templates used to evaluate colorimetric values on OWE, NSWE and SAWE/ NSAWE

To determine the colour distance, ΔE is calculated according to EN ISO 11664 as following:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3.4 Surface scans

Specimens were scanned with a RICOH MPc6502 to provide photographs with the same level of illumination throughout the experiment. **OWE** samples were scanned before and after various exposure times and **NSWE**, **NSAWE** and **SAWE** samples before and after different weathering techniques were applied. The evolution of samples appearance was observed. The images were collected at a resolution of 600 dpi and colour scans were saved as JPG files.

3.5 Light microscopy

The microscope DM750M from Leica was used to observe solid samples in reflection mode. Magnifications ranged from 100 x up to 1000 x. The Leica LAS software called “Image builder” facilitates the taking of in-focus pictures of rough and uneven surfaces. A trial, using the light microscope (LM) in transition mode, where microtome slices were cut, and dyed, is shown in **Annex III**.

3.6 ESEM

The ESEM Quanta 250 from the company FEI was used for electron images. No prior preparation of the samples was done except for the specimens exposed to **NSWE** which were marked by creating a hole with a small pin in the bottom-left corner of the specimen. This corresponded to the area where surface roughness (SR) measurement were started. The pin hole in the wood surface was easy to find with the ESEM after each weathering stage, the hole was placed in the lower left corner of the picture at 100x magnification.

Tilting the sample by 30 ° facilitates the observation of the roughness of the surfaces. Preliminary experiments indicated that the optimum parameters for taking pictures with the ESEM are: a pressure between 70-90 Pa, a voltage of 5-10kV and a spot size in between 3.5 and 4. The exact settings used for each specimen varied due to wood species, sample size and surface characteristics (roughness, splits, protruding fibres, etc.). Setting the relative humidity of the ESEM chamber atmosphere between 60-80 % was found to be optimal for the observation of fungal hyphae. Photos were mainly taken at 200 x, 400 x and 600 x magnification, however magnifications up to 12000 x were useful in some cases.

3.7 Assessment of the visual observations

Standards such as EN 16492, EN ISO 4628-1 and 4628-4 describe the evaluation of degradation of wood coatings. In this research, however, no coatings or treatments were applied prior to exposure of the samples (except for end sealing). The degradation of wood after the different weathering methods was assessed using a new ranking system described below. This ranking system allowed the visual data collected by scans, light microscopy and ESEM from the **NSWE** samples to be combined for statistical analyses such as principle component analysis (PCA). 3 replicates were considered for each wood species, weathering technique and exposure length.

3.7.1 Levels of observation

- Scans (~10x mag.)
- Light microscopy in reflection mode (10-40x mag.)
- ESEM (100x mag. and further)

3.7.2 Criteria

- Microorganism: Disfigurement by microbes included colour changes such as blue staining and mould growth and was evaluated by means of LM and scanning. The small field of view combined with the grey scale nature of the images obtained from ESEM observation was not conducive for assessing the presence of microorganisms and was therefore not included in the analyses.
- Cracking: The width, length and amount of cracks (voids in-between fibres) were evaluated.
- Defibration: The presence of loose fibres on the exposed surfaces were evaluated.
- Early-latewood differences: The criterion considers the difference in disfigurement of early- and late wood due to microorganisms, cracking and defibration. The criterion indicates the susceptibility of the wood species and is an indicator for the wood variety and density. It is not evaluated when specimens were observed with the ESEM, since pictures were always taken at the same area (starting point of surface roughness measurements) and so early- late-wood boundaries were not always present.

3.7.3 Range for each criteria

Each criterion was evaluated on each observation level. In Table 8 the evaluation range is explained.

Table 8: Assessment of the visual observations: Explanation of the evaluation range from 0 to 5 for each criterion

Evaluation range	*Microorganisms*	*Cracking*	*Defibration*	*Diff. between early-/latewood*
0	None	None	Not visible	No difference
1	very few	very few	Rare	very slight diff.
2	Few	few	Very few	slight
3	moderate	moderate	Easily found	moderate
4	considerable	considerable	Very many	considerable
5	Dense	dense	Everywhere	very marked diff.

3.7.4 Evaluation of results

The sum of all evaluation criteria and levels for each wood species and weathering technique was calculated (50 points are the maximum) and then converted to a percentage.

Table 9: Assessment of the visual observations: Decay classes

Decay classes	*Per criteria*	*Description*
0	0	No signs of decay
1	>0-25	Slight signs of decay
2	>25-50	Degradation patterns, especially visible with higher magnification (>100x)
3	>50-75	Degradation observable at all three levels
4	>75-100	Severely decayed through biotic and abiotic factors, very visible to the naked eye

3.8 FTIR- ATR

ATR was applied to analyse the relative quantities of chemical functional groups and to indicate the variation of cellulose, hemicellulose and lignin contents before and after application of different weathering techniques.

The analysis was conducted on solid wood specimens. One specimen for each **NSWE** weathering technique, exposure length and wood species, so 8 specimens, were analysed in early- and latewood regions.

ATR spectra were recorded employing an ALPHA Bruker spectrometer equipped with attenuated total reflection (ATR) module. The spectra were recorded in the range 4000 - 400 cm^{-1} at a resolution of 4 cm^{-1}, each spectrum representing an average of 24 scans. Spectra from six randomly chosen measuring areas (3 early- and 3 latewood areas) were recorded for each specimen. Each spectrum was further processed for baseline correction and smoothing. An average spectrum of the three individually recorded ones was calculated. This average spectrum was further max-min normalised in the range between 1800- 700 cm^{-1}, whilst the peak at ~ 1023 cm^{-1} showed to be the highest peak within the set range. The OPUS 7.2 software from Bruker Optik GmbH was employed for spectra processing and calculations.

3.9 XRF

XRF spectroscopy was carried out with an X-MET 7500 (Oxford instruments, Model: XMDS2726). Gamma sterilized wood samples were observed and served as a reference. Both the front and back side of the wood specimens exposed to artificial weathering (**SAWE/ NSAWE**) were measured. Douglas fir was examined in early- and latewood areas, oak wood was measured in strongly discoloured areas as well as less visually modified zones.

3.10 Surface roughness

The SR of the **NSWE** specimens were measured with a MarSurf XT 20 perthometer (Figure 19) using parameters as listed in Table 10. The application of the robust Gaussian regression filter is recommended when measuring wood surfaces in order to reduce the bias from wood anatomy (Gurau et al. 2017).

Table 10: Settings of the MarSurf device to measure the surface roughness, recommended by Gurau (2019).

MarSurf settings	
Software	Open Software MarSurf XR20
Standard	ISO, JIS, ASME (N)
Tip radius	2 microns
Tip angle	90°
Measuring speed	0,5 mm/s
Measuring interval	5
Vertical range for the scanning head	±750 µm
Lateral resolution	5 microns
Measuring length	45 mm
Measuring direction	Perpendicular to wood grain direction
Data points	9000
Sampling lengths	1
Filtering cut-off	2,5 mm
Filter	RGRF
Number of profiles	10 in 5 areas
Parameters	Ra, Rq, Rt, Rp, Rv, Rsk, Rku, Rk, Rpk, Rvk, A1, A2, Pa, Pt, Wa, Wt

Sample SR was calculated from 10 profiles traced per specimen. The first trace was made on the lower left corner of the samples where a hole had been made with a pin (see 3.6). The second profile was 4 mm above the first, the third measurement 10 mm above the second and so on as shown in Figure 19.

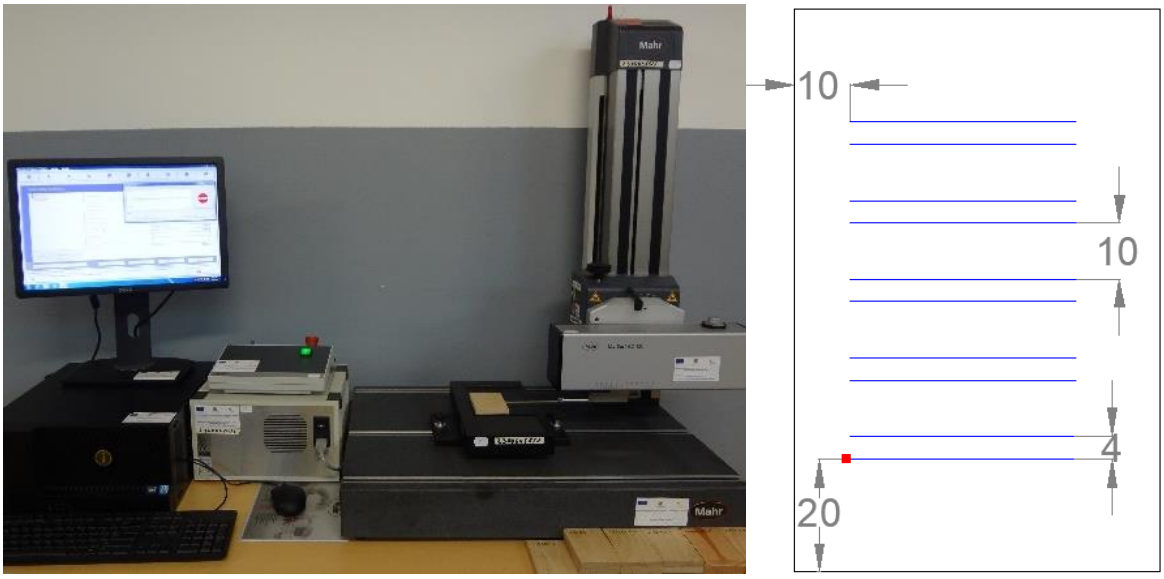


Figure 19: MarSurf device (left), scheme of measuring lines (blue) on samples and measurement starting point (red)

3.11 Microbiological analysis

This section describes procedures to recover, incubate, enumerate, isolate and store microorganisms.

3.11.1 Nutrition agar

The plate count standard method agar (PC-agar) M091 from the company Himedia is specifically made for determining microorganisms in foods, water and wastewater, which includes environmental bacteria. It is composed of tryptone, yeast extract, dextrose and agar. 23.5 g of the powder was diluted in 1000 ml deionised water. PC-agar was used for experimental work, where bacteria was aimed to be developed. In order to develop fungal strains, malt extract agar (ME-agar) was used and prepared in the laboratory. It consisted of 20 g of bacteriological agar Type E A1012 from Biokar diagnostics and 20 g of bacteriological malt extract from Biokar diagnostics A1101 mixed into 1000 ml deionised water. Both agar solutions were stirred in a water bath for 30 min until boiling and subsequently autoclaved at 121 C° and 1 bar for 30 min. Once the agar cooled down to about 50 °C, it was poured into sterile 90 mm petri dishes in a LFB. The now ready to use agar plates were stored for no longer than a month at 6 °C.

3.11.2 Sterilisation and creating a sterile environment

The risk of cross contamination was minimised by conducting all manipulations in an LFB, the work surfaces of which had been previously disinfected with 70 % ethanol. Generally, disposable sterilized utensils, such as drigalski spatulas, cotton swabs, sampling tubes and pipette tips were used. Reusable equipment was sterilized using either a Bunsen burner or autoclaving for 30 min at 121 °C and 1 bar. Sterilization of deionised water was achieved by the same autoclaving procedure.

Wood samples were sterilised by gamma radiation by the company Ionisos with a minimum dose of 25 kGy according to the standard ISO 11137 (2013). Before sealing the codified samples in plastic bags for gamma sterilisation, the samples were stored in a climatic chamber (Vötsch VC150) at 65 % relative humidity and 20 °C for at least 2 weeks.

3.11.3 Recovery of microorganisms to media

As it was not intended to compare the amount of CFU found on samples exposed to various weathering scenarios with each other, but rather to investigate multiple procedures, three different recovery techniques were applied. The different procedures used to assess the amount of CFU in water and/or on wood samples exposed to OWE, NSWE and SAWE/ NSAWE are described below.

3.11.3.1 Surface contact technique

For specimens exposed to **OWE** it was important to choose a non-destructive recovery method in order to evaluate subsequent microbiological contamination of the surfaces with increasing time of exposure. The method chosen was the surface contact technique (SCT) and was performed in accordance to Swedish standard (SCAN-CM 61 2002).

As shown in Figure 20, inoculation of the agar was achieved by pressing the specimens firmly onto agar plates for 5 s. Samples were handled with 70 % ethanol sterilized gloves only and immediately re-placed onto the exposure racks. The plates were then incubated (see below in 3.11.4) and the CFU were counted after a predetermined time (see 3.11.5). On occasion, some fungal and/or bacterial colonies were transferred several times onto new petri dishes in order to gain pure cultures. Consequently, the strains were transferred into tubes for storage and subsequent identification (see 3.11.6 and 3.11.7 for further information).

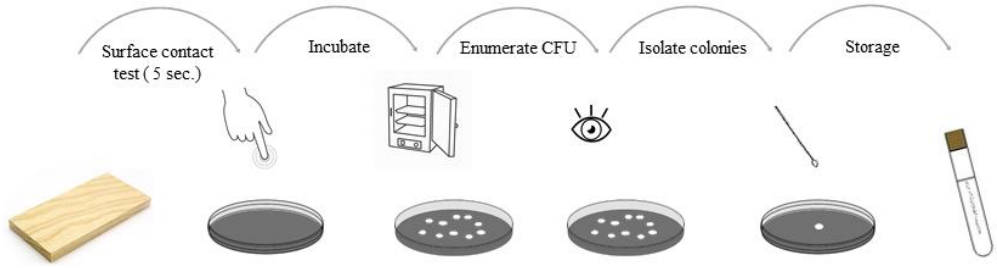


Figure 20: Recovery method from samples exposed to OWE

3.11.3.2 Scratching technique

NSWE specimens were the only specimens evaluated by this destructive recovery technique. This technique was chosen because NSWE samples were not re-exposed after evaluation, therefore a destructive method, which, according to the literature, achieves higher recovery rates compared to the SCT technique, was used. Evaluations were conducted after 6 and 12 weeks of exposure.

A sterilised razor blade was used to scrape off about 5 mg of wood fibres from a defined area such that only the very surface layer is removed. The fibres are placed in 2 ml of sterile deionized water in Eppendorf tubes. The tubes are then vortexed twice for 30 sec with 1 h waiting time in between. Right after the second vortex sequence, 200 µl of the solutions were plated on to PC- and ME- agar. Consequently, dishes were incubated and analysed as described on page 50.

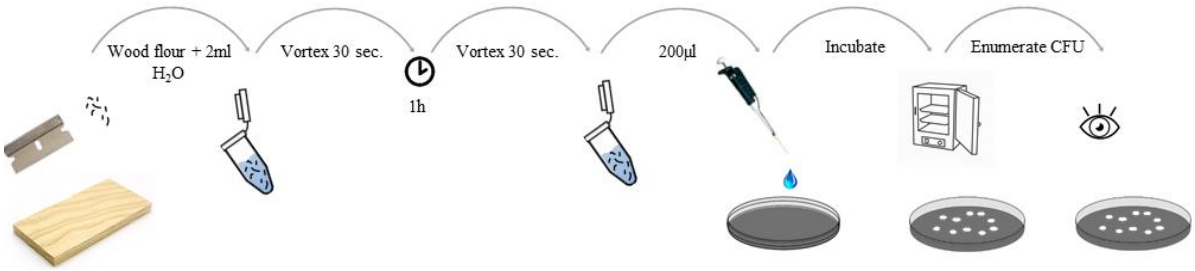


Figure 21: Recovery method from samples exposed to NSWE

3.11.3.3 Solution plating technique

It is imperative that the specimens used in the **SAWE** remain sterile throughout the experiment. Therefore, they could not be manipulated during the experiment. The alternative is to test the water that is circulated in the apparatus to see if it contained any microbes. Certainly, this procedure lowers the risk of cross-contamination of specimens because they are not removed from the test apparatus.

A small sample of water was collected from the SAWE apparatus via the tap in the base. A sterile pipette was used to take 200 µl of the water and place it onto agar plates. The water droplets were evenly dispersed with a sterile drigalski spatula. The subsequent incubation and CFU counting procedures are described in the following sections.

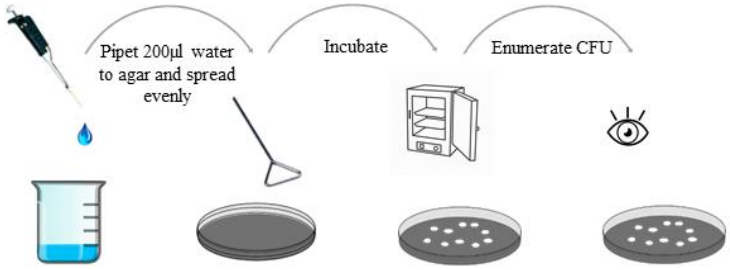


Figure 22: Recovery method from water in the self-built weathering devices

3.11.4 Incubation

The incubation temperature is commonly chosen according to the microorganism to be developed. In this study, the microbes collected were unknown prior to incubation. In general, bacteria development at higher temperatures than those that are optimal for most fungal species. Since only one incubator (Memmert, Model 500) was available for the experiments, both the PC- and ME- agar plates were incubated at a temperature of 32 °C, unless otherwise stated. This temperature is more appropriate for fungi than bacteria, but, it is a reasonable compromise.

PC- agar dishes for bacterial growth were incubated for 24 h and then visually assessed, whereas the ME- agar plates were incubated for 5 days before evaluation.

3.11.5 Determining the CFU

Pictures of the agar plates were taken using a custom-made PhotoBox as shown in Figure 23. A Huawei smartphone camera, with a resolution of 13 MP, was placed on the top of the box and the petri dishes were placed, without cover lid to avoid reflections, underneath. This approach allowed that all the photos had the same distance to camera (magnification) and similar lightning throughout the whole test period. Examples of resulting pictures of PC- and ME-agar plates can be seen following in Figure 23. These pictures were used for manually counting the CFU values. Each solitary colony was recognised as one CFU. Counting of colonies was possible until about 80 CFU. All dishes where the CFU count exceeded 80 were classified as having a count of 100 CFU. The standard variation within replicates was found to be very high. The CFU counts were thus, for several investigations, transformed into ordinal data, whilst 0 represents no growth at all, 1 is moderate growth until 33 CFU, 2 is considerable growth between 34 until 66 CFU and 3 represents everything above and until 100 CFU. This allows to reduce the spread of the data as well as permits a better representation of the data in view cases.

A side experiment to quantify microbiological growth on petri dishes via image analysis is shown in **Annex IV**.

Figure 23: PhotoBox (left) and resulting pictures of PC-agar (middle) and ME- agar plates (right)

3.11.6 Isolation and storage of bacterial and fungal strains

One colony of a bacterial strain was removed with an inoculation loop and placed on a new petri dish in a zigzag on one half of the sterile dish. The loop was then sterilized with a Bunsen burner and used to spread three lines from the inoculated half of the dish to a sterile quarter. The loop was sterilised again and three more lines were drawn from the latest inoculated quarter to the remaining sterile quarter. This helps to ensure a spread of the colonies and isolation of the strain.

Fungal strains were isolated in a different manner. A small piece at the edge of the visible fungal growth was cut and placed in the middle of a sterile petri dish containing ME- agar and incubated.

After several repetitions of the isolation procedure, the strains were moved to 2.5 ml agar tubes from the company Humeau, which allow the strains to be conserved for several months. The storage media is composed of peptone, agar, meat extract and sodium chloride (ref.: 747.028097.02). The tubes were incubated with loose lids at 32 °C for at least 24 h and consequently sealed and stored at 6 °C. A small database of microorganisms recovered from weathered wood surfaces has been established and an excerpt is shown in **Annex V**.

Due to capacity issues at the microbiological laboratory at ESB, only a few strains obtained from the OWE were preserved. A selection procedure was chosen as follows (see Figure 24): At each measurement date, 24 petri dishes were used for SCT (12 on Douglas fir and 12 on oak). From each lot of samples, a maximum of 4 strains were isolated and stored. The colonies were chosen according to their appearance with the idea to isolate as many different kinds of strains. Characters such as form, size, elevation, margin, opacity and colour were crucial for selection. Therefore, in some cases two colonies from the same dish were isolated.

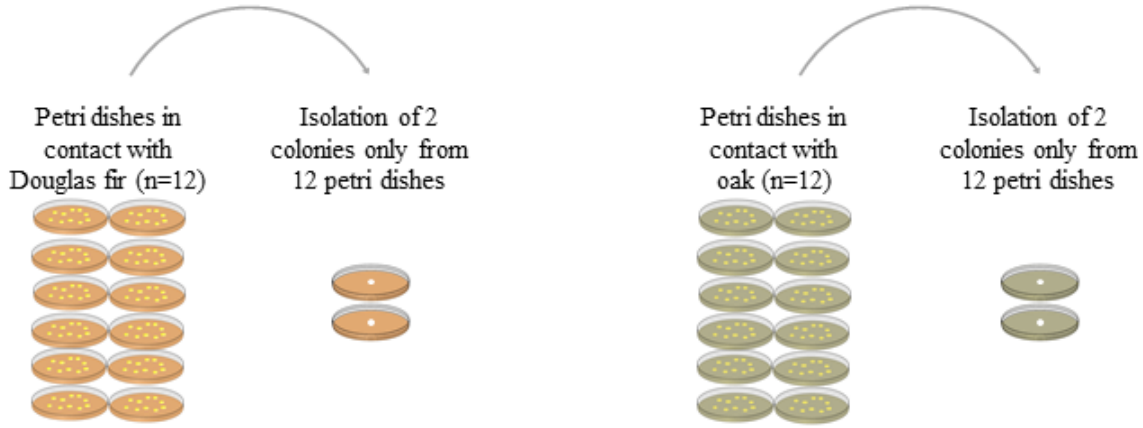


Figure 24: Isolation procedure from strains deriving from OWE at a single measurement date

3.11.7 Identification of microorganisms

A batch of purified samples was sent to the Hospital GHR Mulhouse Sud-Alsace to conduct Maldi-TOF analysis. Moreover a command for a sequencing service was completed with the company Macrogen (https://dna.macrogen.com). View strains were characterized according to their morphological aspects.

4. Chapter: Outdoor weathering experiment

This chapter and those following, up to and including Chapter 8, present experimental works that make up this thesis.

Within this chapter the outdoor weathering experiment (OWE), where environmental degradation factors cannot be controlled, but measured, is described. This experiment was carried out in order to get an insight about the degradation phenomenon in authentic conditions, with focus on microbiological contamination of wood surfaces exposed to an Atlantic climate. Sample preparation, results of the measurements as well as interpretations of findings are presented.

4.1 Material and methods

Information about the origin and initial preparation about the *Quercus petraea* (oak) and *Pseudotsuga menziesii* (Douglas fir) raw material is given in **Chapter 3.1**. Boards were cut with a circular saw and planed to the desired sizes. There are two different sample sizes**:** 375*78*20 mm^3 and 50*50*20 mm^3 (Longitudinal*tangential*radial). Only defect free, i.e. no knots or resin pockets, heartwood was used. Specimens were codified on their cross sections with a permanent marker. The transverse faces of the specimens were then sealed according to EN 927-3 with a hydro-chrome type paint furnished by the company MILESI, in order to avoid excessive water uptake. The paint was applied in three layers with a brush. The specimens were dried for at least 24 h in between layer applications.

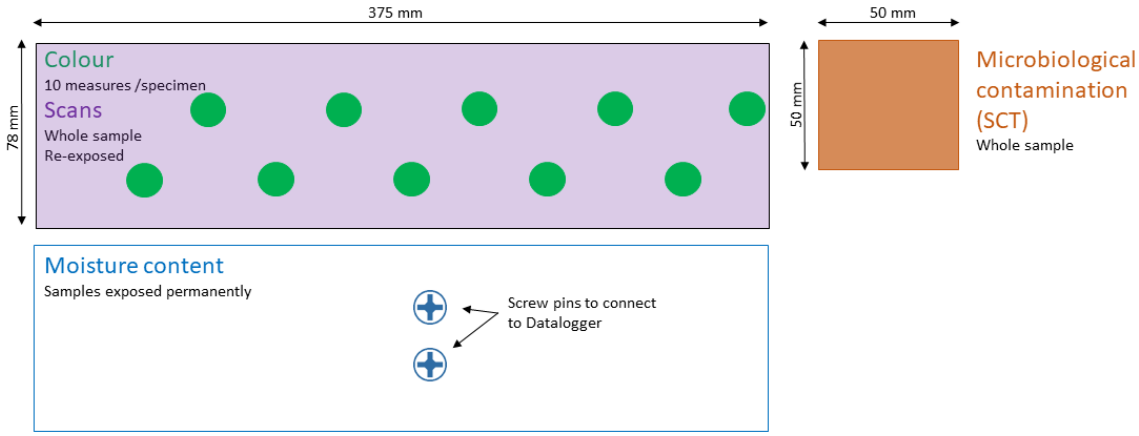


Figure 25: Scheme of the OWE samples and the areas of measurements

As shown in Figure 25, specimens with dimensions of 375*78*20 mm^3 were used for visual evaluation, colour and MC measurements. Smaller specimens with dimensions of 50*50*20 mm^3 were exposed in order to follow the microbial contamination over time via surface contact testing (SCT) because these dimensions allow the specimens to fit in to the petri dishes. These specimens were re-exposed immediately after the different measurement procedures (see **Chapter 3**) were carried out. Two of the large dimensioned specimens of each wood species were constantly exposed in order to

provide MC data throughout the experiment. Please see **Section 3.11.3.1** for precise information on how CFU data were obtained from OWE samples.

4.1.1 Outdoor ageing procedure

The samples were exposed according to the standard EN 927-3 (EN 927-3 2006), facing south, with horizontal wood grain alignment and inclined at an angle of 45 ° from the ground.

4.1.1.1 Exposure site

Figure 26: Geographical position (left) and a picture (right) of the outdoor exposure site at ESB campus (right)

The location of the exposure site on the rooftop of the building is shown in Figure 26. Stainless steel frames were mounted on ESB´s roof and samples were fixed to the display using double-sided adhesive Velcro tape on the back side of the specimens. This fixing system does not require any drilling into the samples, prevents corrosion through screws and avoids water traps. Moreover, samples can easily be removed and fixed on the frame again.

4.1.1.2 Exposure lengths

A set of specimens was exposed from the 15.07.2019 onwards and measurements were carried out until the 12.10.2020, this group is termed the summer batch as the exposure start was during summer. A second set of small wood specimens, termed as winter batch, was exposed three months later (28.10.2019-12.10.2020) and served to measure only the microbiological contamination.

4.1.1.3 Meteorological conditions

Weather data were recorded with a weather station Davis vantage pro 2, located right next to the exposure site. Data points were registered every 10 min and stored on a local server and shared via the online platform Weathercloud (https://app.weathercloud.net/d0800467527#profile). Unfortunately, data of several weeks were not recorded because of connection problems to the server. Missing temperature, humidity and rain data was downloaded from the nearby weather station at the air-

port in Nantes (https://rp5.ru). Table 11 gives an overview of the mean values of the measured meteorological parameters during 'summer' and 'winter' exposure. Comparing both exposure periods with an f-one way Anova, all measured parameters, except rain rate and wind direction, show significant differences. Atmospheric pressure and temperature measurements (p-values= 0.00) show lower significant differences (significance level α= 0.05) compared to humidity ($p= 1.16\times10^{-270}$), wind speed ($p= 1.21\times10^{-47}$), rain ($p= 1.42\times10^{-26}$) and solar radiation ($p= 2.62\times10^{-116}$). Figure 27 shows the climate conditions during the whole exposure length (Weekly average values).

Table 11: Mean values of meteorological measurements during OWE. Legend: Temp: Temperature, Hum: Air humidity, Wspd: Wind speed, Wdirect: Wind direction, Pressure: Atmospheric pressure, Solarrad: Solar irradiation

Exposure start	Temp (°C)	Hum (%)	Wspd (m/s)	Wdirect (°)	Pressure (hPa)	Rain (mm)	Rainrate (mm/h)	Solarrad (W/m²)	UV Index ()
Summer	12.6	80.2	2.2	200.4	9405	1.25	0.09	96.1	0.45
Winter	8.8	83.9	2.4	200.4	8234	1.52	0.10	51.5	0.11

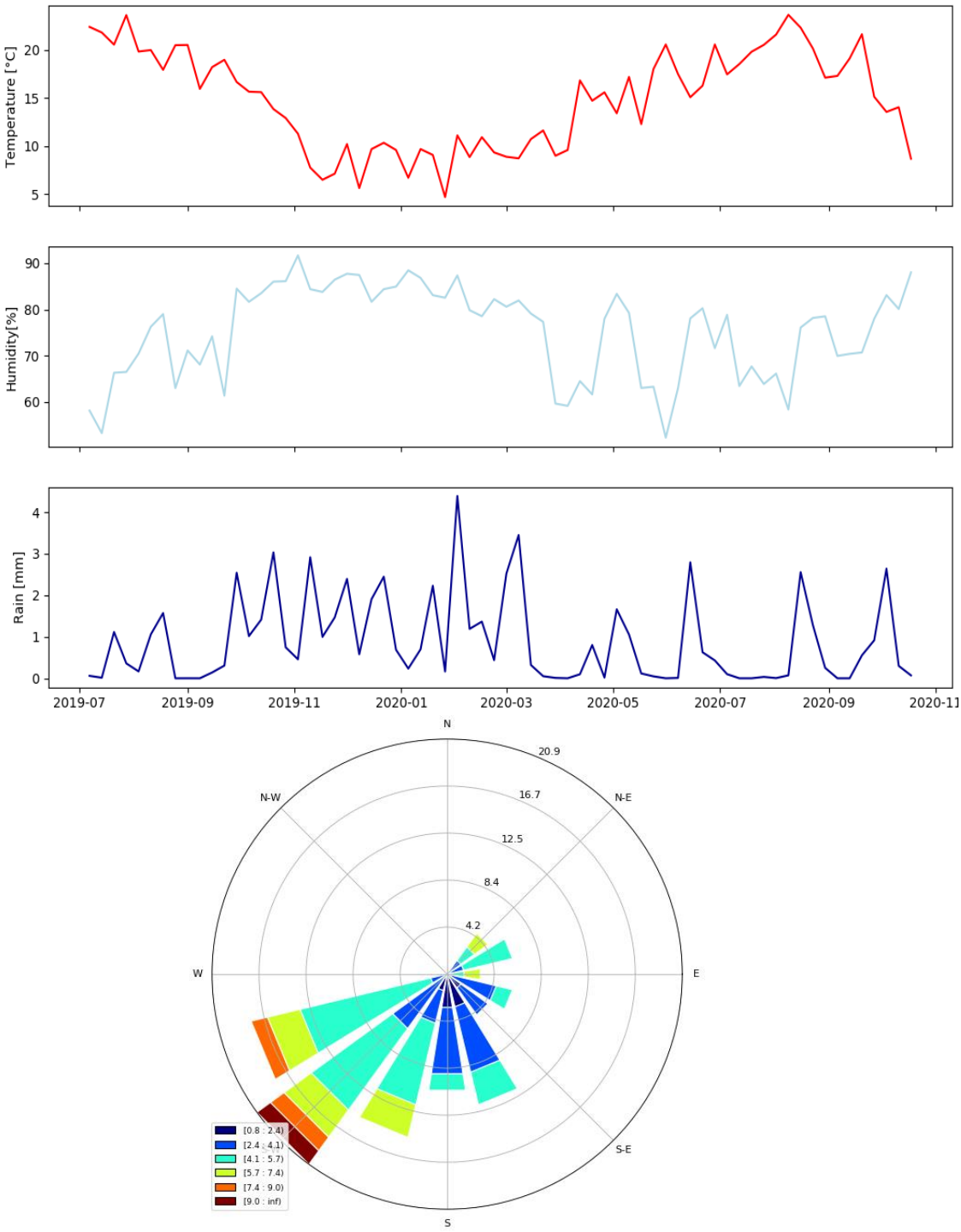


Figure 27: Temperature, humidity, rainfall and wind -direction and speed during OWE

4.2 Results and discussion of OWE

Following results of the measurements performed on outdoor weathered oak and Douglas fir wood are shown. Pearson correlation analysis, principle component analysis (PCA) and clustering were performed in RStudio, the visualisation of the data was achieved using Python 3.6.

4.2.1 Statistical analysis

The OWE experiment helped gather plenty of records. In order to get a first overview of the data, a PCA as well as cluster analysis are shown before results of each applied measurement is observed individually. As the gathered data sets are quite ample and at the same time showing missing values, prior data preparation was necessary.

- The data sets for MC measurements and environmental conditions such as temperature, humidity, solar radiation etc. have missing values. Missing data points were interpolated in Python using the SciPy open-source software module ".interpolate ()" which is a linear interpolation method that adds values by forward filling.
- From the meteorological data in general the mean values were used for statistical analysis. In addition, new variables of the rain [mm] and rain rate [mm/h] data were calculated: "Rain (sum)" and "Rain rate (sum)" representing the total amount of rain prior to the implementation of SCT.
- Correlations between the different variables might be time frame dependent. For example, the observed colour data is likely to be more strongly influenced by the amount of rain failing in the previous 3 hours before colour measurement compared to the average rainfall over the previous week. Likewise, a CFU count obtained on any day is likely to be affected by the weather that immediately preceded the data collection and less so by the historical weather. Following two data sets were created by calculating the mean values considering data only 3 h (df_3h) and another data frame with weekly mean values (df_weekly) before SCT and visual observation.

4.2.1.1 Correlations between the different parameters

The aim of this section is to identify correlations between the data collected from measurements performed on the wood specimens (CFU on PC-agar and colour) and different measured weather parameters. As the amount of variables are quite large respectively to the quantity of measurements performed, assessing correlations prior to the implementation of statistical analysis will help reducing the amount of variables to be investigated. Due to this reason, only CFU count on PC-agar were used to determine correlations. This section explores the data sets df_weekly and df_3h.

The following heatmaps show linear correlations, whilst the spot diameters and colour intensities indicate the coefficient of determination (R^2). Positive correlations are shown in blue and negative

correlations in red. The first column of each map gives information about variables influencing the amount of CFU on PC-agar.

Figure 28 show the Pearson correlation for Douglas fir performed on the data sets df_3h (top graph) and df_weekly (bottom heatmap). Both the 3 h and weekly average data sets show negative correlations between CFU and ΔE*, Hum, MC, Rain and Rain rate. This indicates that CFU recovered from Douglas fir is strongly dependent on the wood moisture level. In addition, the 3 h average data set shows slight positive correlation of the UV index parameter towards CFU counts. The data set df_weekly indicates that wind- speed influences the CFU counts observed in that low wind speeds reduce the observed counts. The wind- speed influences the CFU on Douglas fir, rather on a long term, likely to the fact that through the air circulation the examined softwood specimens tend to dry faster (Cirad 2013) as well as to the fact that wind can act as a carrying agent of spores (Tignat-Perrier et al. 2019).

The data collected from the oak wood samples (see Figure 29) show quite different correlations: Colour coordinates a* and b*, atmospheric pressure, rain (stronger in the data set df_3h), Temp, UV index, Wind -direction (only the data set df_3h) as well as the exposure length.

The colour variable L* as well as the calculated variables “Rain (sum)” and “Rain rate (sum)” did not show correlations towards the measured amount of CFU and were therefore not considered for the PCA. Otherwise, the correlations indicate high importance of meteorological conditions, thus all of the weather variables were included in further data investigations. As the data set df_weekly showed more and stronger correlations between CFU and meteorological variables compared to the data set df_3h, the data set of weekly mean values were used for the following statistical analysis.

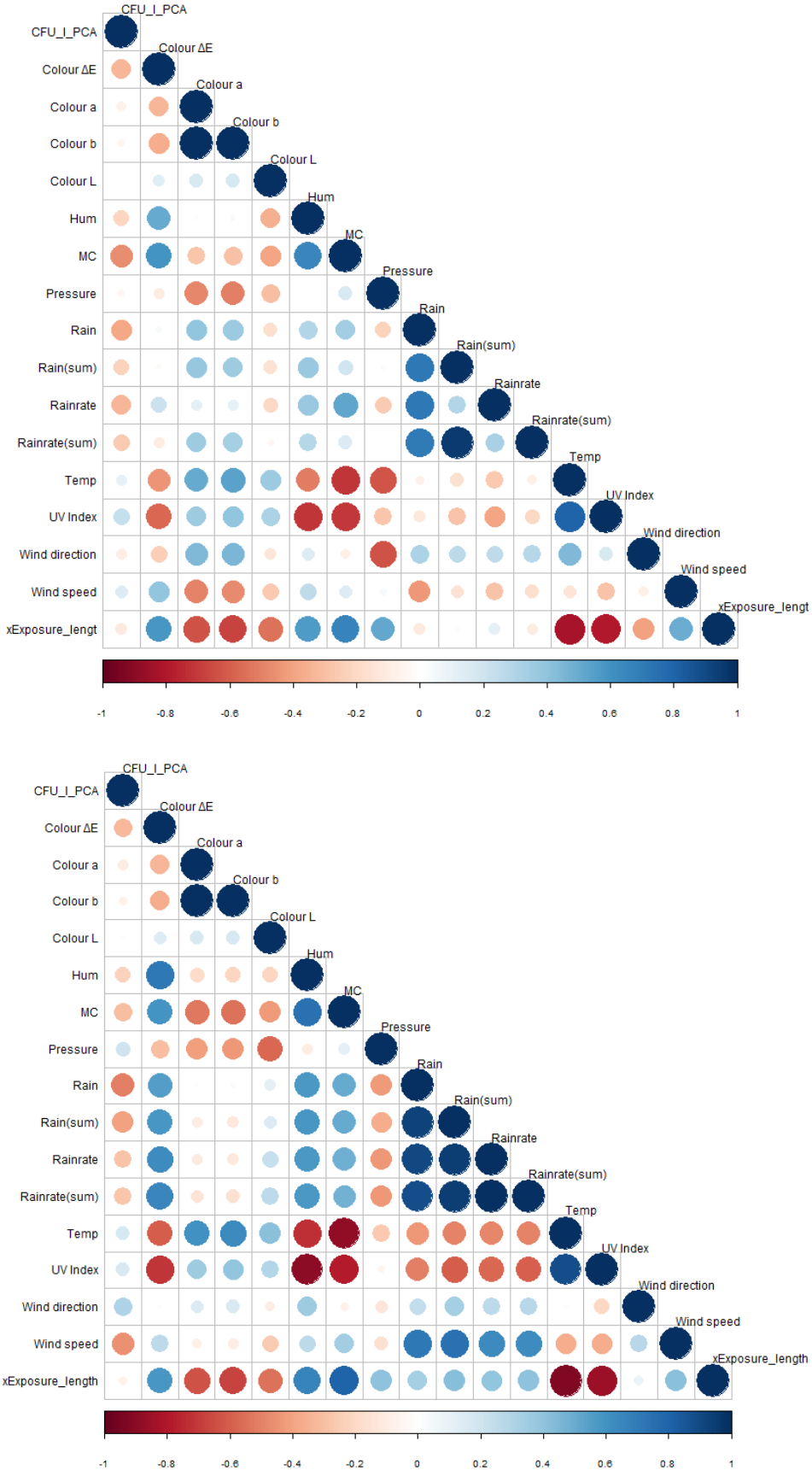


Figure 28: Heatmaps showing Pearson correlation of df_3h (top) and df_weekly (bottom) for Douglas fir. Legend: CFU_I_PCA: Number of CFU on PC-agar recovered from samples exposed from summer 2019 onwards, Hum: Air humidity, MC: Wood MC, Pressure: Atmospheric pressure, Temp: Temperature

Figure 29: Heatmaps showing Pearson correlation of df_3h (top) and df_weekly (bottom) for oak wood. Legend: CFU_I_PCA: Number of CFU on PC-agar recovered from samples exposed from summer 2019 onwards, Hum: Air humidity, MC: Wood MC, Pressure: Atmospheric pressure, Temp: Temperature

4.2.1.2 PCA

The inertia of the dimensions show if there are strong relationships between variables and suggests the number of dimensions that should be studied. The first two dimensions of the analysis express 61.4 % of the total dataset inertia; this indicates that 61.4% of the individuals cloud total variability is explained by the plane (see Figure 30). This percentage is high enough to represents the data variability only on the first 2 axis (Kassambara 2017), therefore only the first plane is investigated.

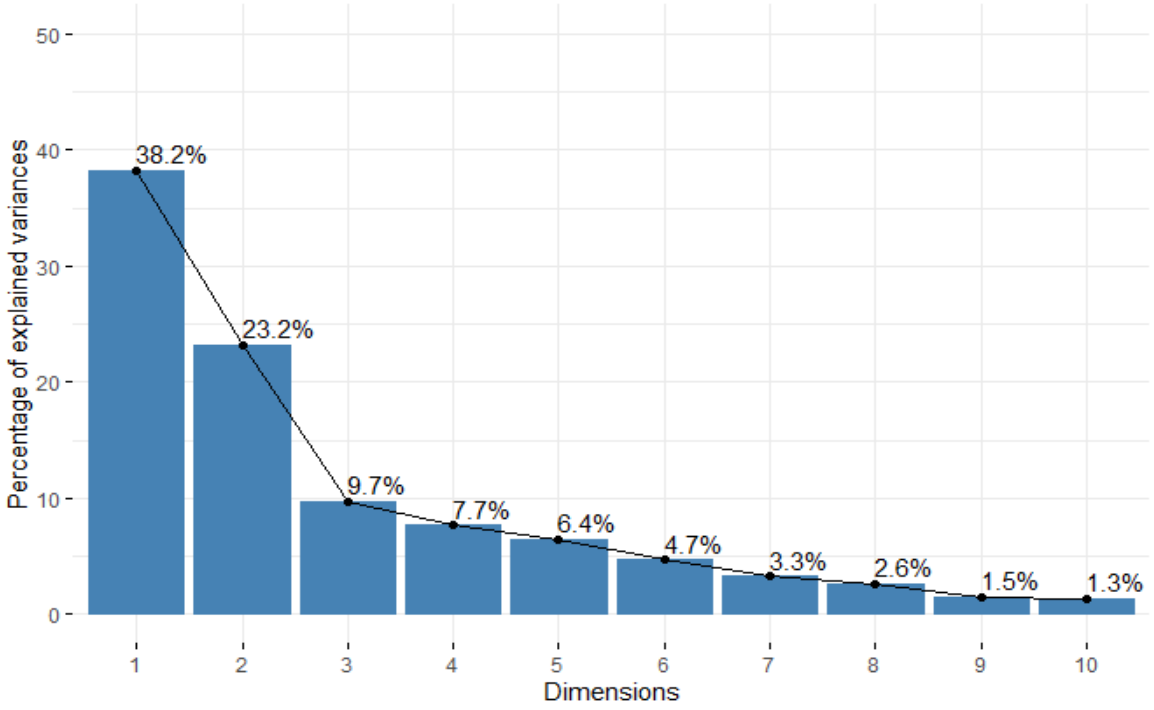


Figure 30: Scree plot of OWE data

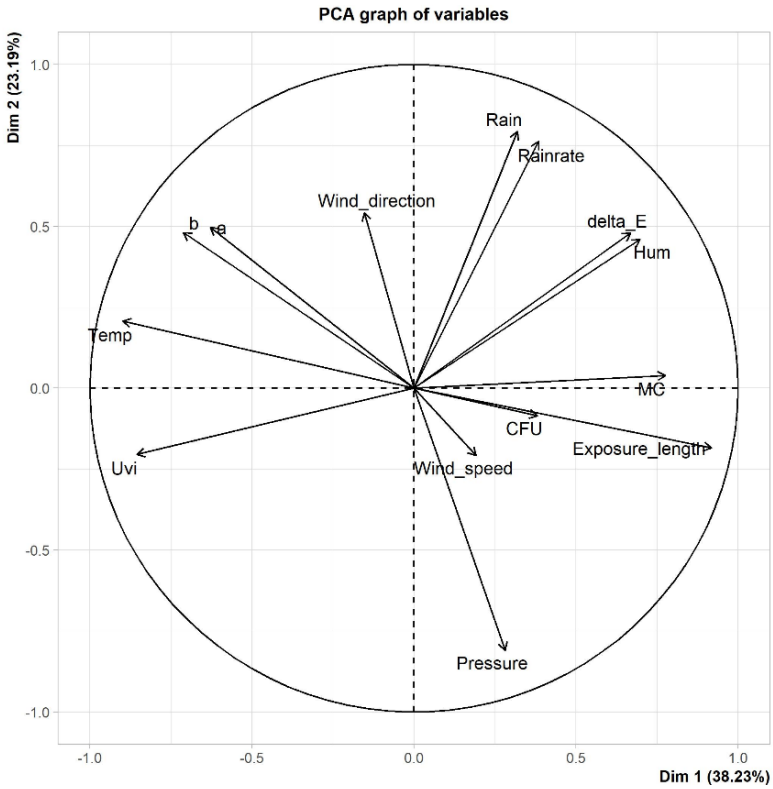


Figure 31: Variables factor map on OWE data. The labelled variables are those the best shown on the first two dimensions

Figure 31 shows the variables factor map on OWE data of the first two dimensions and groups individuals which are impacted by the same variables, the longer the vectors, the higher their influence. The 1st dimension shows two groups of individuals. The first group, in which individuals of both wood species were exposed the longest (132, 181 and 209 days), is sharing:

- high values for the variables MC, pressure, wind speed, CFU counts and air humidity
- low values for the variables b*, a*, temperature and UV Index

The second group, in which the individuals of both wood species were exposed only for 1 and 8 days, so the shortest, is sharing:

- high values for the variables temperature, UV Index, b* and a*
- low values for the variables MC, ΔE*, humidity, rain rate, rain and CFU counts

The 2nd dimension identifies a third group of individuals, including both species, having in common that they were tested with PC-agar. Furthermore their exposure times were 92 and 108 days. This group, with low significance as the inertia of the second dimension is quite low, shares:

- high values for the variables rain rate, rain, ΔE* and humidity
- low values for the variables atmospheric pressure and wind speed

4.2.1.3 Hierarchical cluster analysis

Following, abbreviations for the samples are used. The individuals ID is composed of the wood species (Q= Oak and P= Douglas), type of agar used for SCT (PC and ME) and length of exposure (in days).

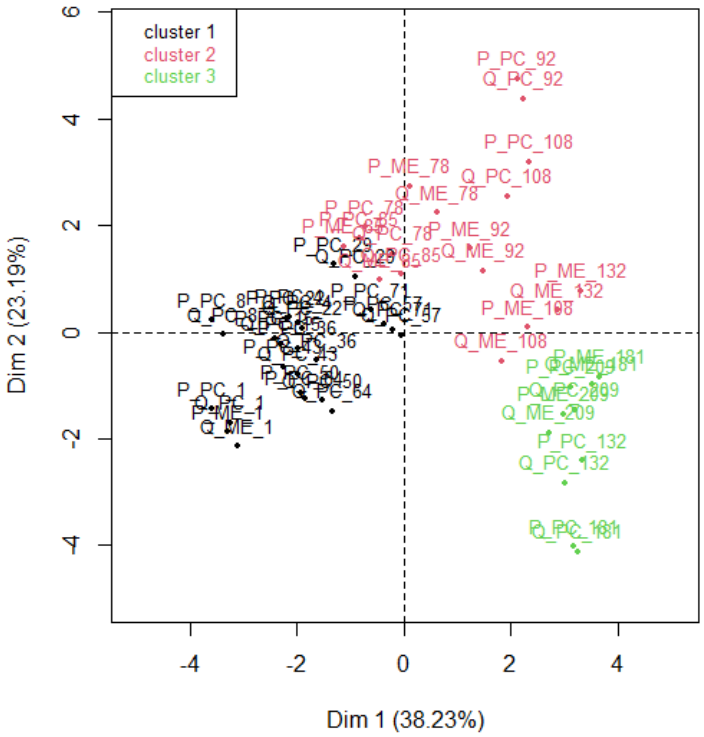


Figure 32: Ascending hierarchical classification of the individuals

Hierarchical classification showed very similar clustering of individuals by exposure lengths, as it was noticed with the PCA. As samples can be grouped according to their exposure length, as visualized in the hierarchical classification of the individuals in Figure 32, rather than by wood species or the agar-type used, meteorological conditions seem to influence the individuals the most. Moreover are these clusters evidence for the increasing change of samples properties with length of exposure, the type of wood species seems to be of less importance. Following, visualisation of the data was plotted to show the evolution of the samples over time.

4.2.2 Surface scans

Figure 33 shows an example of the visual changes of one specimen for each wood species. A summary of the scans performed on OWE specimens can be seen in the attached file **OWE_Scans.pdf**.

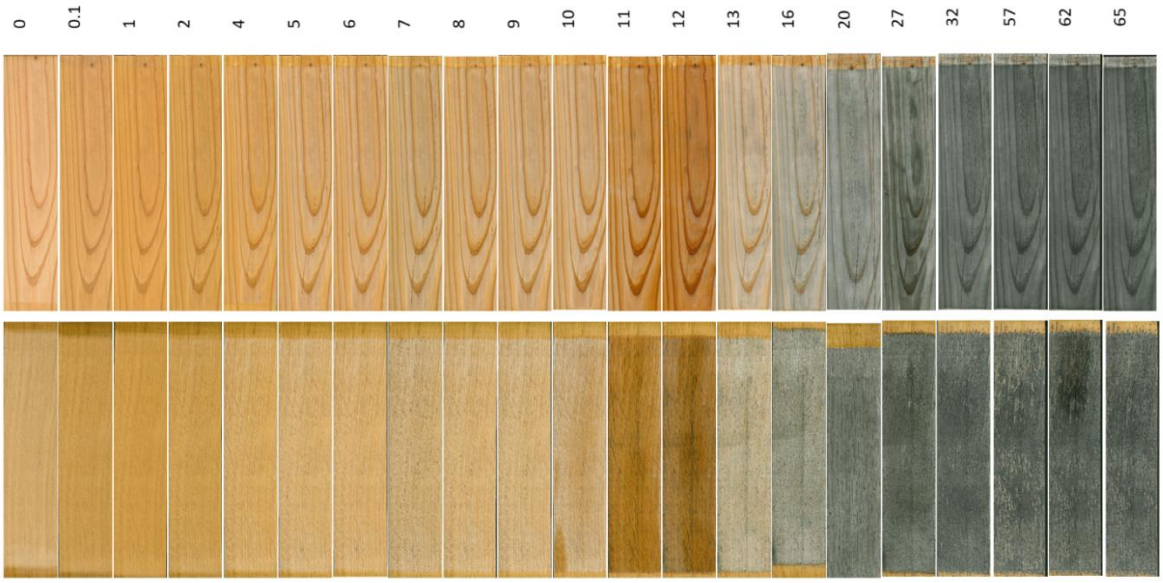


Figure 33: Scans of OWE Douglas fir (upper row) and oak (bottom row) wood after different exposure length [weeks]

A slight darkening and pronounced yellow tone of the samples can be observed after only 3 days of exposure and is attributed to photochemical breakdown (Sandoval-Torres et al. 2010; Žlahtič and Humar 2016; Volkmer et al. 2016). After 7 weeks samples start to grey, specimens exposed for 11 and 12 weeks show high contrasts and the following weeks the greying process is in full swing. The greying indicates specifically the break down and leaching of chemical bonds such as lignin (Feist 1989; Huang et al. 2012a). Furthermore, the darkening and increase in contrast of the samples during weeks 11 and 12, are likely to be related to a rise in MC (Baar et al. 2019), as during this period weather records showed rain.

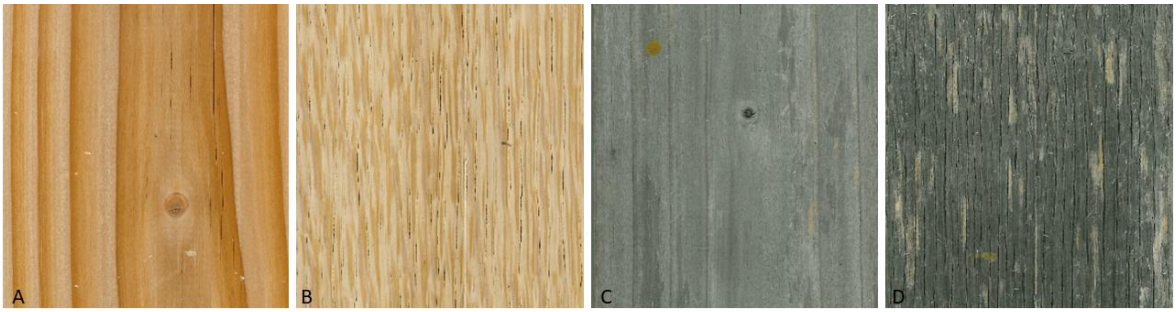


Figure 34: Scans of OWE Douglas fir (A, C) and oak wood (B, D), exposed for 7 weeks (A, B) and 12 months (C, D)

As shown in Figure 34, cracks that developed after only a few weeks of exposure are more pronounced in Douglas fir (A) compared to oak (B). Cracking of the samples is clearly visible, however, it becomes less apparent if MC rises due to wood swelling. Micro-cracks are formed during the drying of wood, moisture-induced stress and photochemical reactions lead consequently to the propagation of cracks (Sandberg 1999).

After about half a year, the wood samples started having fuzzy surfaces, degraded fibres detach from the surfaces. As shown in Figure 34, this phenomenon is especially visible in Douglas fir samples (C). Later, bigger bits detach from oak wood (D), resulting in patchy surfaces. Swelling and shrinking

leads to mechanical tensions in the wood boards and accelerate the detachment of fibres (besides environmental conditions such as UV irradiation, temperature and microbiological contamination). Once the fibres sticking off the surfaces are broken apart (e.g. accelerated mechanically through wind), un-degraded layers below come to the fore (D).

Darkening of the surfaces and staining patterns are visible from week 13 onwards and appear more irregular after about 4 months of exposure, which indicates degradation due to microorganisms in already advanced stages (Lie et al. 2019).

The specimens were scanned almost immediately after removing them from the exposure racks. Consequently, their MC varied throughout the experiment. MC is known to impact the visual aspect of wood surfaces, however the method was helpful to follow up visual changes with time on each specimen.

4.2.3 Wood moisture content & air humidity

Especially the wood moisture content (MC) measured with the Datalogger device failed to collect data during certain periods. Following, the raw data (non-interpolated and missing values were dropped) is presented. Daily mean values of MC for both wood species, as well as the air humidity (Hum) during OWE are shown in Figure 35.

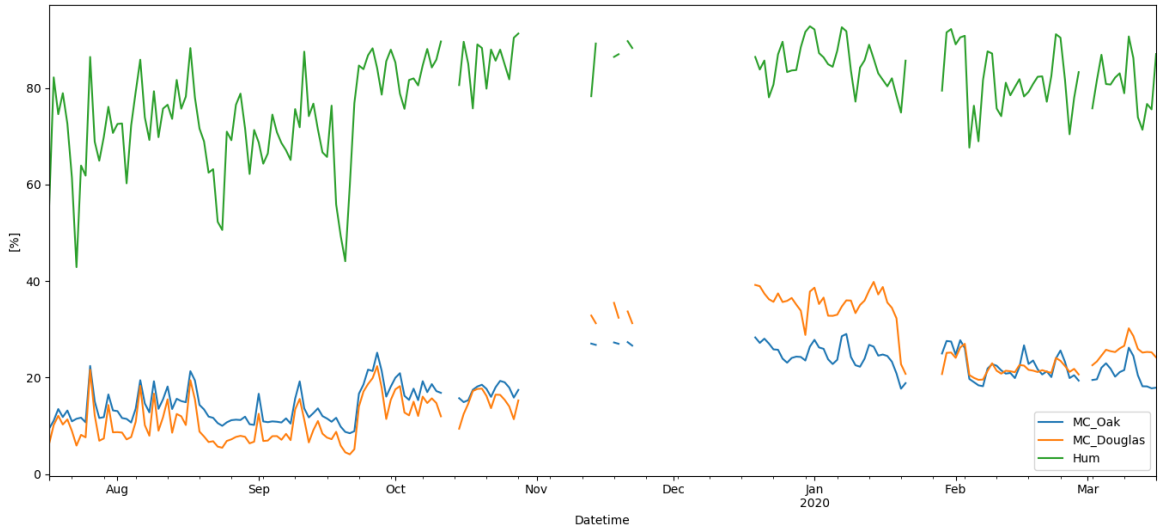


Figure 35: Wood moisture content and air humidity during OWE

The Pearson correlation coefficient of the measured Hum and MC of Douglas fir was established at 0.55 and at 0.58 for oak wood, showing a moderate positive correlation. This indicates that MC can partially be explained by Hum, however cannot be replaced by Hum only. It can be observed that the investigated softwood species is more sensitive to moisture changes. A standard deviation for Douglas fir´s MC was calculated and is with 10.18 almost the double compared to the standard deviation calculated for oak wood´s MC with 5.56.

Table 12: Mean, min and max values of the measured wood moisture content and air humidity during OWE

	MC_Oak	***MC_Douglas***	***Hum***
Mean	18.6	19.3	78.7
Min	8.5	4.1	42.9
Max	29.1	39.8	92.8

As shown in Table 12, mean MC was measured just below 20 % and the Hum reaches from 42-93 % whilst MC ranges from 4- 40 %. Therefore degradation processes during OWE are strongly increased (Volkmer et al. 2016) and the wood is said to be susceptible to microbiological attacks (Dirol and Deglise 2001).

4.2.4 CFU

A summary of pictures taken from the incubated petri dishes can be seen in the attached file **OWE_Petri_dishes.pdf**. Mean values (n=6) of ordinal CFU data were calculated. Figure 36 and Figure 37 show the CFU growing on PC-agar (indicating bacterial growth) and ME-agar (representing fungal contamination) recovered from both wood species, which were exposed from summer 2019 onwards (summer batch). In general, the CFU counts on ME-plates were higher compared to PC-agar. The amount of CFU on PC-plates does not provide any information whether there is more growth on Douglas fir or oak wood, a hint towards the importance of environmental conditions rather than nutrition source (wood). The higher fungal than bacterial contamination on OWE exposed samples may be a true reflection of microbial activity on wood surfaces, but it is not certain as the SCT method may preferentially recover fungal strains and spores. For example, microscopically, bacteria tend to be smaller compared to fungi, so they may be able to enter into the wood surfaces to deeper layers and are thus not recovered via pressing the agar-plates onto the specimens.

The same may be true for the differences in the observations between the two wood species. Higher CFU values of fungal strains were observed on oak compared to Douglas. Rather contrary findings were expected, since antimicrobial effects of oak were expected to reduce the CFU, thus these effects may only consider bacterial strains. Moreover, strains were thought to be able to enter into the big vessels in oak wood and thus cannot be recovered via the SCT. A higher CFU on oak may be explained by a rougher surface, where spores and strains can more easily attach to. Furthermore, moisture levels on wood surfaces, which varied throughout the measurements may have falsify recovery rates as well. With increasing MC, recovery rates tend to decrease, however these observations are not significant. Due to these doubts an experiment to verify the SCT method was carried out and will help clarify if the applied technique is suitable for further investigations. The experimental work to verify the SCT technique is shown in the following **Chapter 5**.

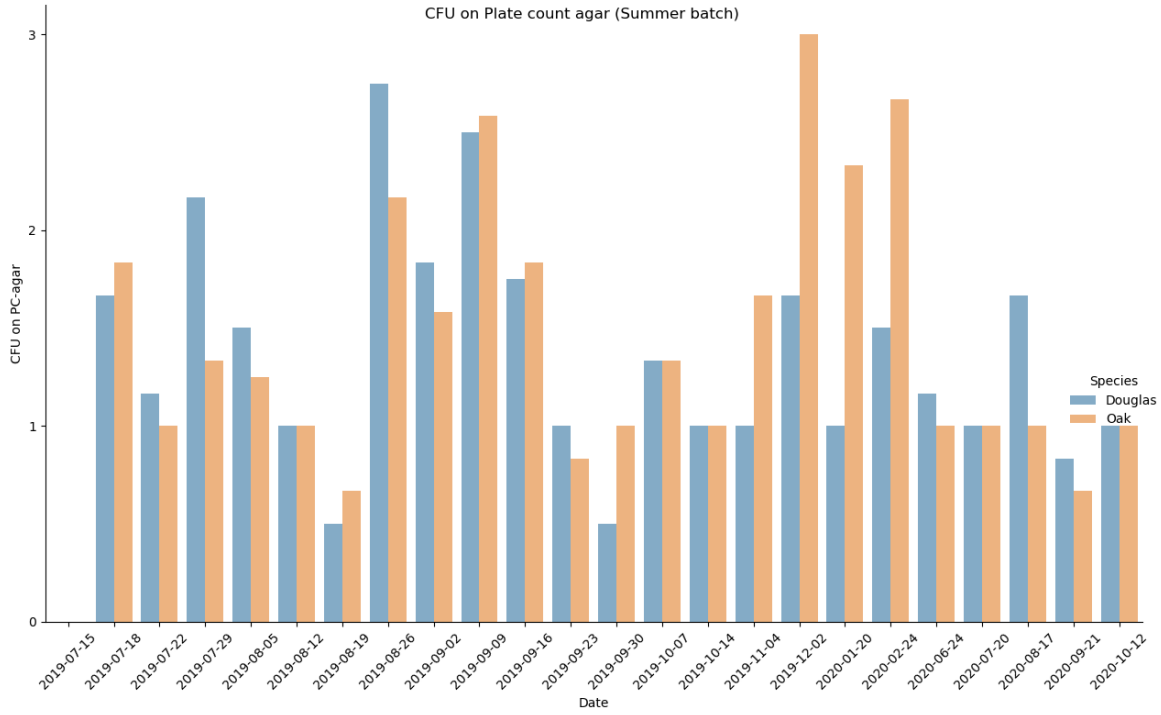


Figure 36: CFU on PC-ager recovered from OWE samples exposed from summer onwards

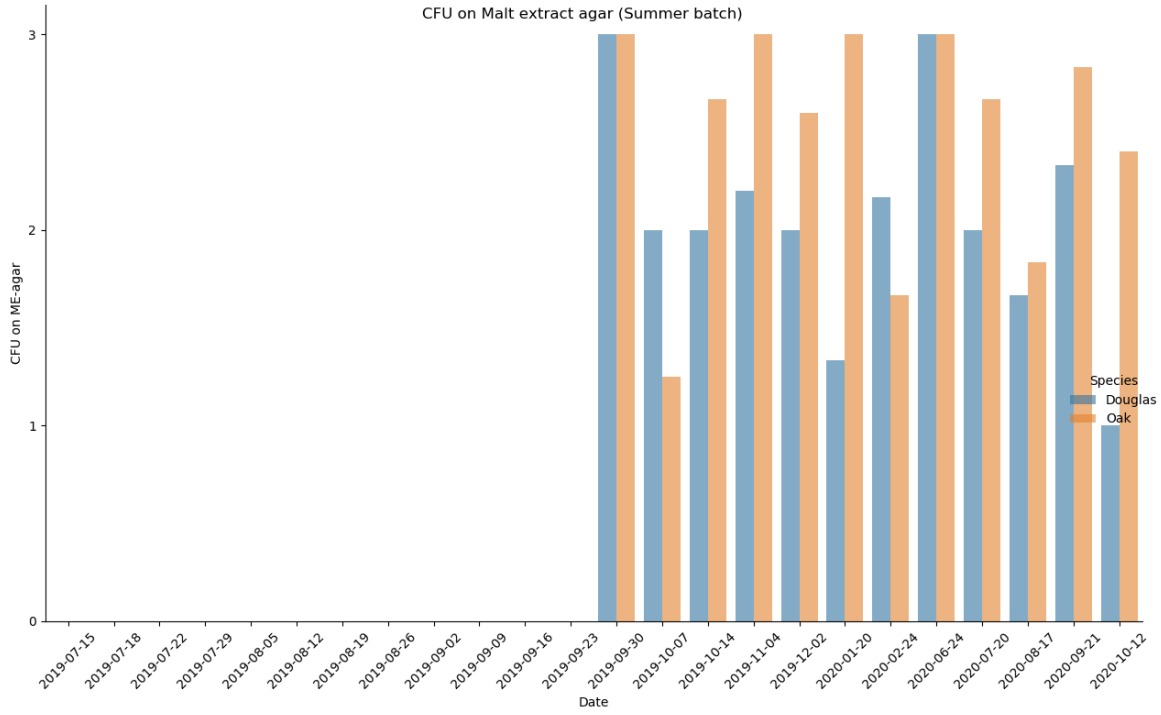


Figure 37: CFU on ME-ager recovered from OWE samples exposed from summer onwards

Comparing the amount of CFU from samples, where the exposure-start was in summer to those which were first exposed in "winter" (see Figure 38 for CFU on PC-agar and Figure 39 for ME-agar), showed in several cases higher values on samples that were exposed for longer periods, which might be explained e.g. by the fact that through increasing photodegradation of the wood, nutrition are more easily available to microorganisms, however no specific trends can be observed. The amount of CFU recovered from wood surfaces varies from measurement to measurement, most likely linked to weather conditions as well as cyclic reproduction of the microorganisms.

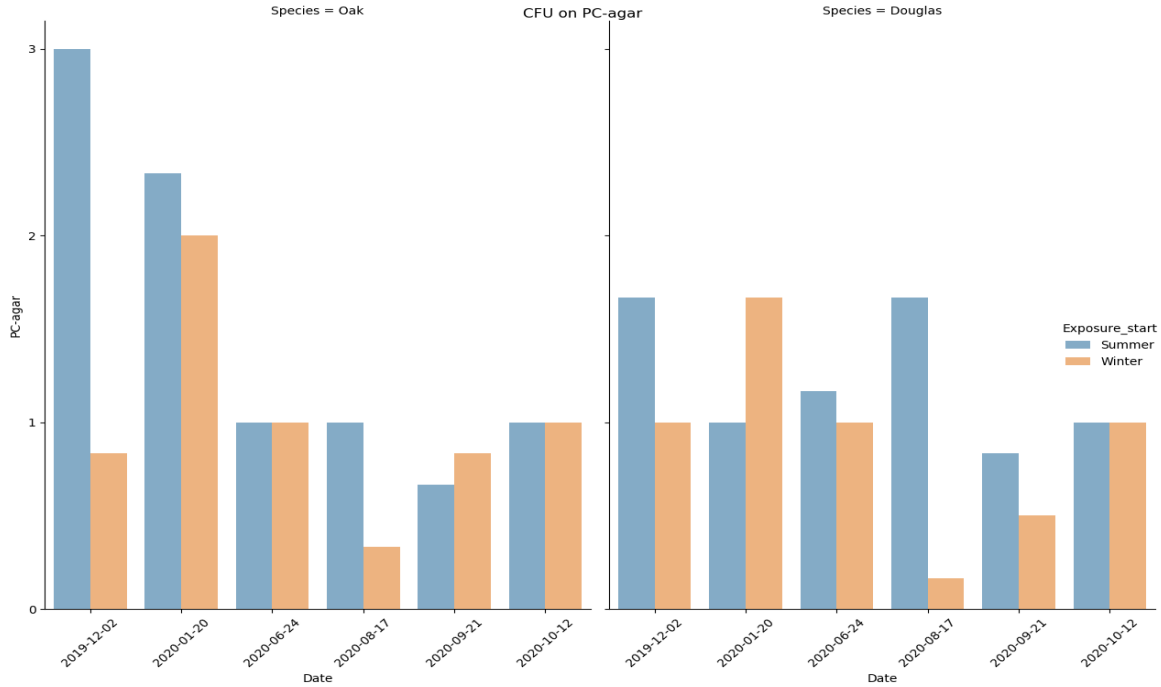


Figure 38: Comparison of CFU on PC- agar from samples exposed from summer onwards and later (winter batch)

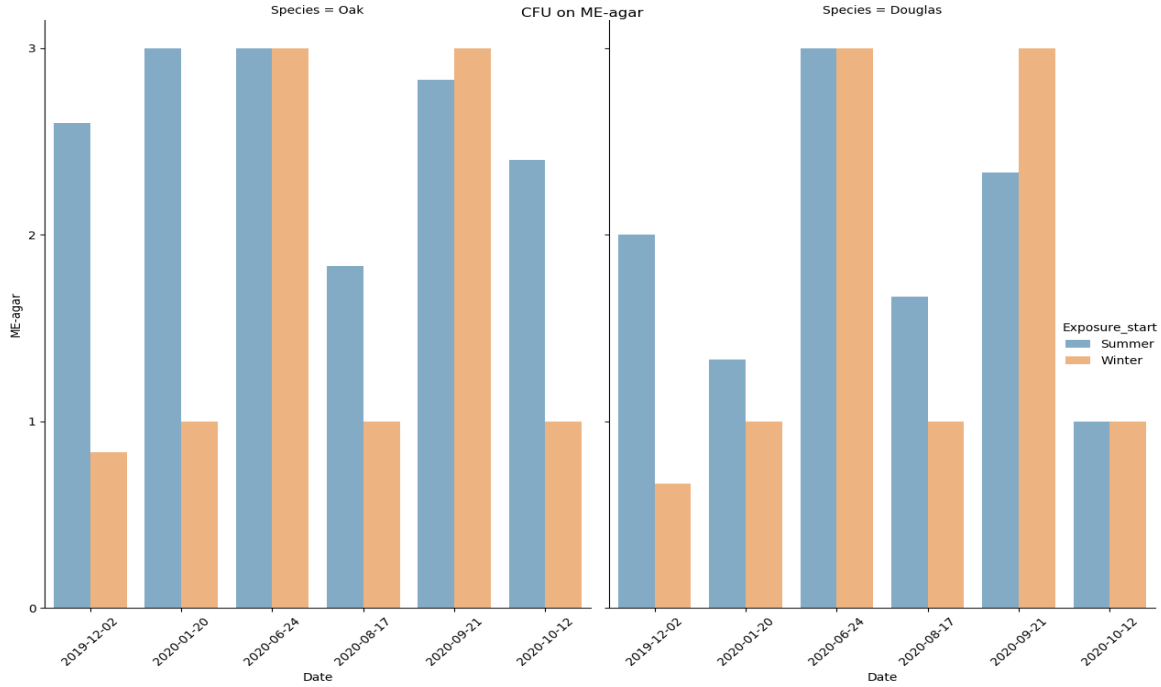


Figure 39: Comparison of CFU on ME- agar from samples exposed from summer onwards and later (winter batch)

A comparison of CFU on PC- and ME-agar plates at specific exposure lengths, rather than dates, of "summer" towards "winter" samples show again higher contamination of samples that started to be exposed in summer. Figure 40 shows the CFU on PC-agar. This observation leads to the assumption that during the summer month August meteorological conditions were favourable for microbial growth. Once certain microbial communities invade the surfaces, a good foundation; one may name it microflora; for further microbial invasion may be set and biofilms start to create. The reproduction of strains by cell division and sporulation is a continuous process if conditions are not too harsh. However, as the SCT is likely to be related to the MC on wood surfaces, a comparison between

"summer" and "winter" samples at same exposure lengths increases the bias as the SCT were not carried out at the same days and MC levels of the two sample batches may have been very different.

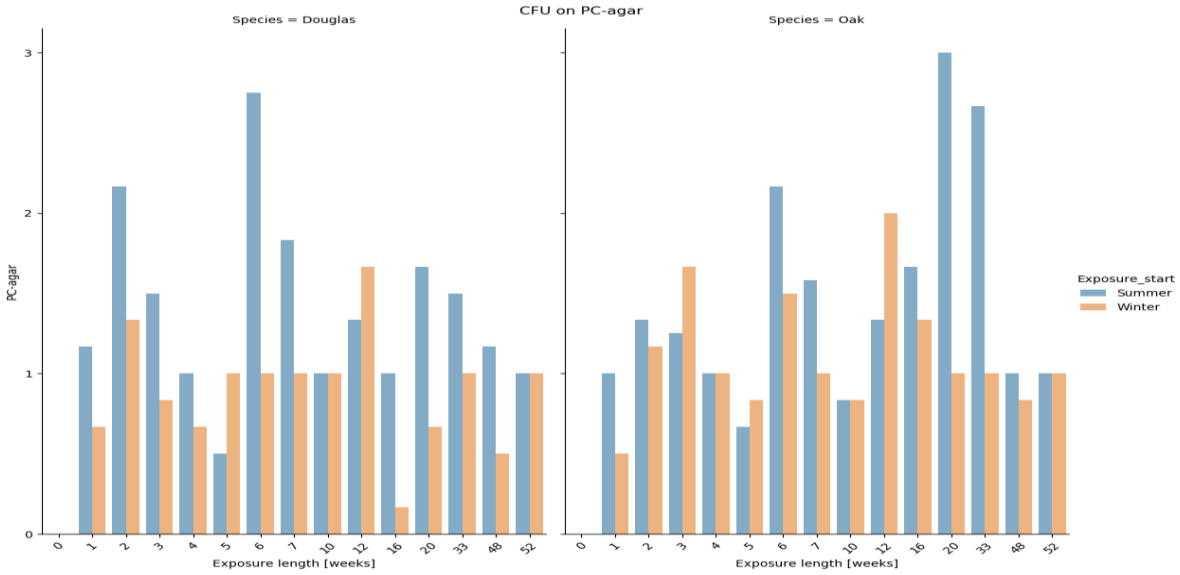


Figure 40: Comparison of CFU on PC-agar at specific exposure length from samples exposed at different times of the year

The enumerated CFU counts were used for linear regression analysis (LR). As shown in Figure 41, CFU on PC-agar decreases slightly and CFU on ME-agar increases with exposure time. This is true for both investigated wood species exposed from summer onwards as well as from winter. A possibility for this phenomenon is the availability of nutrition for the organisms which is rather augmenting with increasing exposure length as through photodegradation and other abiotic mechanisms low molecular weight products become more accessible. Especially for fungi this might enhance hyphae's ability to intrude into the wood cell walls as the cell structure loosens and cavities in between arise.

Another explanation are antagonistic effects of fungi towards bacteria which have been reported by many researchers in the past (Smith 1980; Gramss 1987; Gramss et al. 1999). A rapid decrease of the pH through decay fungi, as e.g. oxalic acids may be released, can be damaging to many bacteria (De Boer and Van der Wal 2007). Moreover the production of free radicals of fungi can be harmful to wood invading bacteria if their protection mechanisms such as anti-oxidative activity are not sufficient (De Boer and Van der Wal 2007). However, antagonistic effects in the reverse sense have been observed as well (Eriksson et al. 1990; Frey-Klett et al. 2011; O'Callahan et al. 2012). As the interactions between basidiomycetes and eubacteria seems to be strongly depending on the strains, knowing the microbial communities that are manifesting on the investigated surfaces seems indispensable.

Moreover, a strong correlation between exposure length and temperature (p= 0.83) was observed, which indicates a high importance of the prevailing temperature towards the growth of fungi and bacteria.

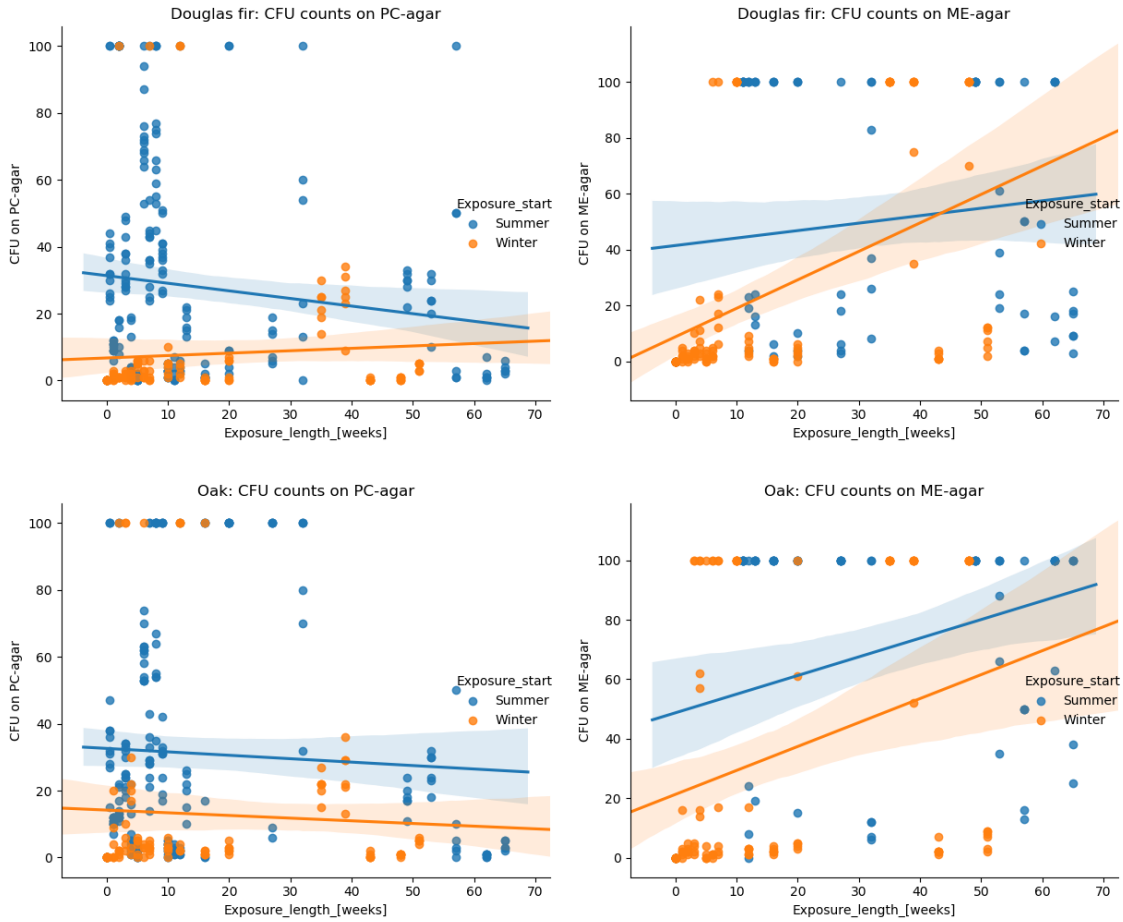


Figure 41: Linear regression of CFU on PC- (left) and ME-agar (right) from Douglas fir (top) and oak (bottom)

4.2.5 Colour

Following graphs show the evolution of the colour coordinates L*, a* and b* as well as the calculated colour change ΔE*. Each data point represents an average (n=120). The colorimetric data was interpreted by taking in consideration the scans as well, as shown in e.g. Figure 33 on page 64. In general, with exposure time, the L*, a* and b* values decrease, representing a darkening of the surfaces with increasing exposure to outdoor weathering (Kránitz et al. 2016; Lie et al. 2019).

The observation of the sample´s colour was not always done at the same MC, as specimens were re-exposed directly after evaluation, in order not to interrupt the weathering process. The specimens were dried, during rainy test days, until no liquid water was present on the surfaces, before colour coordinates were measured. Very remarkable peaks throughout the exposure and specifically until exposure week 20 were noticed. Those peaks may therefore be related to an inconsistent MC throughout the measurements as fluctuations in colour are known to be correlated to the MC of the wood samples as liquid water in the cell lumen causes mostly a darkening of the wood (Baar et al. 2019). Prevailing MC irregularities hindered an observation of correlations between colour coordinates and MC in this experiment, however a side experiment, shown in **Annex I**, confirms these correlations.

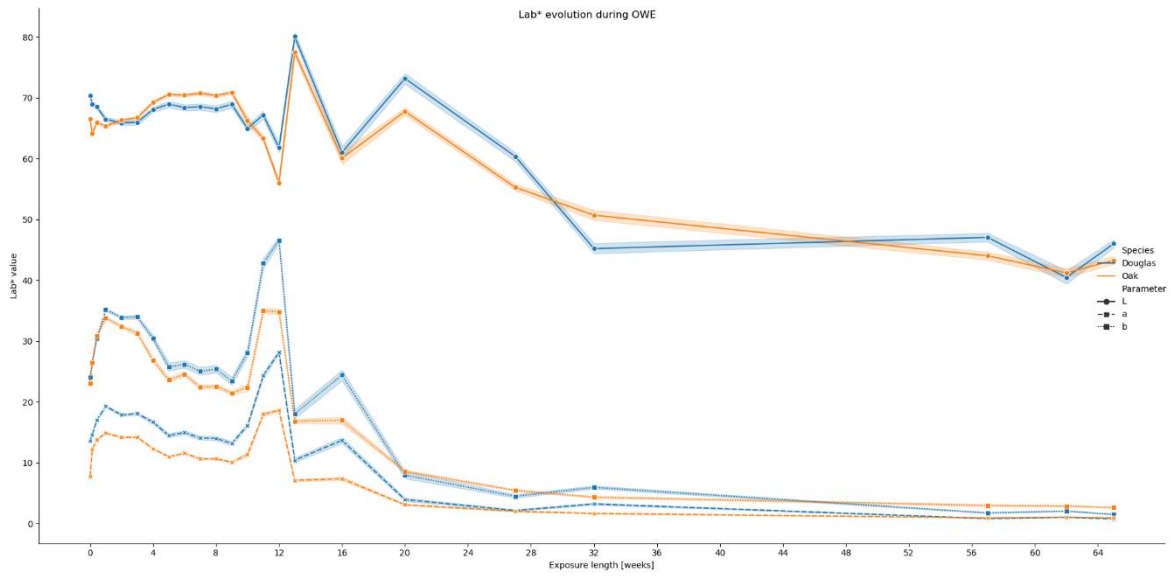


Figure 42: Evolution of the colour coordinates a and b* during OWE*

The a* and b* values are highly correlated, L* values basically show peaks when a* and b* show valleys and vice versa. The L* value representing the brightness of the samples, shows a peak after 5 weeks which may on the one hand be linked to inconsistencies of the surface MC and on the other hand may be explained by a pronounced leaching of lignin resulting in a bright cellulose rich surface layer. The evolution of the brightness parameter shows valleys after 12 weeks indicating a darkening of the surfaces, which is likely to be related to an elevated MC. The valleys after 16 weeks however seem to go hand in hand with a pronounced development of fungi and therefore darkening of the surface, as the scans in Figure 33 show dark spots appearing on the whole surfaces. Peaks after 13 and 20 weeks of exposure indicate very bright surfaces when samples were partially dried before observation.

Colour values, presented in Figure 42, of Douglas are basically higher compared to a*and b* values of oak. This indicates that the softwood species may be more susceptible to outdoor weathering compared to oak wood. Oak wood however shows more severe colour changes at the very beginning of exposure, likely due to the fact that higher amounts of extractives are present, therefore a greater range and quantity of substances, responsible for the colour of the material, are altered in a wide range of mechanisms, e.g. UV decomposition, hydrolysis, oxidation and free radical attack. The peak after 1 week may be explained by an irregularity in MC and therefore the appearance of the colour tones red and yellow become more pronounced. The valley after 9 weeks may indicate an already pronounced degradation of lignin and extractives, especially oak wood shows a more severe change. Peaks at week 12 and 16 may again be related to an increasing MC. After about 32 weeks of exposure, the rate of colour change is relatively low, indicating that the most susceptible chemical structures such as lignin and extractives have been modified and/or leached from the surfaces.

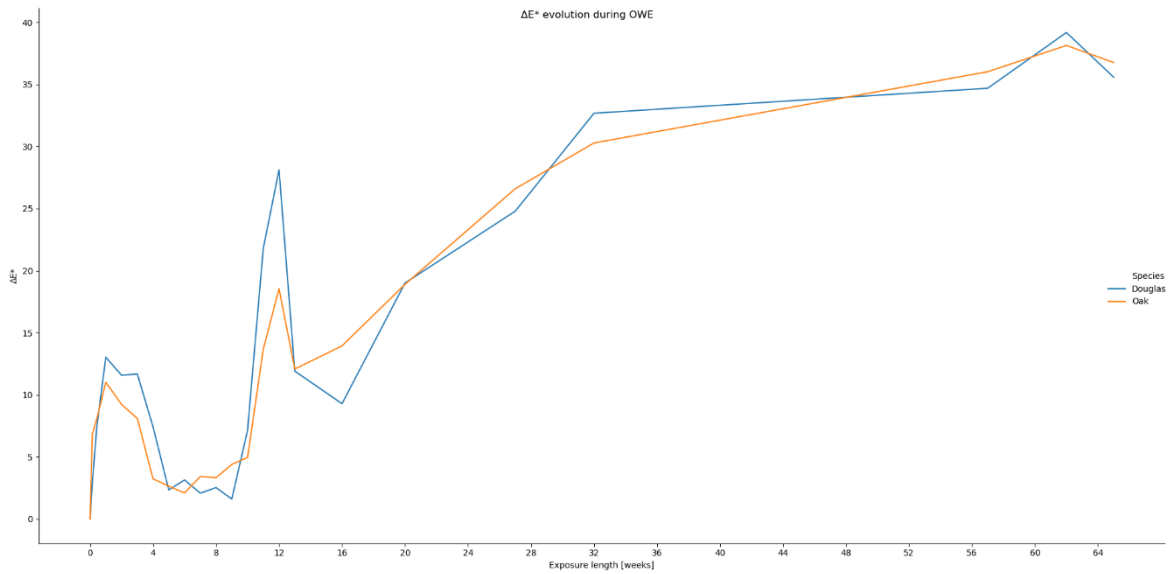


Figure 43: Evolution of the colour change ΔE during OWE*

Looking at the general colour change ΔE*, presented in Figure 43, a severe colour change can be noticed starting from about the 16th exposure week onwards and appears mainly due to darkening of the samples (L*) as well as decrease of yellow tones (b*), demonstrating that lignin is strongly degraded from that stage onwards and moreover the appearance of stain fungi. As shown with the scans in Figure 33 on page 64, the surfaces appear very grey from this moment onwards.

Especially Douglas fir response, in a long term, to a higher extent to changes in MC. Moisture dynamics in Douglas fir are more important compared to oak wood, likely due to a less dense wood structure of the softwood specie. There is no linear evolution of the colour with time, especially at shorter exposure lengths. High correlation with weather conditions, especially MC, rain and rain rate were found.

As the colour parameters indicate, eventually both wood species have a similar colour and therefore appearance. To the naked eye, from a distance, it becomes more and more difficult, the longer the samples are exposed, to differentiate between those two wood species. Nevertheless, the exposure conditions in this standardised weathering experiment do not quite correspond to conditions where wood is used for cladding. As shown in Chapter 1.1, various parameters, such as shadings through balconies, distance to ground, etc. will result in dramatic differences in the appearance of the wooden facades and especially impact the homogeneity of the surfaces.

The boxplots in Figure 44 and Figure 45 reveal that with increasing exposure length the colour change data becomes more dispersed. As demonstrated with the scans, degraded fibres on the wood surfaces detach from the surface exposing the relatively sound wood underneath. This process enhances the inhomogeneity of the wood and may justify the increasing dispersion of the data with time. Comparing the two wood species with each other, Douglas fir shows higher dispersion in general, whilst the lower density of this softwood species as well as a more severe distinction between early- and latewood areas may explain this observation.

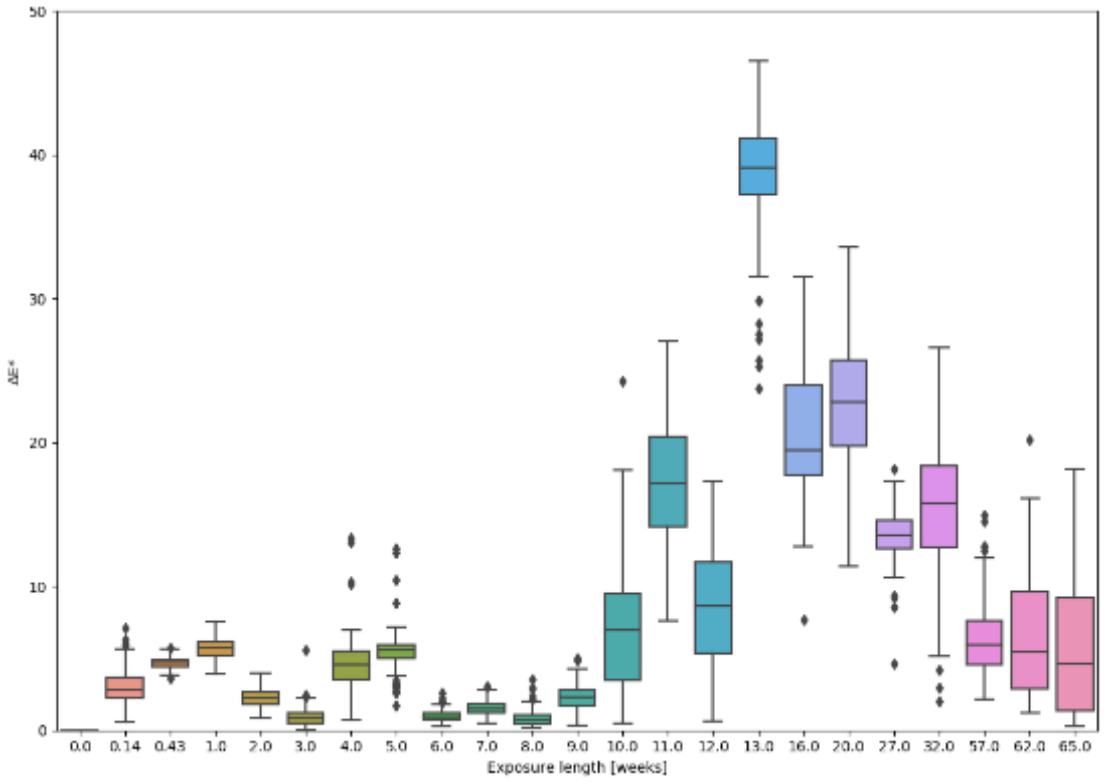


Figure 44: Boxplots of ΔE values with time for Douglas fir*

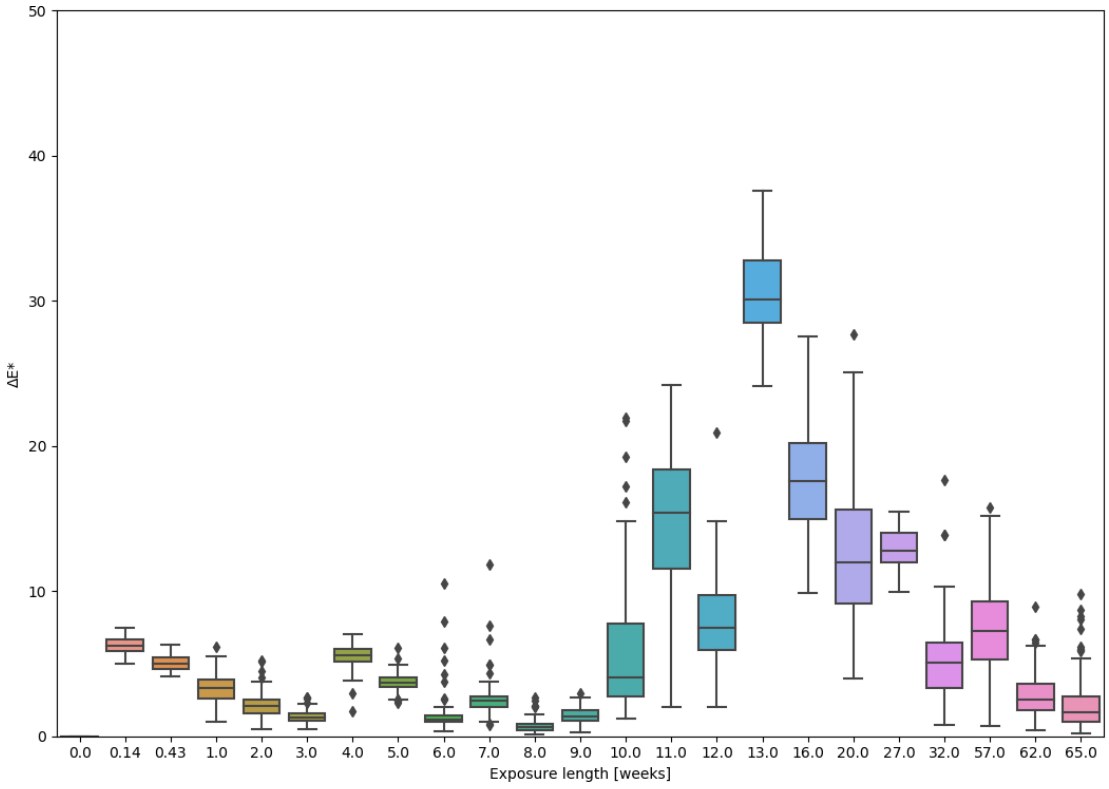


Figure 45: Boxplots of ΔE values with time for oak wood*

4.3 Summary and outlook of OWE

Fast colour change (only few days) was observed in both wood species, thus the chemical degradation process seem to be already in motion in very early stages of outdoor exposure.

The darkening of the samples which enhances with increasing exposure length is furthermore correlated to high MC. Another factor for the increasing dark colour of the samples is related to the growth of moulds.

Colour coordinates a* and b* can be correlated to the CFU counts on oak. This is possibly linked to the appearance of moulds on the surfaces. This finding leads furthermore to the assumption that bacteria may as well contribute to the chemical degradation process of oak.

Cracking is visible, on a macroscopic level, after only view weeks of weathering.

Degraded fibres sticking off the surfaces (appearing after about 6 months of natural exposure) are broken apart and wood surfaces appear fussy. Layers below come to the fore which may encounter problems for further measurements as rather un-weathered layers underneath are thereafter observed.

More fungal than bacterial strains were recovered from naturally exposed wood samples. This observation is a hint towards antagonistic effects of wood degrading fungi towards environmental bacteria.

With increasing time of exposure, bacterial contamination (quantitative) tends to decrease, and fungal invasion increases. This observation leads again to the assumption that antagonistic interactions between fungi towards bacteria are present on wood surfaces in natural environments.

Observations of the CFU counts lead to the assumption that the conditions prevailing at the start of exposure are decisive for the microflora and thus biotic degradation of wood.

Comparing the two wood species with each other, oak wood showed higher fungal contamination compared to Douglas fir. However, the method applied to enumerate the CFU may have falsified these findings. The following **Chapter 5** shows further investigation of this recovery technique.

In any case, many bacterial and especially fungal colonies were recovered from outdoor exposed wood surfaces. It remains unclear at this stage whether spores just happen to land on the surfaces or if these organisms affect the wooden matrix or interact with each other.

The PCA indicates that the meteorological conditions, such as temperature, air humidity, wind speed, atmospheric pressure and UV irradiation are the most decisive for bacterial and fungal growth on wood surfaces. This experiment did not allow to state statistically significant conclusions about the importance of microorganisms as too many interfering meteorological parameters prevail. Due to these reasons the experiment presented in **Chapter 6** , where abiotic degradation factors are controlled, was performed.

5. Chapter: SCT- Method verification

Airborne bacteria are ubiquitous, in outdoor environments their concentration may be affected by factors such as weather (precipitation, temperature, …), location, date and time as well as atmospheric composition due to e.g. urban pollution (Fang et al. 2007; Smets et al. 2016). Bacteria happen to accumulate on particles, which can be found in clouds and consequently be transported by wind and rain (Kaushik and Balasubramanian 2012). These particles can accumulate on wood surfaces and, as a result, potentially influence the weathering of the surface.

The range of microbes on a wooden façade will be dependent on the range of microbial species present in the locality. Clearly, spores might travel a long distance before settling on a surface, but the probability of their presence on a given surface is likely to diminish with distance between the organism that produces the spore and the wood piece in question. In addition, the potential for the spore to germinate and colonise the wood will be dependent on the compatibility of the spore to the surface and the prevailing meteorological conditions. A survey of the range of microbes on a wood surface is potentially useful in understanding wood weathering.

There are many different microbial recovery methods, however, to our knowledge, there is no standardized method for wooden surfaces. For wooden surfaces, literature describes multiple possible methods including, blotting/surface contact, grinding, scratching, swabbing and elusion methods. The bacterial transfer rate of SCT method is lower compared to some other methods such as grinding or stomaching, likely because the SCT is sampling only the immediate wood surfaces (Ismaïl et al. 2015). However, the SCT method is a non-destructive method and samples can be directly re-exposed after testing, which was of big importance during the OWE. In addition, studies have shown that the surface MC supports the microbial survival (Koch et al. 2002; Schönwälder et al. 2002; Milling et al. 2005), thus enhances the recovery rate by different methods. Likewise, the amount of CFU found on outdoor exposed wood samples were expected to be higher during rainy seasons compared to dry periods. The findings in Chapter 4, however, tend to show reverse results. Therefore, the aim of this investigation was to study the influence of surface wetness on the microbial recovery via SCT from Douglas fir compared to a non-porous material.

5.1 Material and methods

The experiment was executed under a laminar flow bench in order to help ensure sterile conditions.

5.1.1 Specimens

Sterile Douglas fir and poly methyl methacrylate (PMMA) samples with a surface of 25 cm^2 were investigated. Tangential wood surfaces were tested. Douglas fir samples were gamma sterilized and the PMMA surfaces were disinfected with 70 % ethanol. All samples were stored for at least 24 h in

the LFB before testing in order to attain constant moisture contents. PC-agar was used as the transfer and incubation medium.

5.1.2 Experiment plan

Table 13 shows the different treatments applied to the specimens prior or after inoculation. 3 replicates of each sample were examined as follows:

- Dry samples are inoculated, SCT applied immediately after inoculation
- Dry samples are inoculated, SCT is applied after drying
- Wet samples are inoculated, SCT applied immediately after inoculation
- Wet samples are inoculated, SCT is applied after drying

Table 13: Experimental plan of SCT verification test

Material	Wetting	Inoculation	Drying	Sample ID
Solution	Solution plated directly on dishes and spread homogeneously			Solution
Douglas	no	yes	no	Douglas
Douglas	no	yes	yes	Douglas + drying
Douglas	yes	yes	no	Douglas wet
Douglas	yes	yes	yes	Douglas wet + drying
PMMA	no	yes	no	PMMA
PMMA	no	yes	yes	PMMA + drying
PMMA	yes	yes	no	PMMA wet
PMMA	yes	yes	yes	PMMA wet + drying

5.1.3 Wetting and drying procedures

Samples were submerged for 24 h in a sterile, deionised water filled petri dishes for wetting. In order to dry the surfaces, inoculated samples were left in the LFB for 1 h, the time to let water droplets evaporate.

5.1.4 Bacterial strain and dilution of the solution

Staphylococcus aureus (SA) was used as the inoculum strain. One to two colonies of SA were removed from a 24 h old petri dish with an inoculation loop and stirred into distilled sterilized H_2O. This original inoculum was adjusted to a McFarland of 0.5. Following the stock solution was mixed for 10 min with the help of ultra-sonication at 20 kHz at a temperature of 30 °C. For each dilution step 1 ml of the solution was transferred to a 9 ml H_2O tube. Prior to dilution steps, the solution was vortexed for 30 sec. Bacterial solutions diluted $1:1*10^5$ and $1:1*10^6$ were used, to ensure countable CFU between 30 and 300 on the petri dishes.

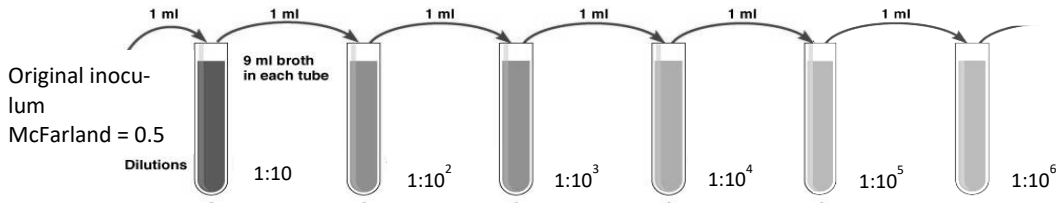


Figure 46: Dilution steps of the Staphylococcus aureus inoculum

5.1.5 Inoculation

The concentration of the inoculum plated directly on the dishes had a 10 x lower concentration compared to the inoculum used on the surfaces. This enabled CFU values to be in a countable range on all dishes. To determine the amount of cells/ml in the used inoculum, 200 µl of the solution were plated directly on the petri dishes and spread with a sterile drigalski spatula. The Douglas and PMMA surfaces were inoculated with 200 µl of the solution by spreading the inoculum in small droplets across the whole surfaces as shown in Figure 47.

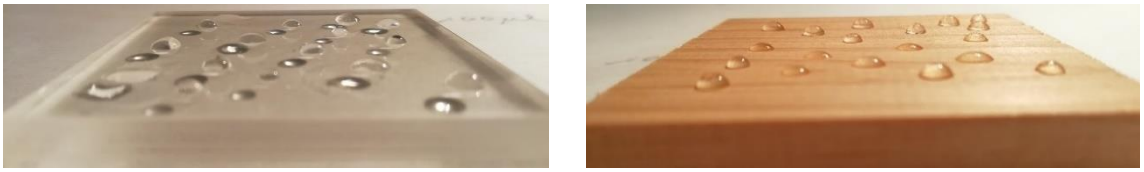


Figure 47: Dispersion of bacterial suspension on PMMA and Pseudotsuga menziesii surfaces

5.1.6 SCT and CFU enumeration

To conduct the SCT, the petri dishes were pressed firmly with three fingers onto the specimen surfaces for 5 s. After 24 h incubation of the dishes at 36 °C, the CFU was counted manually from photographs taken of each dish.

5.2 Results

Figure 48 shows pictures of the dishes after incubation. A few of the dishes show a dense accumulation of colonies, the CFU could not be accurately determined for such dishes. The mean value of 3 replicates was calculated, if possible (3 Na values in the data set). The number of CFU on the solution dishes was counted and the recovery rate (RR) of this value was assumed to be 100 %. Following the RR of the SCT was determined.

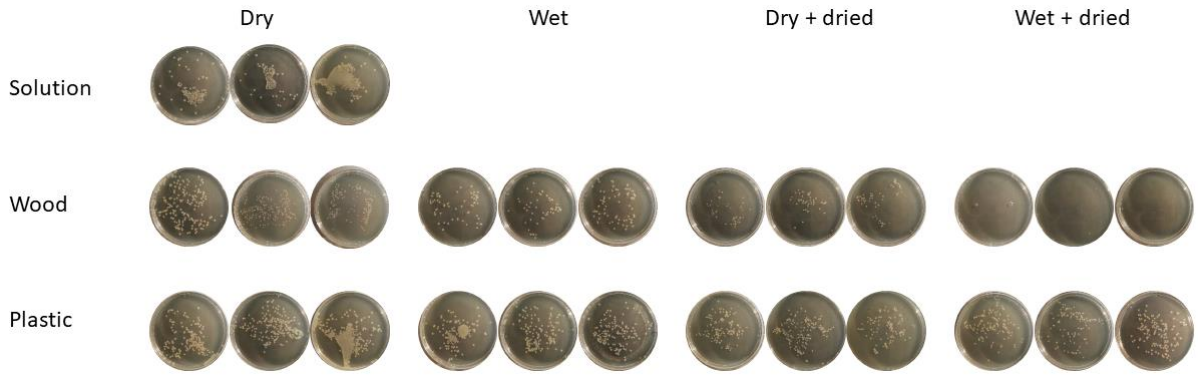


Figure 48: SCT verification experiment- Pictures of the petri dishes after plating, spreading, SCT and inoculation

In **Fehler! Ungültiger Eigenverweis auf Textmarke.** the number of CFU and the calculated RR are shown. CFU values show that the standard deviations (SD) of replicates are low. The highest SD was found in the solution dishes, followed by the plastic sample which has been dried before SCT.

Table 14: Number of CFU and RR of SCT verification experiment

Application of 200 µl inoculum	Sample ID	CFU values (1:10^5)	CFU (Mean value)	RR [%]
Solution diluted 1:10^6 plated (x10 was calculated directly)	Solution	2300	2570	100
		2840		
		na		
Solution diluted 1:10^5 and applied on surfaces before SCT	Douglas	228	217.3	8.5
		213		
		211		
	Douglas + drying	60	53.3	2.1
		42		
		58		
	Douglas wet	65	65.7	2.6
		55		
		77		
	Douglas wet + drying	0	1.0	0.0
		2		
		1		
	PMMA	217	231.0	9.0
		245		
		na		
	PMMA + drying	173	154.7	6.0
		177		
		114		
	PMMA wet	na	169.5	6.6
		174		
		165		
	PMMA wet + drying	147	141.3	5.5
		124		
		153		

In general, from PMMA surfaces more colonies were recovered compared to Douglas fir specimens. Both, wood and plastic samples show the same trend for all different testing procedures. The highest recovery was achieved from dry samples followed by wetted samples that were not dried after inoculation. Dry samples which have been dried after inoculation show lower CFU but still higher than the wet samples that have been dried before SCT. The RR values from PMMA were always observed to be higher than from the equivalent Douglas fir samples.

5.3 Discussion

The repeatability and reproducibility of the SCT is low compared to other microbiological methods (Salo et al. 2000). Even repeating the tests multiple times, only limited data sets could be established within this work.

5.3.1 Douglas vs PMMA

The RR from both materials seem quite low. One explanation for this is that bacteria are able to adhere to surfaces (Salo et al. 2000) which is even more pronounced on porous materials (Tomičić et al. 2017). Another is that the agar is water soluble and so a wet surface might influence the "tack" of the agar.

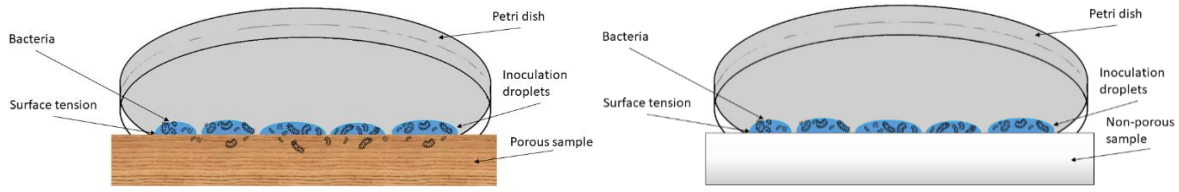


Figure 49: Scheme of inoculated wood and plastic surfaces

A 0.5 % lower CFU on wood compared to PMMA can, possibly, be explained by the greater porosity of wood. The PMMA served as a non-porous reference material. With a density of between 1.12 and 1.18 g/cm3 (Domininghaus et al. 2008) PMMA is twice as dense compared to Douglas fir wood (Cirad 2012). Bacteria might be able to penetrate into the wooden cells, but not into the PMMA. Other research has shown that the wood surface type (cross or longitudinal section) can impact the bacterial flow into the wood matrix as well. Cross sections tend to show lower RR compared to longitudinal cuts (Prechter et al. 2002; Maneewan 2019). Not only porosity but also anatomical structure of the different wood species is likely to affect the RR.

5.3.2 Wetted surfaces

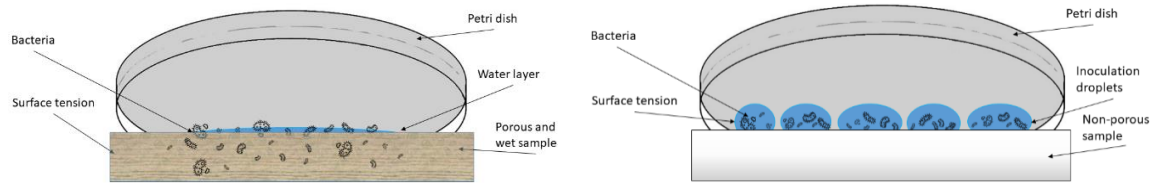


Figure 50: Scheme of inoculated wet wood and plastic samples

It was noticed when inoculating the wet Douglas samples, that the droplets dispersed immediately into the water already on the wood surface. This was not the case PMMA samples. The water uptake of PMMA is with 30 mg/2 inch discs after 24 h (Domininghaus et al. 2008) very low compared to hygroscopic wood. Even though plastic and Douglas fir were stored in the same conditions, the water on the surface started running off after taking the samples out of the water storage. Less water was

present in the non-porous samples. Therefore a significantly higher CFU on wet PMMA compared to wet Douglas can be explained. Through more water the suspension is diluted to a higher extent. Another contributing factor is the potential for the water to flush the strains in to the wood cell structure thus rendering them inaccessible to the agar plate. A lower RR from wood surfaces with higher humidity contents was also observed by Coughenour et al. (2011).

As in the dry samples, the water on top of the moist surfaces might create surface tension so that cells cannot be easily transferred to the agar plates. This effect might be reinforced on the moisture saturated wood samples. The slightly higher CFU in dry PMMA compared to wet plastic might be explained by the fact that a water film on the wet surfaces is thicker and prevent agar to "touch" the bacteria or the "tack" of the agar is reduced again. Alternatively, the difference may be due to chance as the differences observed are not statistically significant at the 5% level.

5.3.3 Surfaces dried before SCT

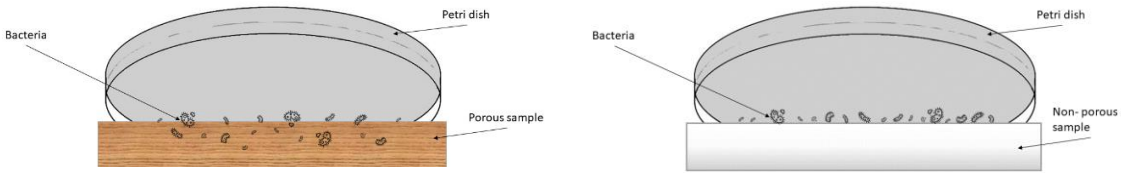


Figure 51: Scheme of inoculated wood and plastic samples dried before SCT

SA might survive longer on drying plastic compared to wood, which has been confirmed by a study using Methicillin-Resistant SA (Coughenour et al. 2011). SA has the ability to survive up to 90 days in dry conditions (Lidwell and Lowbury 1950; Rountree 1963; Hirai 1991), thus the fact of drying the SA inoculum for 1 h on the surfaces might not impact the survival of bacteria. It was reported, however, that evaporation leads to desiccation and reduces cell viability especially on non-porous surfaces (Kusumaningrum 2003; De Cesare et al. 2003; Moore et al. 2003).

Furthermore bacterial cells are reported to adhere to wooden surfaces (Bonfante and Anca 2009; Soumya et al. 2011; Tomičić et al. 2017), which as well can explain a higher RR on PMMA compared to Douglas fir.

A significantly lower CFU on dried wood compared to dried PMMA might be related to antimicrobial properties of wood (Schönwälder et al. 2002; Hedge 2015) as well as to the dispersion of bacteria into the cells. A higher contact angle on dry samples might allow the inoculum to spread and penetrate less into the material compared to when wet.

Moreover the optimum pH- values of 6.5- 7.2 for bacterial growth (Achinas et al. 2019), whereas the acid pH-value of Douglas fir with 3.4 - 4.4 (Rückert 1986) might reduce microbial growth.

5.3.4 Wetted surfaces dried before SCT

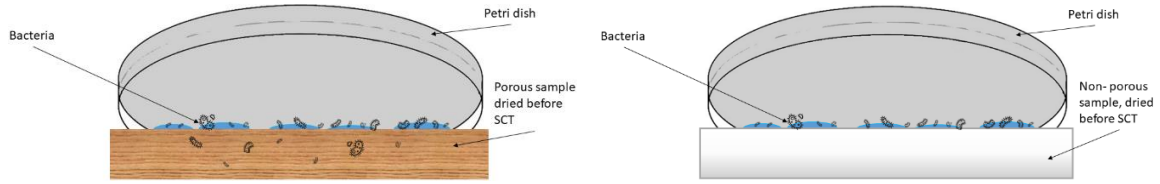


Figure 52: Scheme of inoculated wet wood and plastic samples dried before SCT

Almost no bacteria were recovered from wood specimens that were initially wetted and dried before SCT was performed. The saturated wood surface gives ample opportunity for the bacteria to be flushed or diffuse into the cell structure. This is probably amplified by the wetting that cases the water front to recede into the cell structure, taking the bacteria with it. There is a much lower possibility that such a mechanism can play a role for the PMMA specimens. Otherwise, the formation of biofilms on liquid surfaces is reported to form a distinct macroscopic floating infrastructure different to biofilms on solid matrixes (Magana et al. 2018) may play a role as well. It is not clear if a biofilm can be formed within 1 h, however the bacterial matrix enclosed in a water layer which was slowly evaporating, might have led to a biofilm with greater adhesion to porous surfaces.

5.4 Summary

In this experiment, on Douglas fir a lower RR was found compared to PMMA. The same phenomenon was detected for other wood species (Ak et al. 1994; Schönwälder et al. 2002; Milling et al. 2005; Khalid et al. 2020), this experiment confirms the same observations for Douglas fir as well.

These findings show that microbes are not easily transferred from wood surfaces, which in turn, implies that there is potential for wood to be used in hygienically sensitive areas such as hospitals, food processing and packaging industry. At the same time this experiment is a good initiative to investigate different recovery techniques from wood surfaces to a deeper extend with the aim of applying and standardising methods used for e.g. claddings in order to study wood degradation caused by biotic factors.

The RR depends on humidity content of surfaces to be tested. Possible explanations are:

- Water surface tension
- Water film preventing the contact between agar and bacteria
- Bacterial adhesion to surfaces
- Presence of water changes the “tack” of the agar
- Water/rain transfers strains away or into the wood surface layer

RR falls with the porosity of the surfaces. This is possibly due to:

- Increasing surface roughness (SR) is reducing the actual area of the surface in contact with the agar

The time between inoculation and SCT has an impact on the RR. Possible reasons are:

- Penetration of the inoculum into the cells of the surface
- Drying of the inoculum increases bacterial adhesion to the surfaces
- Antimicrobial effects of wood due to extractives
- Acid pH-values of wood

5.5 Conclusions and perspectives

Pre-serial dilution steps revealed that the concentration of a stock set to a McFarland of 0.5 can vary between 6 $*10^7$ cells/ml and 1.15 $*10^9$ cells/ml. In order to obtain significant results at least 10 essays need to be carried out.

The *SA*, as a gram-positive coccus bacterium appears in grape-like clusters, meaning that the anaerobic bacteria often aggregate in one area. With the use of ultra-sonication of the bacterial suspension a homogeneous solution could have been achieved. Other test strain bacteria, such as *Escherichia coli* or *Bacillus subtilis* need to be investigated as well.

The water stored (wetted) wood samples achieved their FSP whilst the impact of the wetting on PMMA properties and especially on its FSP is probably negligible. In order to test the impact of the presence of water on the RR from plastic, an advanced wetting method, where the formation of a water film on both materials is comparable, needs to be established.

Even though the risk of cross-contamination might be enhanced through the presence of biofilms (Schönwälder et al. 2002; Tomičić et al. 2017), biofilm formation on wood surfaces might also have protective properties (Sailer et al. 2010) against microbial communities or weathering effects. More investigations on protective effects of biofilms on wood surfaces need to be carried out.

Nevertheless, the presented experimental work demonstrates that the SCT method to recover microorganisms from wood surfaces is depending on factors that cannot be controlled when samples are exposed to continuous weathering. Investigation of CFU is dependent on the applied recovery technique. Whether non-destructive or destructive recovery techniques, which are more or less sensitive to MC and surface properties such as SR, are used, may have a significant impact on the obtained results.

6. Chapter: Artificial weathering to control some abiotic factors

This chapter describes the setup of an experiment in which some abiotic parameters are controlled but not the biotic ones. This experiment is subsequently called the non-sterile weathering experiment (NSWE). Specimens were weathered in QUV weathering machines as well as in natural conditions. Previous work demonstrates that the conditions in QUV devices are not sterile (Buchner et al. 2019), which led to the naming of this experimental chapter. It was suggested in Chapter 4, that meteorological conditions are decisive for the degradation process of wood, however, exposure to natural conditions does not allow to set and control exposure parameters. During outdoor weathering, temperatures and irradiation forces are fluctuating, wind direction and speed are unpredictable etc. therefore weathering in artificial setups was conducted. Through this experiment, samples exposed to various artificial as well as natural conditions can be compared with one another, whilst the focus was set on the investigation of microbiological contamination of wood surfaces. The effect of leaching on wood was studied by eliminating spraying cycles of one of the applied artificial weathering techniques. Moreover, through the application of different UV irradiation forces, it was hoped to better understand the importance of photodegradation on wood surfaces. Sample preparation results as well as interpretations of findings are shown in this chapter.

6.1 Material and methods

Quercus petraea (Q), *Castanea sativa (C)* and *Pseudotsuga menziesii* (D) were the wood species to be observed on the tangential surface (See **Chapter 3.1** for further information). The sample racks of the QUV device require a minimum specimen size of 95 × 63 mm. In order to seal the gaps in the fittings with the samples (taking shrinking and swelling into account), a sample size of 100 × 65 × 10 mm^3 (Longitudinal*tangential*radial) was chosen. Samples were cut with a circular saw and planed after conditioning at 65 % rel. humidity and 20 °C, in accordance with ISO 554 (1976). Three replicates were for each combination of wood species, weathering technique and exposure length were tested. Figure 53 shows in which areas the NSWE specimens were assessed by difference techniques.

Methods to characterise wood degradation of specimens exposed to non-sterile weathering tests are presented in **Chapter 3**. Due to the fact that various recovery techniques were aimed to be investigated within this work as well as due to findings presented in **Chapter 5**, a different microbiological recovery method was applied (see **section 3.11.3.2**). MC and microbiological contamination were assessed right after exposure of the specimens. Prior to surface roughness, ATR measurements and visual observations such as scanning, colour measurements, LM and ESEM observation, the samples were conditioned for at least two weeks in a climatic chamber from Vötsch VC150 at 65 % relative humidity at 20 °C.

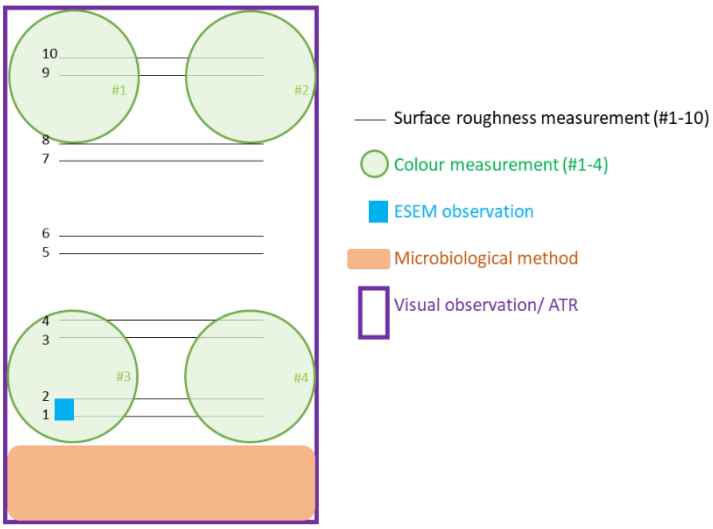


Figure 53: Scheme of a NSWE specimen, showing the areas of measurements

6.2 Weathering techniques

Samples were aged artificially through exposure in QUV weathering machines as well as naturally through the exposure outdoors.

6.2.1 Artificial weathering

Two artificial weathering test devices were used. The UV-Test from the company Atlas, which allows spraying cycles and the Q- Lab from the Q- panel company, without spraying option. UV- 340 lamps served as light sources in both apparatuses. Deionized water was used for spraying as well as for condensation cycles. The amount of water running through these systems are quite high, especially when spraying cycles are applied. In order to reduce consumption, water in the reservoir was recycled and changed only when contamination through discoloration or particles was evident. Wood specimens were placed with vertical grain direction, in the gap of the sample racks and covered with a metal plate from behind which was then fixed with a metal spring as shown in Figure 54.

Figure 54: From left to right: Atlas weathering machine with water reservoir and spraying system, Q-lab apparatus, and sample racks to mount the specimens

In Table 15 machine settings of the 3 artificial weathering techniques are listed. All techniques start with a 24 h condensation cycle. Following, alternating UV irradiation and either spray or idle cycles were applied 48 times. Subsequently the condensation cycle starts again and so forth. Specimens with the ID UVSC, were exposed to UV light, spray and condensation cycles, as proposed in the standard EN 927-6. Two other weathering cycles were modified from the standard as follows: UVC samples were only exposed to UV light and condensation and samples with the ID UVAC were the same except exposed to a lower light irradiation compared to UVC. All specimens were removed from the devices after the 24 h condensation cycle.

Table 15: Set conditions during NSWE weathering cycles in the QUV

Weathering technique/ ID	***Step***	***Function***	***Specification***	***Time [h]***
EN 927	1	Condensation	45±3 °C	24
(UVSC)	2	Sub cycle step 3+4 (48x)		144
	3	UVA-340	60±3°C, 0,89 W/m^2/nm	2.5
	4	Spray		0.5
EN 927 without spray	1	Condensation	45±3 °C	24
(UVC)	2	Sub cycle step 3+4 (48x)		144
	3	UVA-340	60±3 °C, 0,89 W/m^2/nm	2.5
	4	Idle		0.5
EN 927 without spray and lower irradiation	1	Condensation	45±3 °C	24
(UVAC)	2	Sub cycle step 3+4 (48x)		144
	3	UVA-340	60±3 °C, 0,67 W/m^2/nm	2.5
	4	Idle		0.5

As shown in Table 16, during the experiment actual values of the irradiation intensity and the black panel temperature (BPT) were assessed. Moreover the water flow rate in the Atlas QUV was measured for one spraying nozzle (out of 6).

Table 16: Measured conditions during NSWE for artificially exposed samples

Weathering ID	***Value***	***Irradiation [W/m^2/nm]***	***BPT [°C]***	***Water spray/ nozzle [l/h]***
UVSC	Mean	0.57	49.01	400
	Max	1.29	60.50	
	Min	0	13.20	
UVC	Mean	0.61	46.94	0
	Max	0.91	61.00	
	Min	0	27.40	
UVAC	Mean	0.67	54.00	0
	Max	0.71	67.8	
	Min	0	29.5	

6.2.2 Natural weathering

In addition to artificial exposure conditions, a batch of samples was exposed to natural weathering (identified by the code N). The specimens were exposed in accordance to the standard EN 927-3 (EN 927-3 2006). Samples were facing south with a 45 ° angle towards the ground. Wood grain alignment

was vertical. Consult **section 4.1.1.1** on page 54 for information about the exposure site, which is visualized in Figure 26. As for the outdoor weathering experiment, specimens were mounted to the exposure frame with Velcro. Meteorological data during natural exposure are shown in Figure 55 and a summary is presented in Table 17.

Table 17: Meteorological data from NSWE samples exposed to natural weathering

Weathering ID	*Value*	*Irradiation [W/m²]*	*Temp [°C]*	*Humidity [%]*	*Rain rate [mm/h]*
N	Mean	123.8	15.8	77.2	0.1
	Max	947	33.6	97	64
	Min	0	3.9	26	0

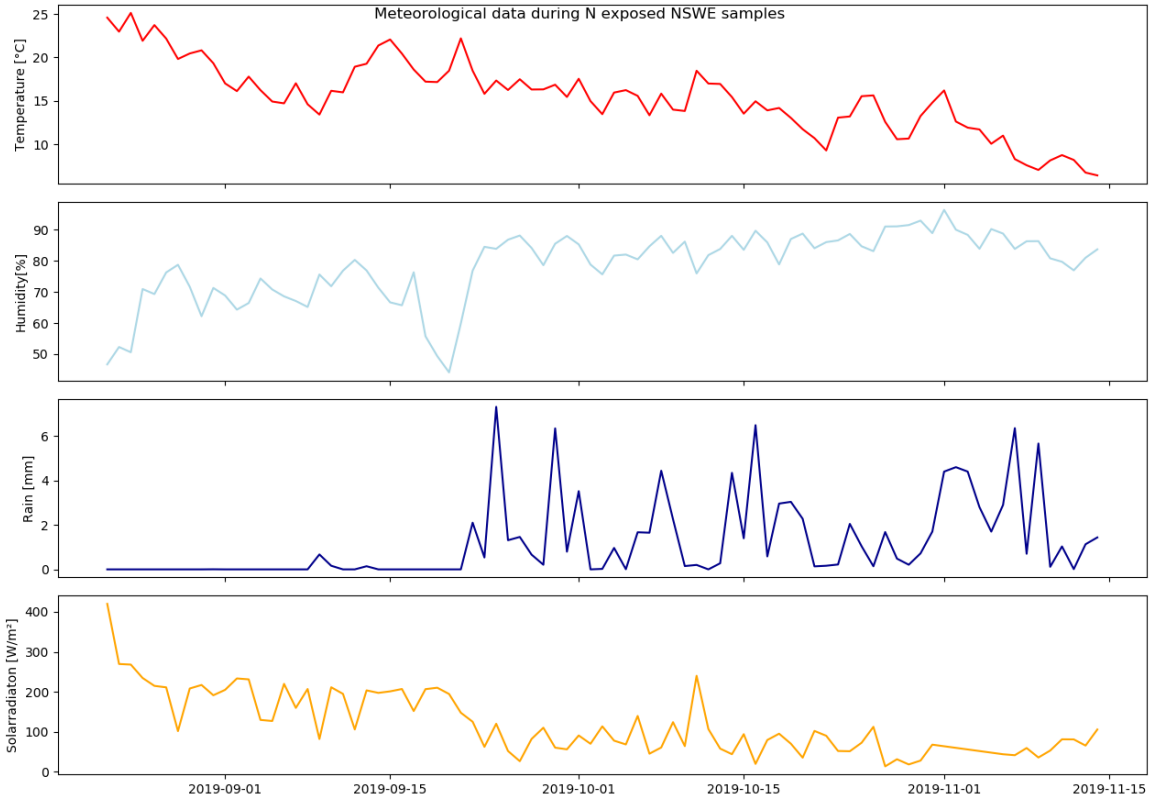


Figure 55: Meteorological data during N exposed NSWE samples

6.2.3 Exposure lengths

The artificial weathering standard EN 927 proposes exposure length of 12 weeks. As it was assumed that degradation processes take already part in early stages of exposure, in addition shorter weathering length were chosen. For both, artificially and naturally exposed wood, half of the samples were exposed for 6 weeks (I) and the other half was observed after 12 weeks (II) weathering. Specimens exposed to natural weathering were exposed from the 22.08.2019. Half the specimens were removed from the racks the 03.10.2019 and the other half was exposed until the 14.11.2019.

6.3 Results and discussion of NSWE

Principle component analysis (PCA) was done in RStudio, the analysis of CFU was executed with the software Tableau, colour data was treated in Python 3.6 and ATR analysis was performed with the software Opus 7.2. But first, a short discussion about the applied weathering techniques is given.

6.3.1 Weathering techniques

During outdoor exposure, predominant meteorological conditions are uncontrollable and difficult to predict. The prevailing weather at the start of a natural weathering cycle is likely to have a significant effect on the way the degradation process of wood may evolve. It is easy to imagine that a natural weathering experiment that starts in the summer could give different results to one started in the winter even if all other factors are kept constant. Setting-up and starting many natural weathering trials at different times of the year is rather impractical and may not give the expected results because of the vagaries of the weather. These are reasons why accelerated artificial weathering techniques are often preferred. In addition, it is clear that comparisons between natural and artificial weathering are difficult as e.g. comparing UVA-340 lamp to sunlight intensities is due to different units not possible.

Exposure of wood samples to normalized weathering machines still entails problems though. Those machines have a high water consumption, especially when spraying cycles are applied, therefore the water is often recycled and reused. With increasing exposure length, the degradation of wood becomes more severe and an increasing number of wood particles enter into the water circuit. These particles lead to clogging in the pump and eventually a shut off of the device. The water must be exchanged on a regular basis and the filters cleaned. This normally means that the cycle must be paused temporarily during the cleaning.

Biofilms were found to develop in the water reservoir and tubes during this research. Anytime a slimy layer was visible to the naked eye, the water circuit was cleaned and refilled with fresh deionized water. This water exchange is likely to reduce the number spores and microbial strains present in the system and this could influence the weathering observed. The experience of this research shows that the investigation of the development of microorganisms using standardized weathering devices is impracticable. It also demonstrates that weathering in outdoor environments is indispensable in order to study the impact of microorganisms during wood degradation.

The ambient temperature in the laboratory played a decisive role for the temperature settings in the apparatus, e.g. during summer season, higher temperatures were reached. This demonstrates that recoding of the prevailing conditions is required, since they deviate from the set and desired ones.

It was planned to compare the impact of different light intensities on the wood samples, however the desired setting in the QUV devices did not prevail, especially the irradiation intensity deviated to a high extent.

6.3.2 Statistical analysis

Through the NSWE experiment plenty of data was obtained. In order to get an overview of the data, a principle component analysis (PCA) was executed before results of each applied measurement was observed individually. Mean values of between 1 and 10 replicates were calculated, depending on the executed measurement method, as following:

- Surface roughness n= 30, mean calculated from delta values before and after weathering (delta values only for dimensional parameters, the mean values of non-dimensional parameters was used for analysis)
- Colour Lab n= 10, mean calculated from delta values before and after weathering
- ATR peaks [cm^{-1}] n=6, delta values of mean of scans in 6 regions, executing 21 scans each (shown as wavelength number of the absorption band in the PCA)
- CFU counts n=6, mean values
- Visual (for each level and parameter) n=1
- Temperature n=1 (mean value and min value calculated during complete exposure time)
- Spray is a binary variable (0/1)

As the measured data had barely missing values, no prior data treatments were done. Different to the observation of OWE data, prior execution of Pearson correlation analysis on the data set obtained from the NSWE, showed for all variables correlations, depending on the wood species. Therefore, all the variables were integrated, even though the significance of the PCA declines.
Following abbreviations for the samples are used. Samples ID is composed of the applied weathering technique (N, UVSC, UVC and UVAC), the wood species (Q= Oak, C= Chestnut and D= Douglas) and the exposure length (I= 6 weeks and II= 12 weeks).

6.3.2.1 PCA

As shown in Figure 56, the first two dimensions of the PCA analyses correspond to a cumulative total of 53.53% of the total inertia; that means that 53.53% of the variables cloud total variability is explained by the plane. The 3rd dimension explains only 12.3% of the total inertia which is below the average eigenvalue of 33.33% and therefore too little to be kept for further analysis (Kassambara 2017) ,thus only the first two dimensions are investigated.

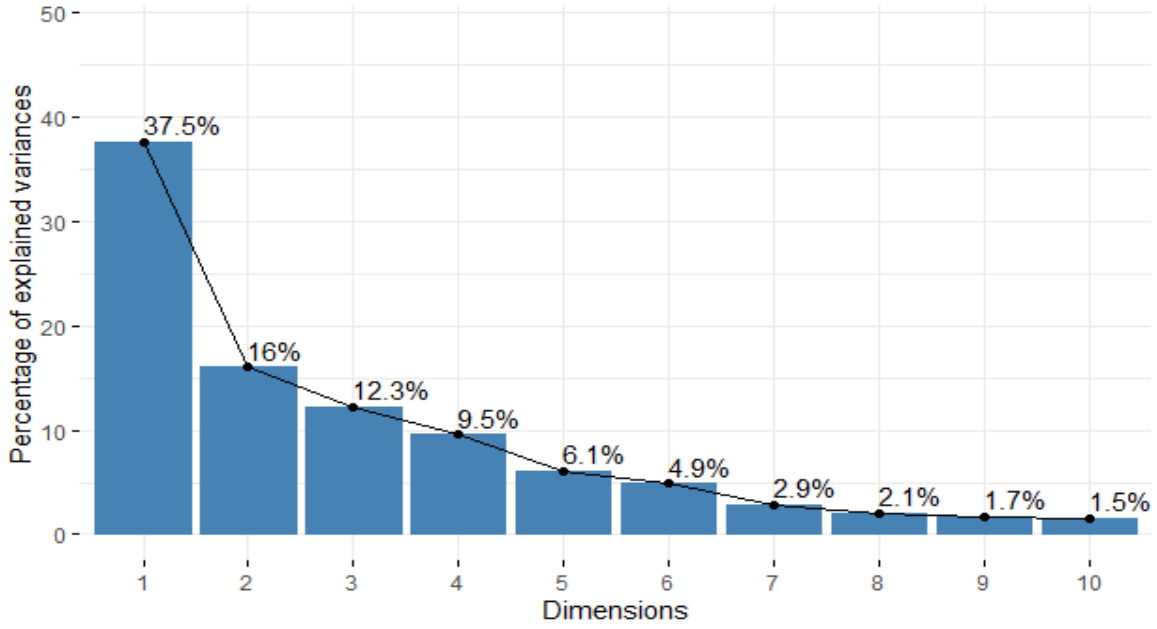


Figure 56: Decomposition of the total inertia – Scree plot of NSWE data

Figure 57 shows the variables factor map on OWE data on the first two dimensions, whilst only the 10 most representative variables are displayed in the graph in order to achieve a better readability of the graph. Unfortunately, the vectors of the CFU counts on Malt-agar and especially PC-agar are not very well presented in this analysis.

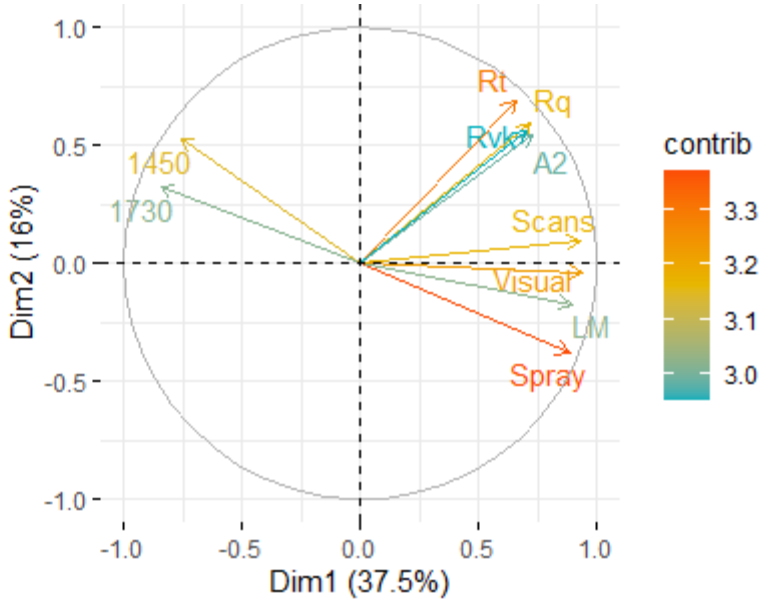


Figure 57: Variables factor map on NSWE data

The 1st dimension opposes two groups, one in which the individuals UVSCDII, UVSCDI, UVSCCII, UVSCQII and UVSCQI stand:

- UVSC exposed samples show high delta values for SR variables such as R_q, A_2, R_{vk} and P_t. Visually, strong differences between early- and latewood degradation are noticeable.

- Low delta values for ATR peaks in the regions 1205 cm^{-1} (Cellulose) and 1265 cm^{-1} (G. lignin) were found for UVSC samples.

Another group in which the individuals UVCQI, UVCDI, UVACCI and UVACQI stand was united:

- Wood exposed to UVC and UVAC weathering techniques show high delta values for hemi-celluloses (regions 1730 cm^{-1} and 1235 cm^{-1}) and lignin peaks at 1450 cm^{-1} and 1430 cm^{-1}.
- Samples exposed to UVC and UVAC show low visual changes. The delta a*, representing the green and red level, shows low values for those weathering techniques.

The 2nd dimension unites the individuals NQII, NCI, NQI, UVSCCI and NCII:

- N exposed samples as well as the chestnut specimens exposed to UVSC weathering technique show high fungal contamination whilst low temperatures prevailed.
- Low delta values for Lignin (1320 cm^{-1}, 1450 cm^{-1}, 1430 cm^{-1}) and Hemi/Celluloses (1365 cm^{-1}).

6.3.2.2 Hierarchical Clustering

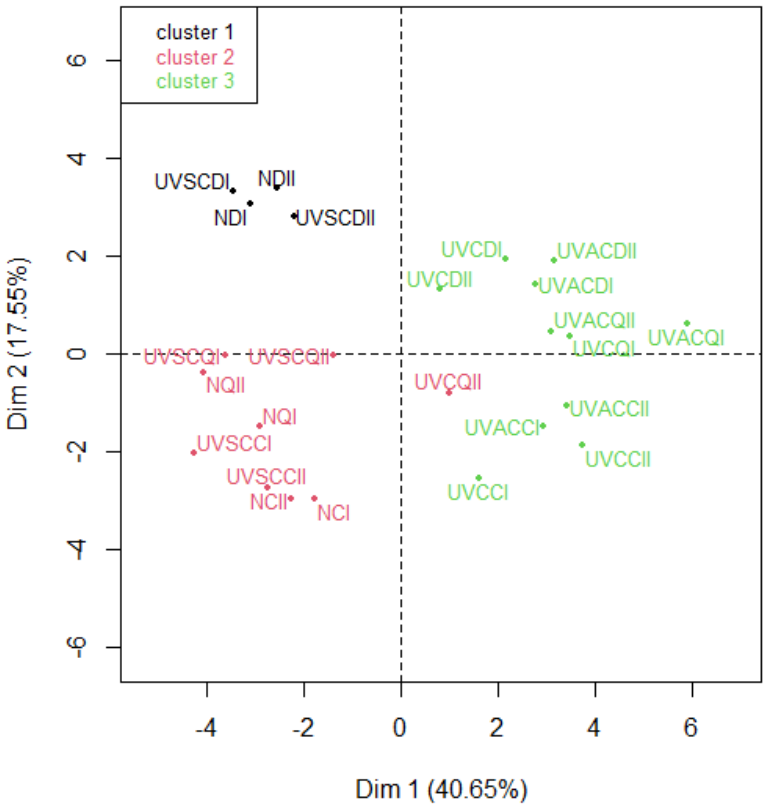


Figure 58: Hierarchical classification of the individuals

The classification of individuals reveals 3 clusters. The 1st cluster is made of naturally and UVSC exposed Douglas fir samples. The 2nd cluster is made of naturally and UVSC exposed chestnut and oak wood. The 3rd cluster is basically made of UVAC and UVC exposed samples of all wood species. These findings show the importance of the different weathering techniques, but also wood species.

Softwood species´ behaviour is quite different towards the two hardwood species. The length of OWE exposure is according to this cluster analysis, rather negligible.

6.3.3 CFU

The graphs in Figure 59 and Figure 60 show the number of CFU (converted into ordinal data) recovered from exposed samples via scratching technique (see **Chapter 3.11.3.2** on page 49). Each value represents an average of 3 measurements.

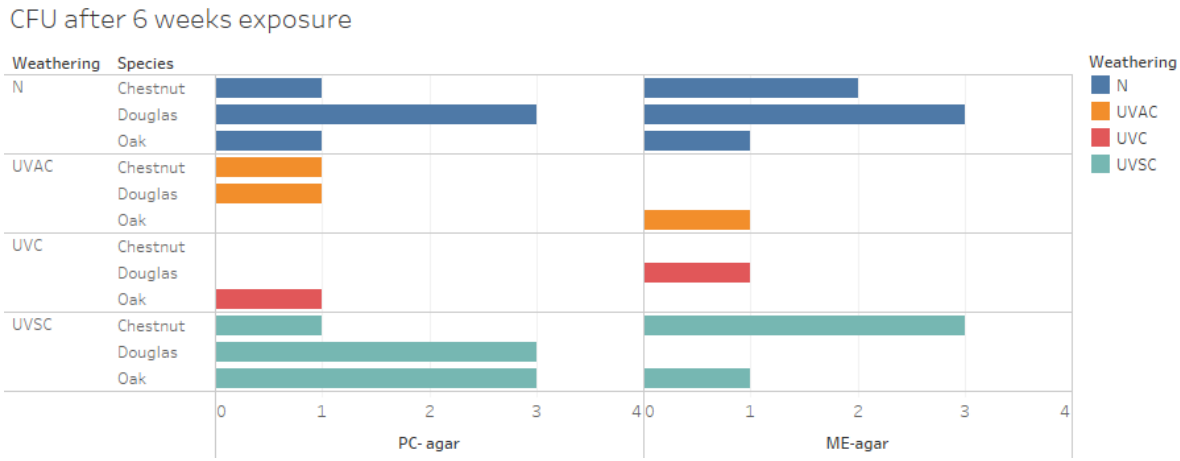


Figure 59: Number of CFU recovered from 6 weeks NSWE exposed samples, growing on PC- agar and ME- agar

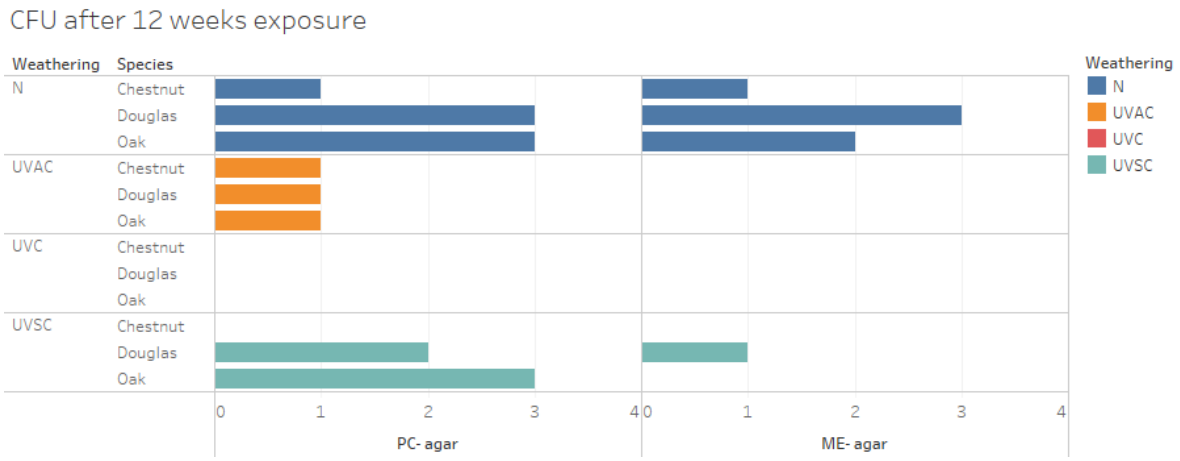


Figure 60: Number of CFU recovered from 12 weeks NSWE exposed samples, growing on PC- agar and ME- agar

The lowest amount of CFU was found on chestnut, followed by Douglas and finally oak. From artificially weathered samples, a higher amount of CFU was observed on PC-agar compared to ME-agar. Most microorganisms were found on N exposed samples, closely followed by UVSC, then UVAC and the least amount of CFU was recovered from UVC exposed samples. Even though no significant trends were observed, naturally exposed samples mainly increase CFU with prolonged exposure times, whilst artificially exposed samples rather decrease the number of CFU with time. Specifically the CFU on ME-agar is very low after 12 weeks of artificial exposure.

The number of CFU recovered from chestnut samples exposed for 6 weeks to UVSC was quite high, whilst otherwise from chestnut only very few microorganisms were recovered. This leads to the

speculation that those petri dishes were cross- contaminated. Otherwise, the higher pH (Kakavas et al. 2018) and concentration of extractives (Fengel and Wegener 1989) and thus, potentially, antimicrobial substances in chestnut wood might explain low numbers of CFU compared to oak and Douglas fir. Moreover, the amount of CFU is likely to be linked to the availability of nutrition. Douglas fir might provide some sugars for microorganisms from the beginning on, whilst in oak wood nutrition are easier to access for microorganisms once the degradation process is in an advanced stage. Moreover, antimicrobial substances in oak will leach the longer the degradation is in process and may become as a consequent more susceptible with time compared to Douglas.

During artificial weathering, more bacterial than fungal strains were recovered. The high resistance of bacteria towards severe conditions, such as high MC or temperature, might explain higher amounts of CFU on PC-agar plates. In unfavourable environments bacteria tend to produce spores which allow them to endure. However, even though the strains are present on wood surfaces does not mean that they definitely metabolise or degrade the wood.

This investigation shows that weathering in artificial conditions allows microorganisms to develop, but not to the same amount and especially the variability of strains are possibly much more important in natural environments. An artificial weathering programme has a predefined set of conditions. If a microbe can develop in those conditions, then it will continue to survive throughout the test and possibly prevent the development of other microbes. Whereas in natural weathering the conditions may change drastically, e.g. extended dry periods, causing limited growth or even death of some fungal and bacterial strains present. At the same time, the wind and rain in a natural environment can be the vectors for providing new colonisation of wood surfaces. The gradual increasing CFU counts on naturally exposed samples and decreasing CFU on artificially exposed samples is indirect evidence of these vectors with time.

6.3.4 Scans

The complete scan collection can be observed in the attached file **NSWE_Scans.pdf**, a selection of specimens exposed for 6 weeks are represented in Figure 61 and exposed for 12 weeks are shown in Figure 62. Douglas and oak wood exposed to UVSC turned grey, even after only 6 weeks exposure. N exposed samples turned rather yellow after 6 weeks and turned grey after 12 weeks. UVC and UVAC exposed samples turned much darker.

Figure 61: Scans of Douglas (upper row), oak (middle row) and chestnut (third row), exposed to NSWE for 6 weeks

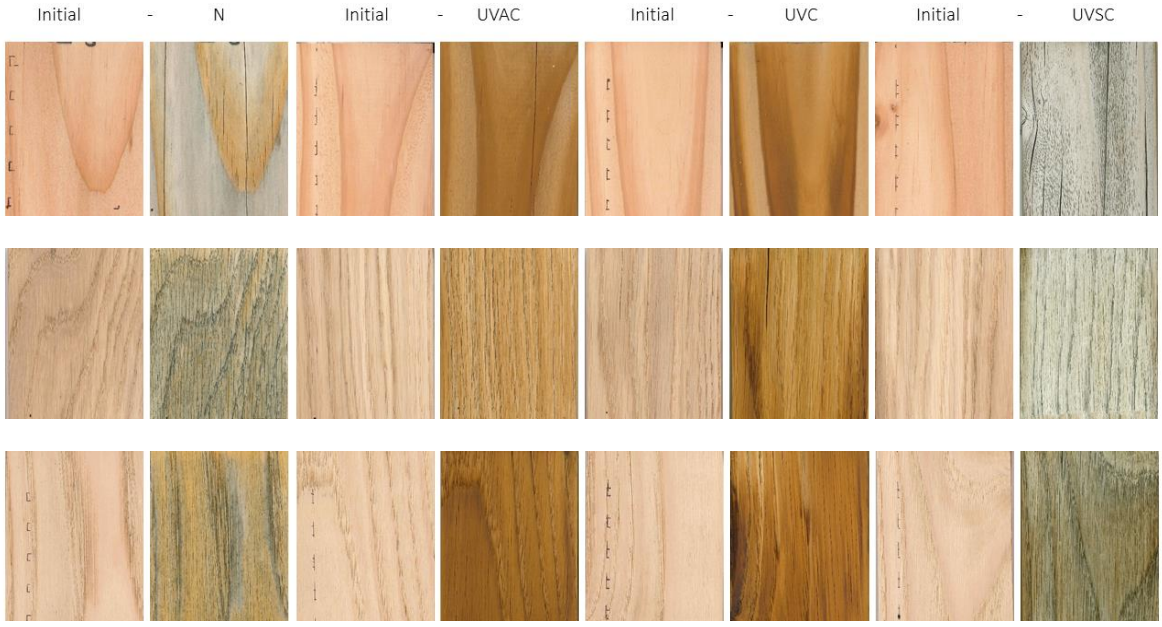


Figure 62: Scans of Douglas (upper row), oak (middle row) and chestnut (third row), NSWE exposed for 12 weeks

The scans revealed important information such as discoloration, cracking and warping. Samples exposed to weathering methods including spraying, where leaching occurs, turned grey whilst weathering techniques excluding spraying turned darker; possibly through the accumulation of chemical degradation products. Chestnut was the least susceptible wood species towards colour change, followed by oak and finally Douglas. UVAC and UVC weathering did not show any major differences on a macroscopic level.

6.3.5 LM

A complete summary of LM pictures can be seen in the attached file **NSWE_LM.pdf**. Following some LM images of Douglas are shown in Figure 63. LM observations of all wood species show that the weathering techniques N and UVSC allow microorganisms to develop whilst UVC and UVAC exposed samples do not show particles on the surfaces. Degradation patterns such as cracking, defibration and discoloration were visible and more obvious in UVSC and N exposed samples compared to UVAC and UVC exposures.

The picture of N exposed Douglas (upper left image) shows early-wood on the left, and late-wood on the right. The very obvious difference in colour indicates a much faster lignin degradation in early-wood. Douglas exposed to UVC for 6 weeks showed yellow deposits on the surfaces, which disappeared again when exposure length increased. Pronounced cracking; especially along ray-parenchymatic cells; was observed in hardwood samples.

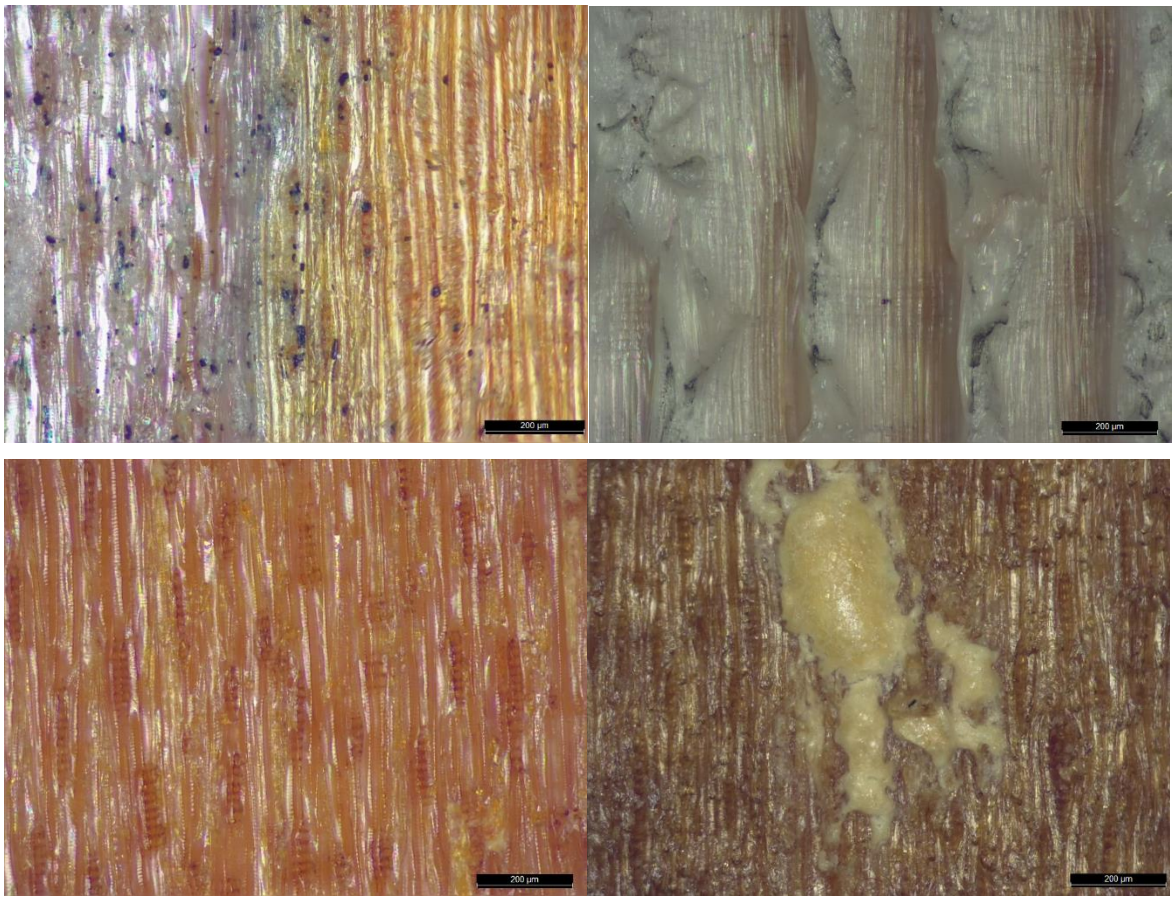


Figure 63: Douglas exposed for 12 weeks to N (upper left), UVSC (upper right), UVAC (lower left) and UVC (lower right)

Observations with the LM in reflection mode provide relatively low-quality images. Preparation of slices and application of dies helps, and makes it possible to visualize fungal and bacterial spores. Only in N and UVSC exposed samples were spores spotted. In UVSC and, especially in N weathered samples, the amount of spores rose drastically with increasing exposure length. Moreover, anatomical degradation patterns could have been defined more precisely in comparison to scans. Cracking,

which was more evident in N and UVSC exposed samples; was often spotted along parenchymatic cells within the hardwood species. Douglas exposed for 6 weeks to UVC showed yellow deposits, which disappeared again after 12 weeks. The yellow deposits are likely to be related to accumulation of extractives (such as resins), which are subsequently removed again through chemical breakdown, leaching and erosion.

6.3.6 ESEM

A collection of ESEM pictures from NSWE specimens can be seen in the attached file **NSWE_ESEM.pdf**. As described in **Chapter 3.6**, a pin hole was created on the specimens in order to track the same area of the samples after exposure. Figure 64 shows an oak specimen before and after 6 weeks exposure to UVSC. The pin hole is visible in the lower left corner of the images. Generally talking, samples exposed to N and UVSC showed more severe degradation patterns such as cracking and defibration, UVAC and UVC exposed specimens show have a few cracks and rarely particles sticking off the surfaces. The longer the exposure the more the wood structure loosens.

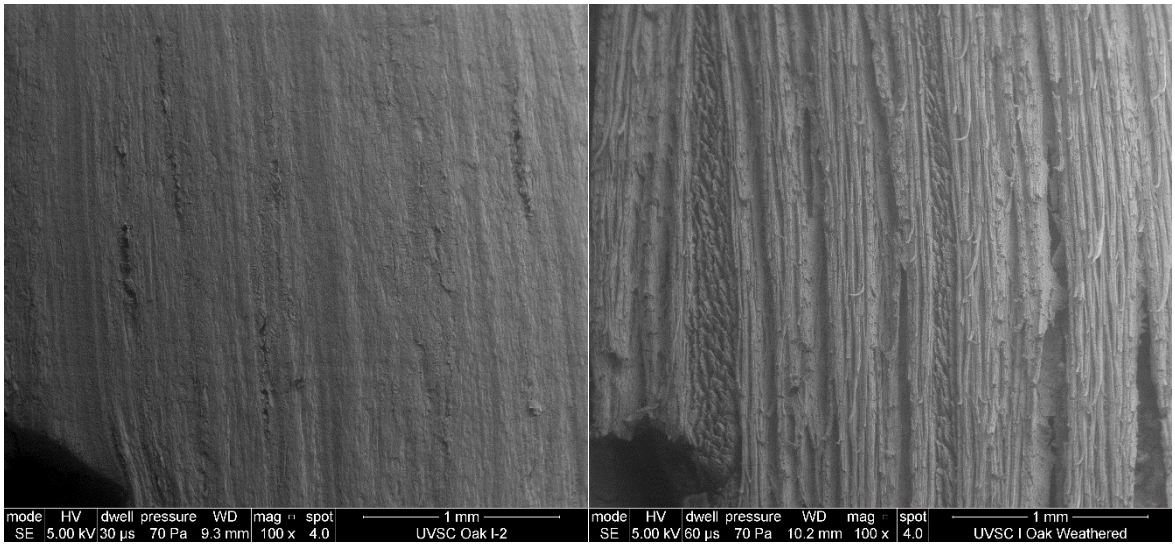


Figure 64: Oak wood before (left) and after 6 weeks exposure to UVSC (right).

Creating pin holes on the wood surfaces was a simple and efficient means to identify and examine the exact same areas during the weathering cycles. The ESEM makes it possible to observe anatomical degradation patterns on a microscopic level, however, detection of microorganisms was less evident, probably due to the fact that ESEM images are in greyscale. Particles such as starch or other pollutants cannot be easily distinguished from spores. It was noted that higher magnifications do not necessarily provide more information.

6.3.7 Assessment of the visual observations

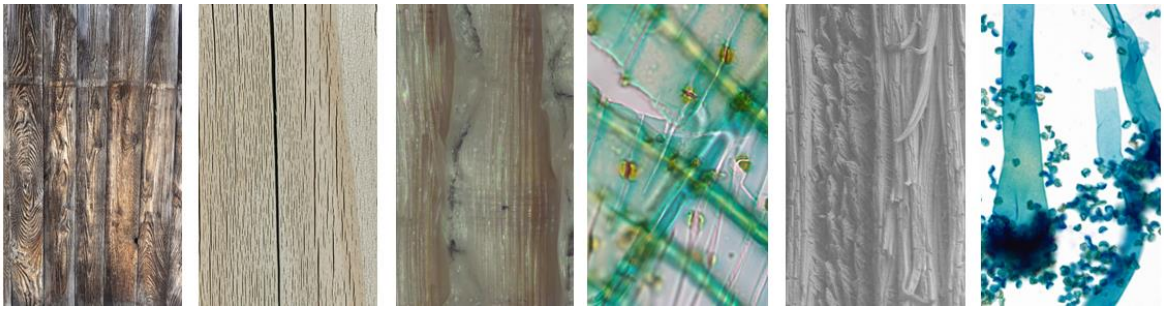


Figure 65: Visual observation with the naked eye, macroscopic and microscopic

As presented above, the visual observation of wood surfaces was carried out at different magnification levels. Observations via ESEM, LM and scans on weathered samples showed that each level reveals different information. Therefore, an assessment of the degradation stage of the samples was carried out (see 3.7 for details) and samples were categorised in decay classes as shown in Table 18. Most samples exposed to UVSC were ranked in the most severe decay class followed by N exposed samples. The chestnut specimens exposed to N for 6 weeks were surprisingly ranked in decay class 2. UVAC exposed Douglas fir showed least degradation patterns.

Table 18: Assessment of the visual observations: Decay classes of NSWE exposed samples

Species	Weathering	Length [weeks]	Sum of criteria [%]	Decay class
Oak	UVSC	12	89.3	4
Chestnut	UVSC	12	85.7	4
Douglas	UVSC	12	81.1	4
Oak	UVSC	6	77.0	4
Oak	N	12	74.5	3
Douglas	UVSC	6	74.0	3
Chestnut	N	12	72.0	3
Chestnut	UVSC	6	70.1	3
Douglas	N	12	69.8	3
Oak	N	6	58.3	3
Douglas	N	6	58.0	3
Chestnut	UVAC	12	54.9	3
Oak	UVC	12	51.9	3
Chestnut	UVC	12	48.3	2
Oak	UVAC	12	42.5	2
Oak	UVC	6	39.9	2
Chestnut	UVAC	6	39.6	2
Chestnut	UVC	6	39.4	2
Douglas	UVC	12	37.3	2
Chestnut	N	6	34.3	2
Douglas	UVC	6	32.7	2
Oak	UVAC	6	31.1	2
Douglas	UVAC	12	24.8	1
Douglas	UVAC	6	17.9	1

It became evident that visual observations at different magnification levels provide slightly different information and are complementary. The implementation of decay classes by the combination of the three visual observation methods proved to be a useful tool in order to integrate the data into statistical analysis. Visual observations are generally subjective and strongly dependent on the operator, however within this research observations were carried out by one operator only and are therefore comparable. Still, a few challenges were encountered: One difficulty was the fact that different hard- and softwood species with different anatomical features were compared with each other. Moreover, it was challenging to rate the appearance of cracks and the intensity of defibration, since the cutting direction of the samples has a big influence on these properties. Cutting direction of the observed samples varied and may have falsified results to a certain extent. UVSC exposed samples showed most severe degradation, followed by N weathering. A clear difference between UVC and UVAC exposed samples could not be seen. Chestnut samples exposed to N weathering for 6 weeks were ranked in decay class 2 only, demonstrating chestnut´s resistance towards short outdoor exposures. Douglas exposed to weathering techniques excluding leaching showed quite low visual changes, compared to the investigated hardwood species. This phenomenon might be explained by a higher cellulose contents in Douglas fir compared to the hardwood species, since cellulose is known to be thermally stable.

6.3.8 Colour

Mean values of 12 colour measurements (4 replicates on 3 specimens) were calculated. The evolution of the Lab* colour coordinates over time as well as ΔE* are presented in Figure 66 to Figure 69. Slight translucent areas around the evolution lines demonstrate standard deviations.

Highest standard variations were observed for UVAC, followed by N and UVSC, and finally UVC exposed samples. The observed softwood showed significantly higher standard variation compared to the two hardwood species. Most data are normally distributed, Douglas fir however shows bi- or even multimodal distributions. Following each wood species was observed:

Douglas fir exposed to UVSC and N shows less change in lightness compared to artificial weathering techniques excluding spraying (UVC and UVAC). Lightness is decreasing the most after UVAC weathering. Values a* and b* decrease after UVSC and N whilst for UVC and UVAC a* and b* values increase. 12 weeks exposure to UVSC shows highest colour change, followed by UVAC exposure. The lowest change in Δ E* was observed after 6 weeks N weathering.

Oak wood after 6 weeks exposure to N increases drastically the brightness, a slight increase can also be observed in UVSC exposed oak samples, whilst UVC and UVAC exposed samples decrease the brightness. A* and b* values rise after 6 weeks of exposure, except after exposure to UVSC. After 12 weeks the parameters drop slightly, except for N weathered samples the decrease is drastically

after 12 weeks. The colour change ΔE* after 6 weeks was observed to be the lowest in UVSC, followed by N, UVC and the highest change in oak was found in UVAC weathering. After 12 weeks, ΔE* values are approaching quite similar changes.

Chestnut samples behaved very similar to oak wood. The brightness parameter L* however decreased to a higher extent. Following each colour parameter is described:

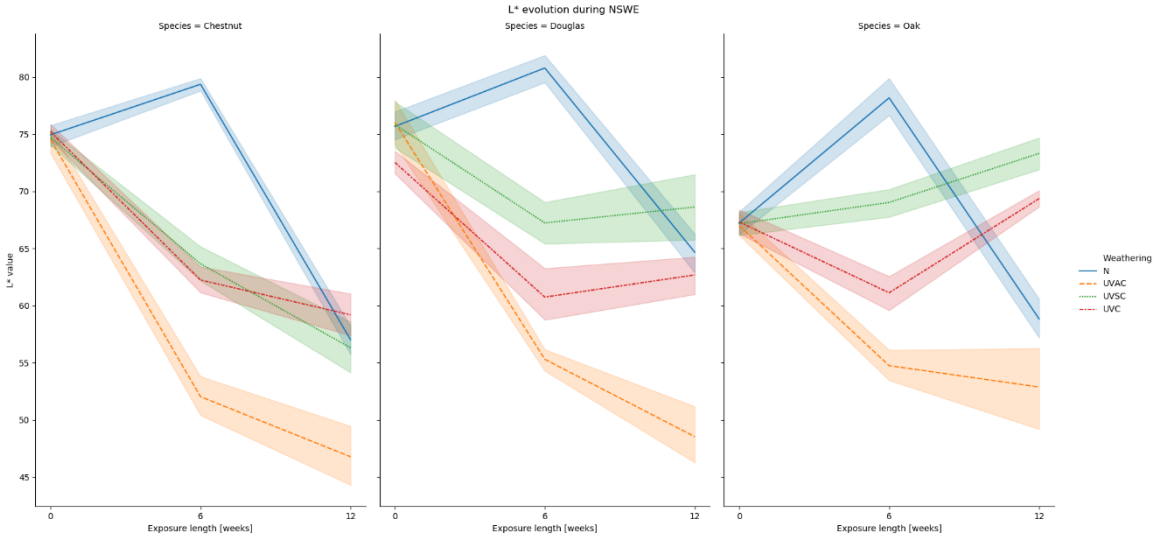


Figure 66: Evolution of L during NSWE*

The L* coordinates decreased after 6 weeks artificial exposure, whilst brightness in N exposed samples increased first and dropped after 12 weeks. UVAC exposed samples showed the most severe drop in L*.

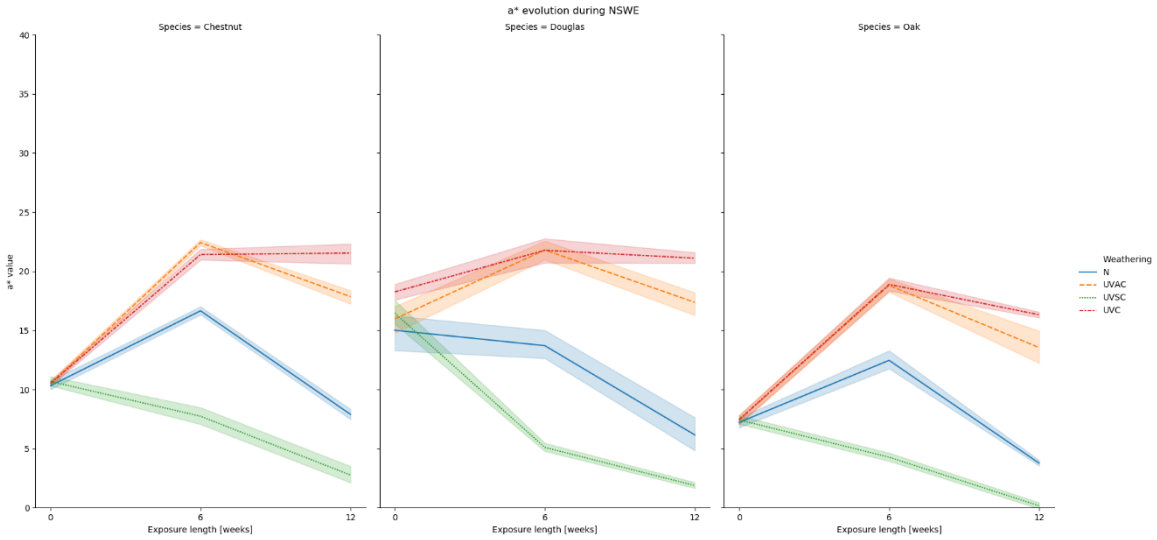


Figure 67: Evolution of a during NSWE*

The a* parameter decreased in N (except oak and chestnut after 6 weeks) and UVSC exposed samples. In UVAC and UVC exposed samples a* increased after 6 weeks and rather decreases in later stages of exposure.

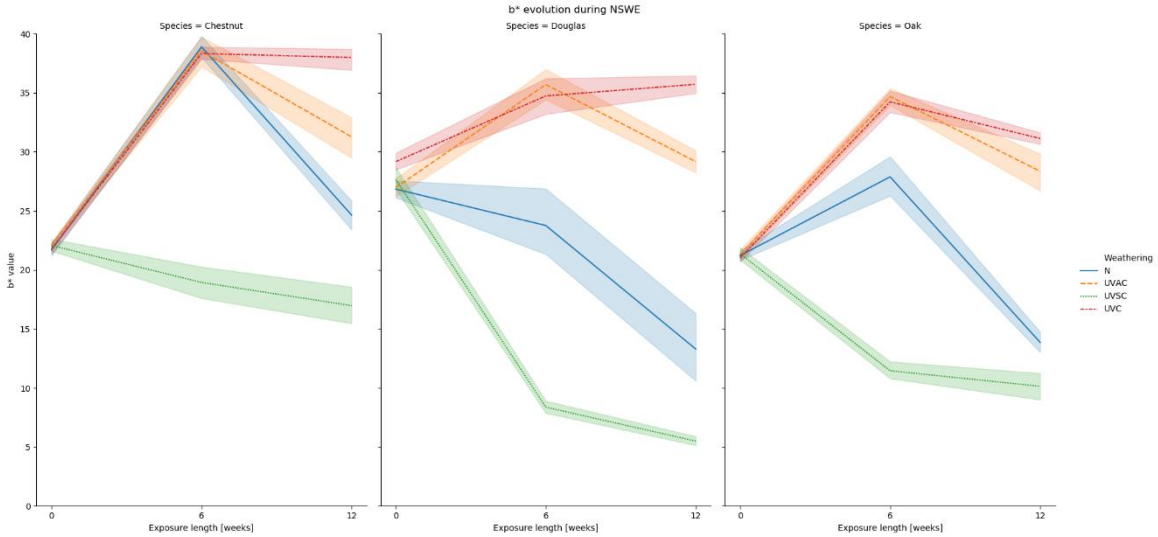


Figure 68: Evolution of b during NSWE*

The parameter b* behaves quite identical to a* (except that chestnut exposed naturally for 12 weeks increases the b* value slightly compared to the initial coordinate.

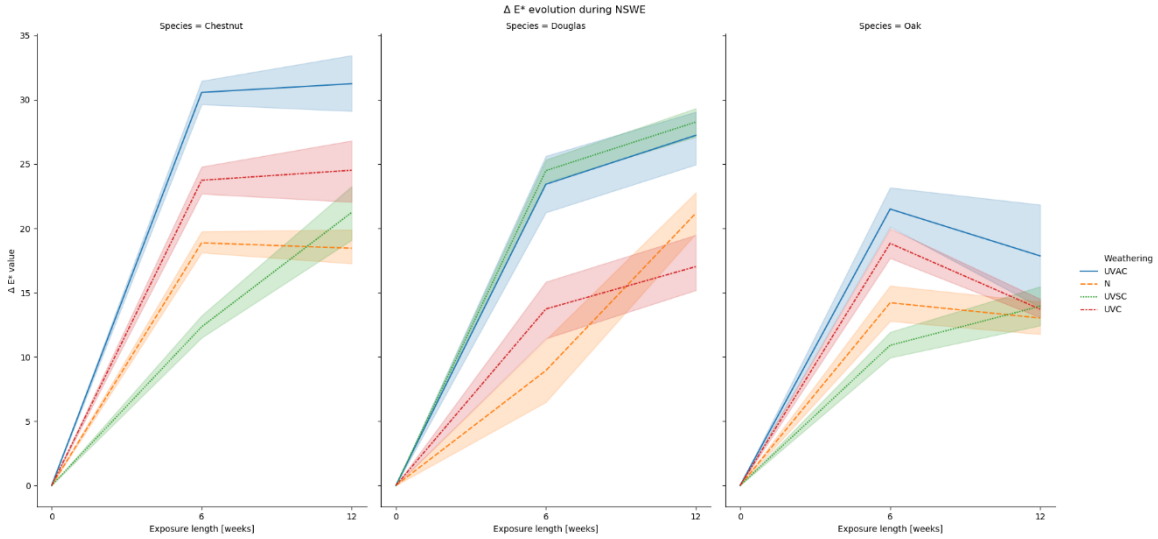


Figure 69: Evolution of ΔE during NSWE*

Observing the ΔE*, UVAC weathered chestnut seems to be most and oak wood exposed to UVSC the least susceptible within the tested wood species.

The colour evolution due to natural exposure was quite different to artificial weathering techniques. According to colour coordinates, UVSC weathering was the closest to N exposure. UVSC was the most severe weathering technique for Douglas fir, demonstrating susceptibility of softwoods towards spraying. UVAC, where slightly higher temperature conditions prevailed compared to UVC, evoked harshest changes to oak and chestnut, showing susceptibility of hardwoods towards thermal degradation. The brightness L* of chestnut was affected the most, and oak wood the least. The b* colour coordinate was equally affected the most in chestnut; except after UVSC weathering. According to Fengel and Wegener, chestnut has compared to oak and Douglas the highest lignin content (1989)

which may explain the significant drop in the b* colour coordinate. Lignin is very susceptible to photodegradation and less impacted by wetting cycles (such as UVSC) compared to UVC and UVAC. A significant increase of the a* colour coordinate was observed in UVC and UVAC exposed samples.

6.3.9 Surface roughness

An explanation about SR parameters can be seen in **Annex II**. Each SR value represents a mean value of 30 measurements (10 measurements on 3 replicates). The evolution of the SR parameters as well as the profiles were visualized in Python. Following the evolution of some selected parameters are described. The translucent areas around the evolution lines represent the standard deviations. In the attached file **NSWE_SR.pdf**, graphs of all SR parameters are shown. Moreover, a comparison between SR profiles and scans can be seen in the attached file **NSWE_Scans vs SR profiles.pdf**.

6.3.9.1 Evolution of the roughness parameters

Evolution of the arithmetic mean roughness is shown in Figure 70. Highest R_a values can be observed in UVSC samples followed by UVC exposed samples. Highest standard deviations were equally found in UVSC, followed by UVC exposed samples. Hardwood species increased the R_a value to a higher extent compared to the softwood species. The one way-ANOVA of R_a values showed significant differences between all weathering techniques and exposure lengths.

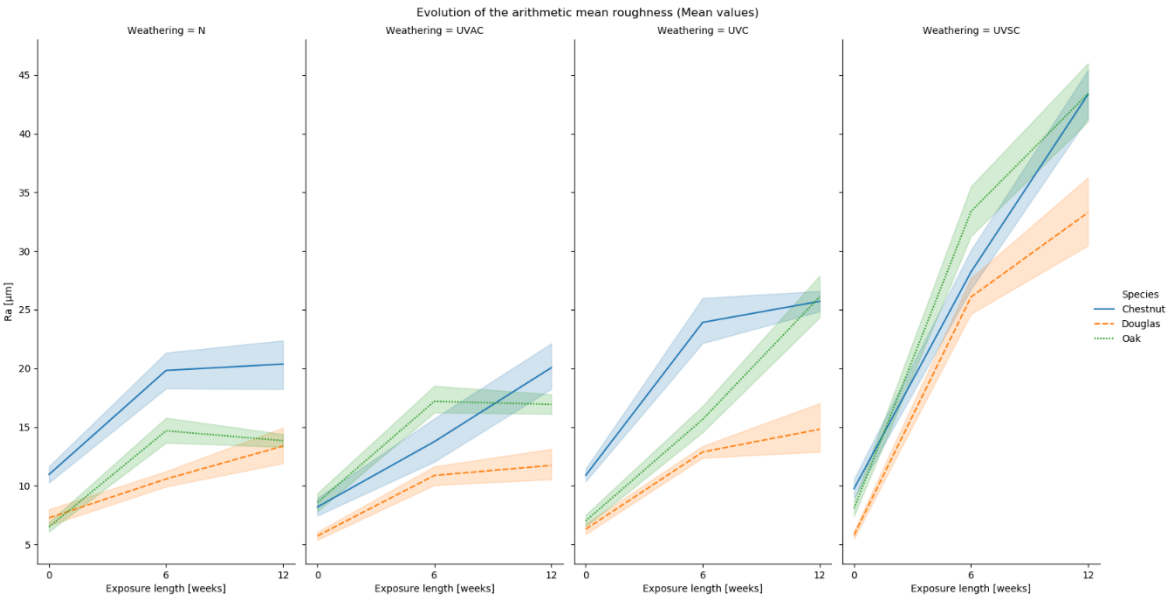


Figure 70: Evolution of the SR parameter R_a due to NSWE

The R_{sk} values are shown in Figure 71. Highest standard deviations were found in Douglas samples, especially after exposure. Very low initial values for both hardwood species were measured. Chestnut and oak have large vessels, i.e. deep valleys and so both have negative R_{sk} values at the start. R_{sk} values of 12 weeks exposed hardwoods changed less compared to 6 weeks exposure, except UVSC exposed samples.

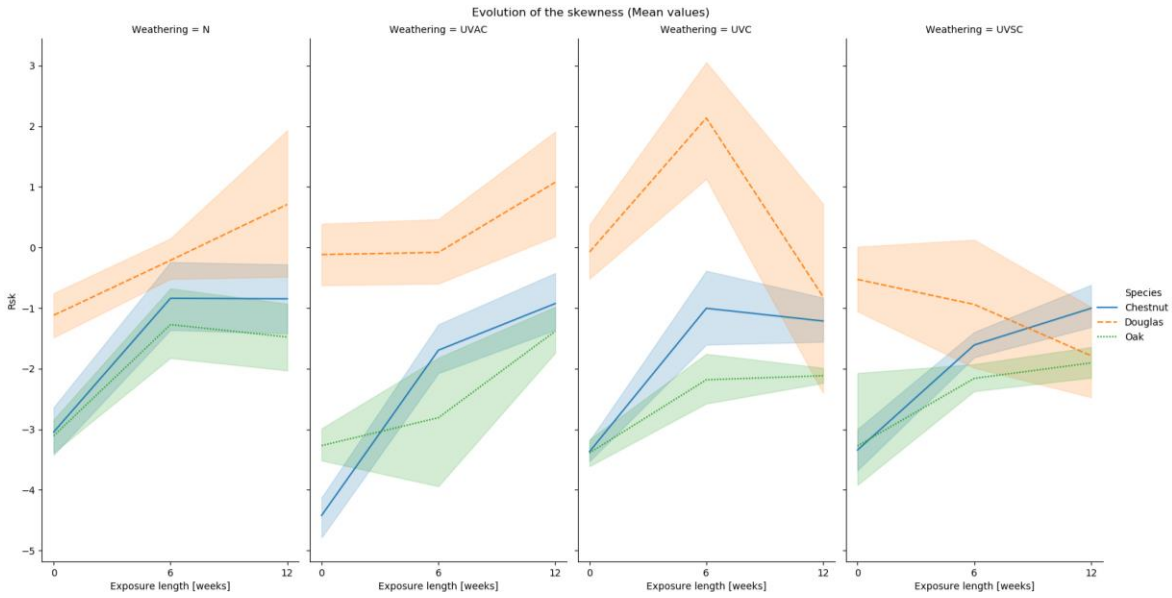


Figure 71: Evolution of the SR parameter Rsk due to NSWE

Highest A_2 values were observed in UVSC samples. UVC exposed samples show slightly higher values compared to UVAC and N samples. The evolution of the profile valley area is shown in Figure 72.

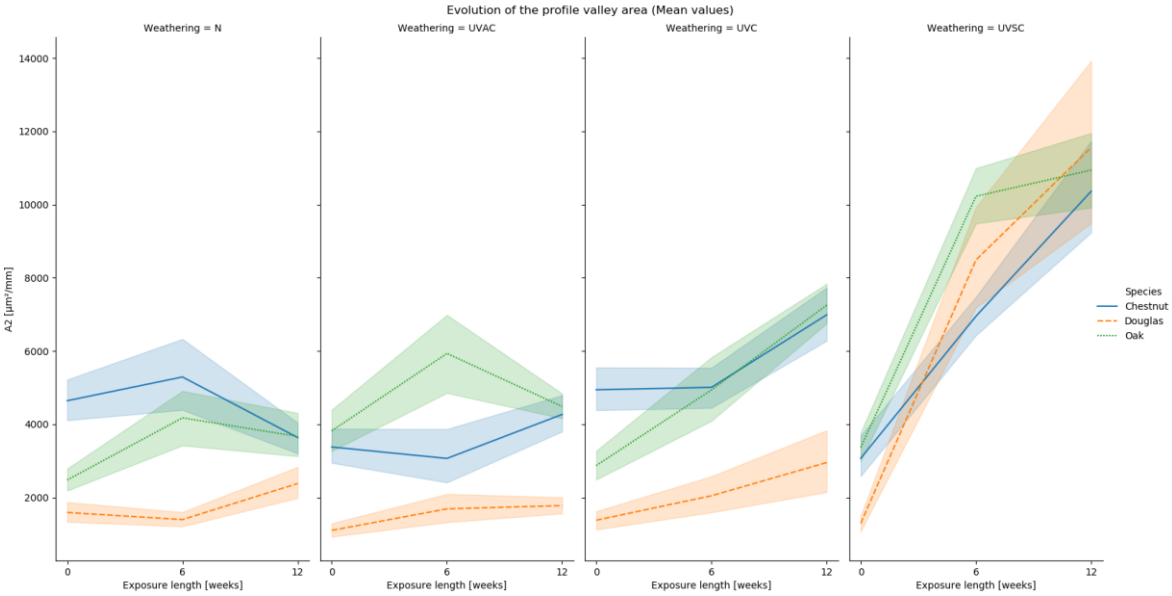


Figure 72: Evolution of the SR parameter A2 due to NSWE

6.3.9.2 Scans and profiles

A subset of the data presented in the previous section is presented here again with the objective of linking the profiles to photographs of taken at the same time. Figure 73 shows surface scans after weathering and the measured profiles before exposure in grey, and after 12 weeks of weathering in red (Distance between ticks on the y-axis show 0.2 µm profile depth). The roughness parameters R_a, R_{sk} and A_2 are investigated following:

R_a: - Roughness in Douglas increases more in earlywood compared to latewood

- Areas with big vessels increase drastically in roughness, in Douglas, where vessels there are no vessels, this phenomenon does not occur

R_{sk}: - Valley depth in hardwood species seem to be related to early-wood regions

- Douglas shows deep valleys after exposure in early and late-wood transition areas due to cracks

A_2: - Valleys become rather wide in hardwood specimens

- Very deep but rather narrow valleys create in early- and latewood boarder regions of Douglas
- A_2 moreover reflects cracking in Douglas fir samples

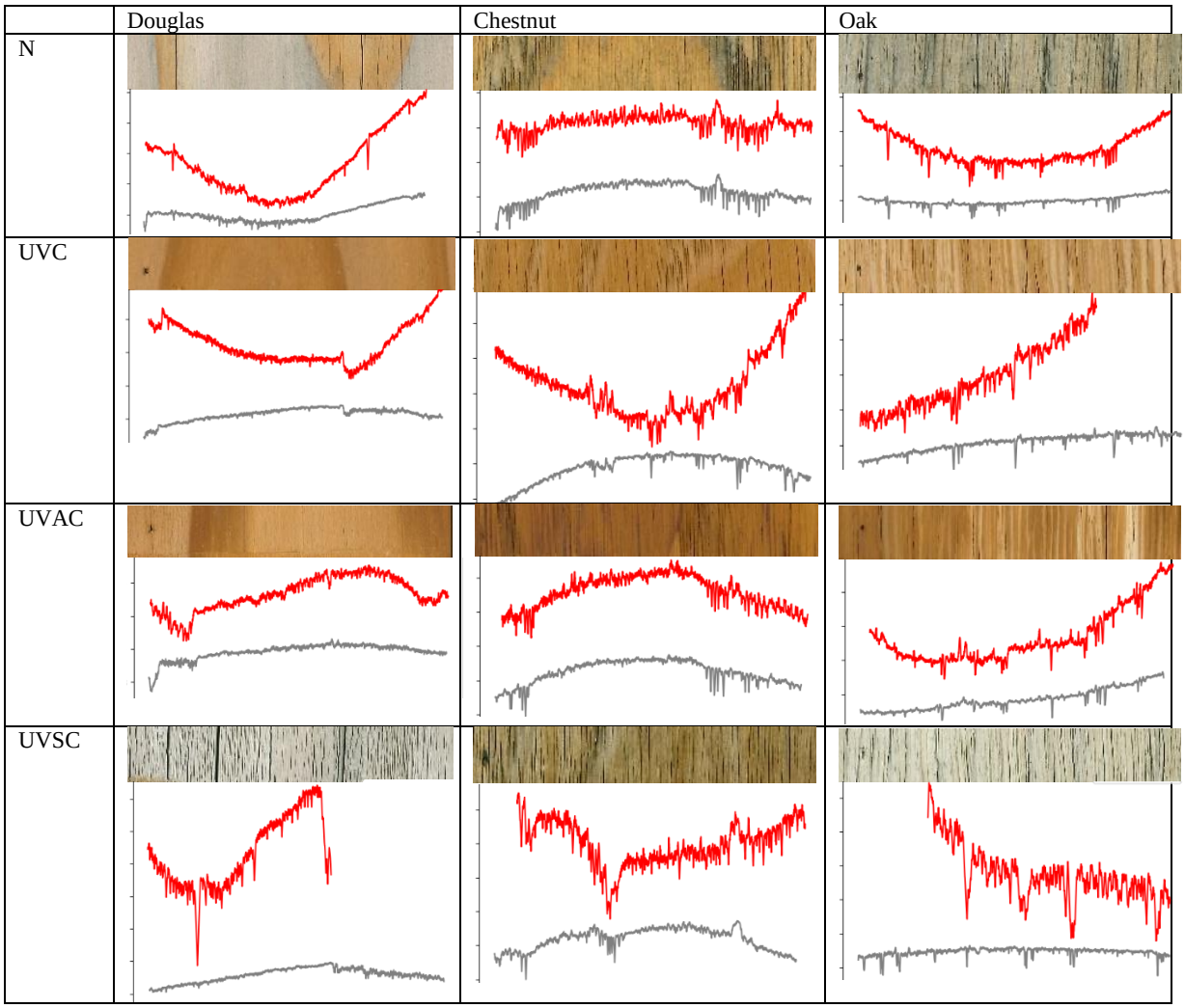


Figure 73: Surface scans with corresponding profiles of NSWE samples

6.3.9.3 Topography

The following figure shows topographies of the three wood species exposed to UVSC. They show a noteworthy difference between hard- and softwood species. Deep valleys correlate with vessel, parenchymatic cells and cracks. Furthermore, early- and latewood areas can clearly be distinguished from one another. Valleys in earlywood vessels enlarge to a high extent compared to latewood vessels.

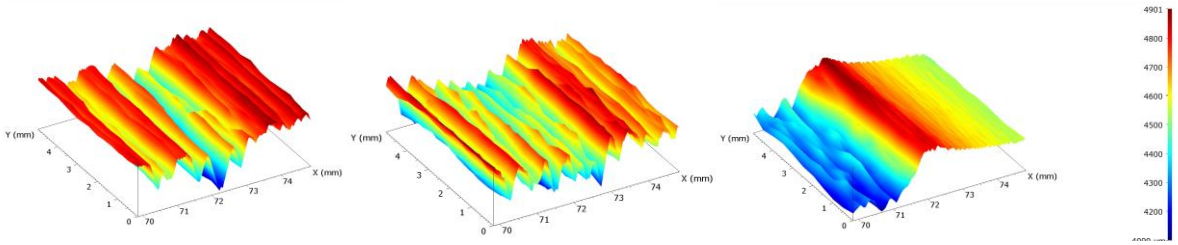


Figure 74: Topographies showing (from left to right) oak, chestnut and Douglas fir samples after 12 weeks exposure to UVSC

6.3.9.4 Summary of the surface roughness investigation

The investigation of SR values show that they are influenced by the application of the chosen weathering technique. It seems to be a good indicator of the state of wood surfaces, especially when comparing different weathering techniques as well as exposure length with each other. However, measuring the SR with a stylus does not provide information on roughness at a microscopic level, it remains unclear which part of the wood cell-walls degrade.

- Observations of delta values revealed that R_a, R_{vk}, R_q and R_k are the least influenced, and R_v and R_t are impacted the most by irregularities deriving from wood anatomy.
- Investigations of mean values of SR parameters lead to the assumption that the weathering technique UVAC is the closest approximation to N weathering conditions. It shall be considered that conditions during N exposure were very dry, with a low rain rate of 0.1 mm/h.
- The SR in earlywood increases to a much higher extent compared to latewood. For a more precise analysis, early and latewood regions shall be investigated separately.
- UVSC weathering, which has a spraying and therefore leaching step, has a marked effect on most SR parameters; more so than N, UVC and UVAC weathering.
- Profiles show a significant increase in profile height after weathering which is strongly related to warping of the samples. Especially UVSC samples tend to deform. However, warping depends strongly on the cutting direction of samples as well. For future investigations I strongly recommend a very precise sample preparation with very low divergence of the observed cutting direction.
- Outliers and high standard variations are likely to be related to cutting direction of samples.
- The continues increase of the skewness in Douglas wood exposed to N and UVAC might be explained by the increasing fuzziness of the softwood species, whilst decreasing R_{sk} values might indicate the progressive appearance of cracks.

- Oak and Chestnut have vessels that appear as valleys in SR profiles. The size of these valleys seem to increase during exposure, which would make one think that the R_{sk} would fall further, but it does not. The reason is that the surface develops many peaks, which has a marked effect on the R_{sk} value.
- The comparison between scans and profiles helped showing accuracy of the stylus measurements. Major defects such as big cracks are not always well captured with the stylus. High peaks, as in the transition of early to latewood in Douglas fir do not let the stylus come back to the surface rapidly, therefore the profile shows peaks rather round shaped than thin tipped.
- ESEM observations help understand the SR evolution on an anatomical level only to a minor extent. N exposed samples show deposit of particles that are not related to wood; however, they aren't captured by the SR measurements. UVSC samples show a lot of detached fibres on the surfaces and contribute to higher SR parameters.

6.3.10 ATR

ATR measurements in early- and latewood regions were compared with each other and did not show significant differences, expect for the peak at 900 cm^{-1}, which showed lower signals in earlywood regions compared to latewood. Therefore, the data from the spectra of early- and latewood, were combined in subsequent analyses.

Comparison between 6 and 12 weeks exposures showed same trends of degradation. Changes of peak intensities were not necessarily higher after longer exposures. It is assumed that degradation processes are more evident the longer samples were exposed, thus further investigations were performed on 12 weeks exposed samples.

Following figures show the obtained spectra for each wood species and noticeable changes are listed. Colour codes of the weathering techniques are:

Initial (black), N (green), UVC (pink), UVAC (light blue), UVSC (dark blue).

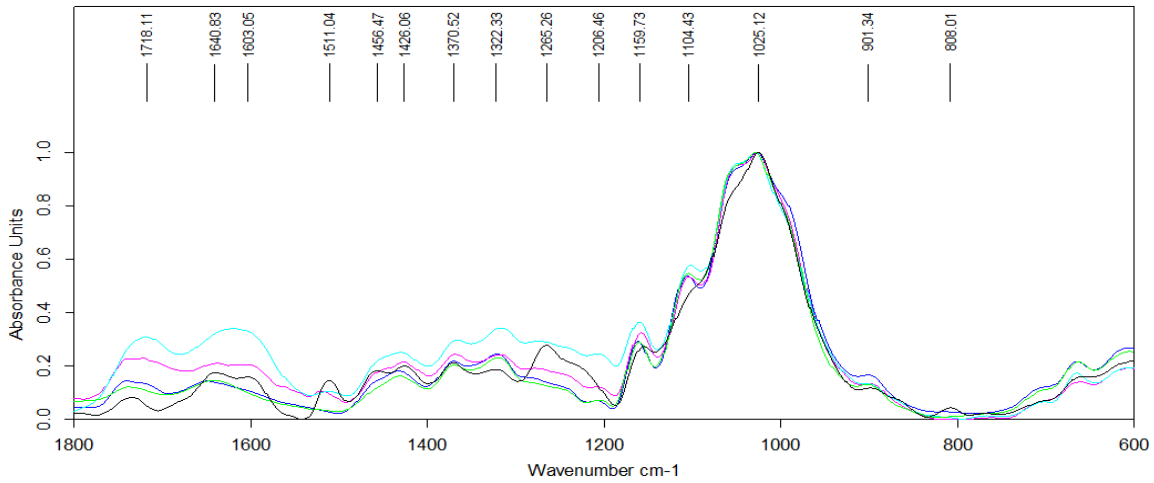


Figure 75: ATR spectra of Douglas fir before and after the application of weathering techniques for 12 weeks

- 1640 cm^{-1} Disappears in UVAC (or fuses with 1603?)
- 1603 cm^{-1} Disappears in N and UVSC samples
- 1511 cm^{-1} Disappears in N and UVSC, signal left in UVC and UVAC
- 1456 cm^{-1} Only shoulder left in UVC and UVAC, N and UVSC almost disappears
- 1320 cm^{-1} Decreases in N samples
- 1265 cm^{-1} Disappears almost
- 1206 cm^{-1} Appears
- 1104 cm^{-1} Shoulder becomes peak
- ~1045 cm^{-1} Shoulder appears

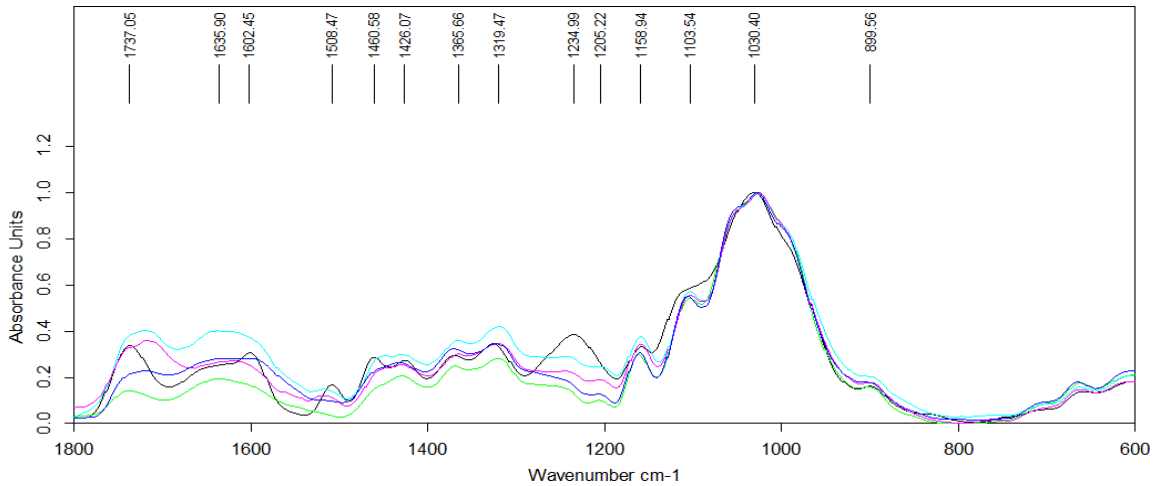


Figure 76: ATR spectra of oak before and after the application of weathering techniques for 12 weeks

- 1602 cm^{-1} Flattens
- 1508 cm^{-1} Disappears in N and UVSC. In UVAC and UVC a small signal is left
- 1460 cm^{-1} Disappears
- 1426 cm^{-1} Flattens
- 1320 cm^{-1} Decreases in N samples
- 1234 cm^{-1} Disappears almost
- 1205 cm^{-1} Appears

- 1103 cm^{-1} Shoulder becomes peak

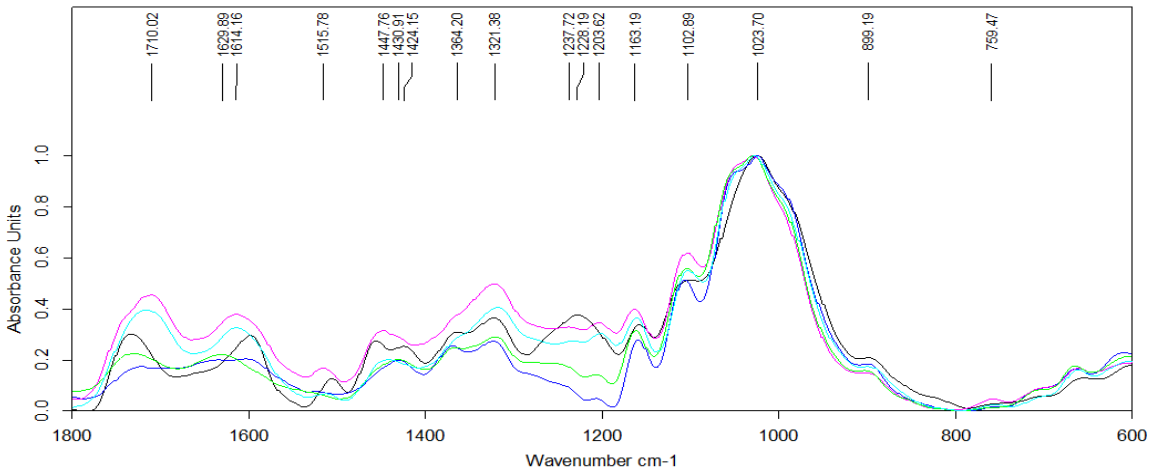


Figure 77: ATR spectra of chestnut before and after the application of weathering techniques for 12 weeks

- 1630 cm^{-1} Small signal only in N and UVSC samples
- 1614 cm^{-1} Disappears almost in N and UVSC
- 1515 cm^{-1} Disappears in N, UVSC and UVC. UVAC stays
- 1447 cm^{-1} Disappears in N and UVSC
- 1424 cm^{-1} Disappears almost in UVC and UVAC
- 1364 cm^{-1} Disappears almost in UVC and UVAC. Small peak remains in N and UVSC
- 1320 cm^{-1} Decreases in N and UVSC samples
- 1228 cm^{-1} Disappears almost
- 1203 cm^{-1} Appears
- 1103 cm^{-1} Shoulder becomes peak
- 1045 cm^{-1} Should appears

The applied ATR measurement method is convenient since no sample preparation is needed; however, ATR spectra give limited accuracies, probably linked to the roughness of the surfaces since rough surfaces have possibly less contact with the ATR diamond (Gupta et al. 2015). Moreover, chemical heterogeneity is increasing with progressing weathering length.

Whether the noticed difference between early- and latewood regions for the peak at 900 cm^{-1} (lower signals in early wood regions compared to latewood) are real or rather related to variability or roughness in samples is not clear. Furthermore, executing the measurements 100% in either early- or latewood showed to be a difficult task. The samples need to be placed exactly above the diamond. Measurements might not always represent only early- or latewood. The peak at 900 cm^{-1} represents cellulose and shows that the thicker cell wall in latewood, and especially the proportion of secondary cell walls increase and therefore the cellulose content is higher in latewood.

Investigating each wood species by its own, N and UVSC exposed samples show very similar changes. This indicates that the applied artificial weathering technique UVSC, which is in accordance

with the standard EN 927, is, on a chemical level, comparable to natural weathering. UVC and UVAC weathered samples show unsurprisingly similar trends as applied weathering cycles were applied with only slight differences in irradiation intensity and temperatures. Still, differences of absorption intensities were noticed, however it remains unclear whether those differences are related to the applied weathering cycles or rather to variations in the samples.

The main differences in FTIR/ ATR spectra between the two groups of unweathered wood species, softwoods and hardwoods, result from the differences in their chemical composition: The content and type of lignin, namely syringyl- guaiacyl lignin for hardwoods and guaiacyl lignin (G.lignin) for softwoods, and the total amount of hemicelluloses as well as the contribution of pentosans (Fengel and Wegener 1989; Wagenführ 1999). The observed differences between the investigated wood species before weathering are evident in the peaks at ~1630 cm^{-1} (Aromatic carbonyls), which only shows in Douglas fir and oak but not in chestnut wood, the peak at ~1265 cm^{-1} (G.lignin), only in the softwood species, the peak at ~1235 cm^{-1} (Acetyl in xylan), which is much more present in the hardwood species compared to Douglas fir and the shoulder at ~1045 cm^{-1} (C-O stretching in secondary alcohols) which is more evident in chestnut and oak than in Douglas fir. Accordingly, a comparison between the different wood species exposed to different weathering techniques is rather difficult. According to the PCA several peaks were found to explain distinction between the different weathering techniques and wood species. The most significant peaks are listed following:

- N exposed samples show a decrease of several peaks associated to lignin, at 1320 cm^{-1}, 1450 cm^{-1}, 1430 cm^{-1}. The decrease at 1365 cm^{-1} (hemi/celluloses) is especially noticeable in the hardwood species.
- N and UVSC exposed Douglas wood show strong decrease for lignin peaks in the peak regions 1265 cm^{-1} (G. lignin) and 1510 cm^{-1} (Volkmer et al. 2016), related to photodegradation (Tolvaj and Mitsui 2005; Lesar et al. 2011) and at 1630 cm^{-1}(aromatic and conjugated ketones) which is associated to absorbed water (Schwanninger et al. 2004). Peaks in the region 1205 cm^{-1} (cellulose) in Douglas fir is strongly affected by UVSC weathering.
- UVC and UVAC weathering techniques strongly increase in the region 1730 cm^{-1} (unconjugated carbonyls) and at 1235 cm^{-1} (syringyl ring) as a result of UV-light induced chemical degradation processes (Timar et al. 2016). Peaks at 1450 cm^{-1} and 1430 cm^{-1} (lignin) remain rather stable compared to samples exposed to weathering including leaching.
- Significant decrease in aromatic ring vibrations of lignin in the peak at 1600 cm^{-1} was found in oak and chestnut samples (Faix 1991; Tolvaj and Faix 1995; Tolvaj and Mitsui 2005).
- C-O deformations of lignin at 1045 cm^{-1} (C-O stretching in secondary alcohols) remains nearly unchanged in oak samples, whilst in chestnut and Douglas a shoulder creates.

6.4 Summary and outlook of NSWE

Even though the lengths of artificial and natural weathering techniques are not quite corresponding to be correlated, all four weathering techniques were compared, and a summary is shown following:

The PCA indicates importance of the different weathering techniques as well as wood species, whilst the length of 6 or 12 weeks exposure is rather negligible.

It is implicit that through the weathering techniques N and UVSC, which evoke leaching through spraying or rain, samples tend to degrade a lot faster and turn grey.

Microscopic observations of the samples revealed to be good tools to investigate the state of degradation. Especially the LM was a helpful and rather quick method to spot fungal attack, compared to ESEM investigation. Fusing the information obtained from scans, LM and ESEM reduces the bias and the implemented ranking system allows to use to data for statistical analysis.

Observation of the colour coordinates may lead to the assumption that UVSC weathering is the closest artificial weathering technique towards natural weathering. Especially after 12 weeks exposure, as suggested by the standard EN 927, the colour changes are quite similar what is in favour for the authenticity of the standardizes artificial weathering technique.

However, CFU counts indicate that with progressive exposure length, naturally weathered samples show more contamination whilst on artificially exposed samples the contamination decreases. Even though these observations are not significant, they indicate that natural weathering cannot be replaced by artificial techniques if biotic factors are considered.

Settings during UVC and UVAC weathering cycles were quite similar, nevertheless, bacterial growth was more enhanced during UVAC weathering compared to UVC exposure, an indicator for the importance of only slight changes in temperature and/or light irradiation forces towards the development of bacteria.

Moreover, the significant alteration of ATR peaks and SR parameters between all applied weathering techniques demonstrate the importance of even minor differences in weathering cycles on wood degradation. Thus, a comparison between different researches seems almost meaningless.

High changes of the SR parameters R_q, A_2, R_{vk} and P_t were outstanding for UVSC exposed samples, indicating the harshness of this weathering technique, however, SR as well as ATR investigations did not allow to specifically acquire insight into biotic degradation.

The applied technique to recover microorganisms from NSWE degraded wood samples via scratching seemed to be less affected by e.g. MC and surface properties compared to the SCT method, however it was difficult to remove wood particles until the same depth on each specimen due to warping and cracking of the wood. For further investigations again a different recovery technique was applied as explained in **Chapter 3.11.3.3**.

Within this experiment, biotic factors were not controlled, therefore weathering in a self-built weathering device was envisaged. The following **Chapter 7** presents the work done to design an apparatus in sterile conditions and in **Chapter 8** the experimental work to degrade the wood samples are shown.

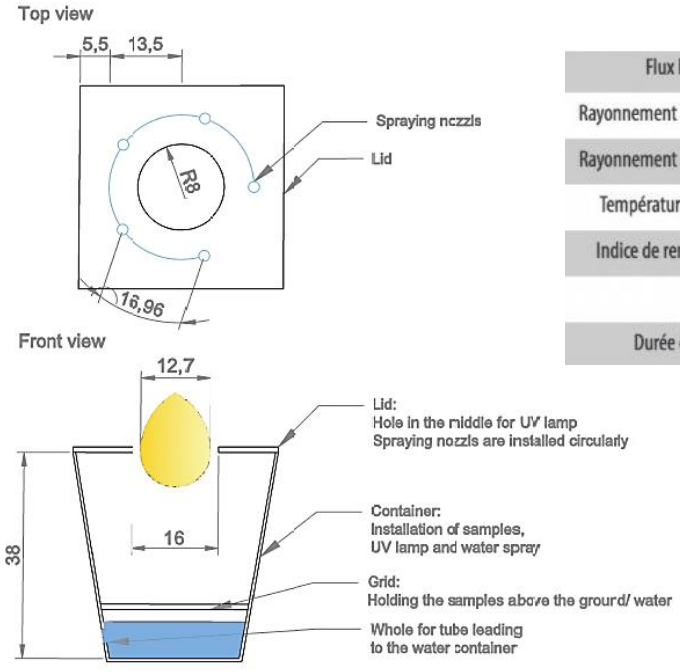
7. Chapter: Approach to design an artificial weathering device in sterile conditions

Standardized accelerated artificial weathering machines allow the control of abiotic factors such as temperature, UV- irradiation and water spraying. As the experiment described in **Chapter 6** clearly demonstrated that fungi and bacteria are present during standardized artificial weathering tests, it was decided to design an apparatus which would allow the weathering of wood specimens whilst controlling the above mentioned abiotic factors as well as the biotic factors, e.g. the presence of fungi and bacteria. Up to now, no standardized test methods have been established for doing so.

This chapter serves as an introduction to **Chapter 8**, which describes the results obtained from the final version of the apparatus built for this purpose. This chapter describes the development of the apparatus and method used for weathering in sterile conditions. It is thought that the results of the development process might be of help to others planning to work in this area. Therefore, three experiments were carried out to evaluate sterility over time and are presented in a chronological order.

7.1 Design of the artificial weathering test device

Figure 78 on the left shows a plan of the sterile artificial weathering setup. The test chamber can be placed in a LFB, which is considered a sterile environment. The samples are placed on a stainless steel grid; with an inclination of approximately 5°, so as to encourage water to run off the upper surfaces; in a plastic container containing 4 l sterile deionized water. The container is covered with a plastic lid that also holds the spraying nozzles, which are uniformly arranged in an arc. A hole in the middle of this lid allows to mount the UV lamp centred above the specimens.



Flux lumineux (lm)	1000
Rayonnement UV-A @15cm (µW/cm²)	250
Rayonnement UV-B @15cm (µW/cm²)	65
Température lumineuse (Kelvin)	4000
Indice de rendu des couleurs (Ra)	>60
Douille	E27
Durée de vie moyenne	6000 heures

Figure 78: Plan of the purpose build artificial weathering test device (left), information about the light source (right)

The use of electricity and water in one setup demands safety regulations such as waterproof bulb sockets. Those were only available for light bulbs up to 100 W, which is the reason why a light source with 100 W was chosen as irradiation source. On the right of Figure 78 detailed information about the UV-A/-B light bulb from `Reptile Systems` are shown. Another benefit with this light source is the fact that maximum irradiation intensity is achieved immediately on switch on. The setup was designed in a centric manner, so the distance from the round shaped light bulb is identical to all the specimens placed underneath.

One of the challenges of designing the test device was to wet the samples homogeneously. Initial setups, where water droplets were used to wet the wood specimens, showed very heterogeneous wetting patterns, and were discarded. An alternative, which was used in the final setup, uses 5 spraying nozzles that generate a fog of water droplets to wet the specimens uniformly. The spraying nozzles require a minimum pressure of 2 ± 0.5 bar and, therefore, the apparatus requires a water pump. A large, powerful pump was found to cause a strong increase of the water temperature, which would clearly affect the weathering and cause too much water loss through evaporation. A 12 v diaphragm pump (SFDP1-030-045-33) from the company SEAFLO with a maximum flow rate of 11.3 l/min and a shut-off pressure of about 3.1 bars was found to work well. Black panel temperature (BPT) and water temperature (H_2OT) were measured with k- type thermocouples connected to a Datalogger and data was collected using the software “TestLink SE520”; all were supplied by TC Direct. The aim was to achieve a BPT similar to the standardized QUV test devices, i.e. not exceeding 60 °C, therefore, the appropriate distance between lamp and specimens was found to be 20 cm.

A tap with two outlets was installed at the bottom of the container. One outlet was connected to the pump, the second tap was used to facilitate sampling of the water. Close to the top of the container, a small camera was fit in order to verify the water level, the functioning of the spraying nozzles and the specimens without opening the test chamber.

The biggest challenge of designing the device turned out to be the sterility in the test chamber. Sampled water pipetted on agar plates showed bacterial growth present after 3 days in this initial version of the apparatus. Therefore, several studies were carried out and are shown following in a chronological order.

7.2 Sterility of water in the presence of wood

The aim of this test was to find out how long a sealed, sterile box, containing water only as a degrading factor (excluding light source), would remain sterile whilst in the LFB.

7.2.1 Procedure

Gamma sterilized Douglas fir samples with dimensions of 50*50*10 mm^3 (Longitudinal x tangential x radial) were placed in plastic containers that had been previously sterilised with 70 % ethanol. About 500 ml autoclaved and deionized water was added to the container so that samples were submerged halfway. This allowed the observation of both surfaces completely submerged in water, where oxygen levels may be low, and humid surfaces, where oxygen levels are probably higher. The sterile container was placed in a LFB and a second "non-sterile" container that also contained autoclaved deionized water and sterilised Douglas fir samples was kept outside the LFB, but, in the same laboratory. After different exposure times (24h, 3d, 4d, 7d… 2 months) the absence/presence of microorganisms was verified by surface contact testing (SCT). Both sides of the specimens (+oxygen/-oxygen) were pressed for 5 sec onto PC- or ME-agar. After 24h (PC-agar) and 5 days (ME- agar) incubation time at 27 °C, the colony forming units (CFU) were counted. Strains were purified, dyed with methylene-blue and observed with a light microscope. Moreover, scans of the wood specimens were performed for visual observation.

7.2.2 Results

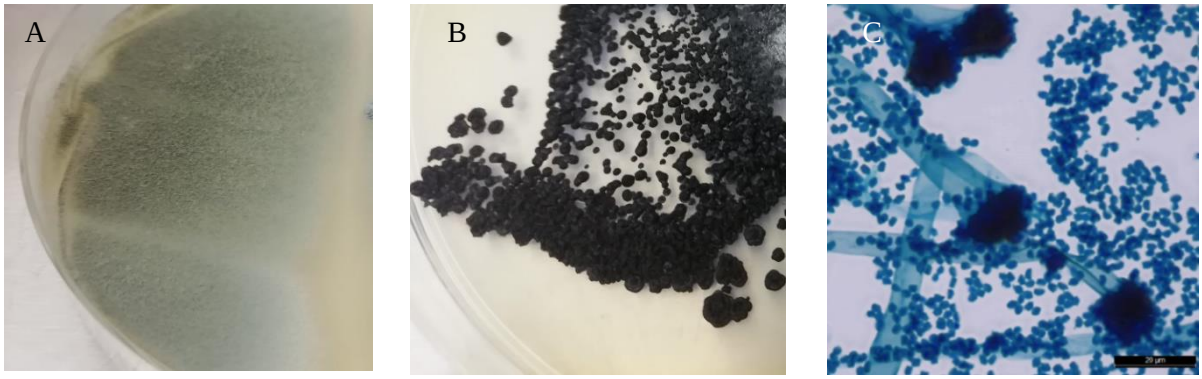


Figure 79: ME- agar in contact with non-sterile Douglas fir's surfaces low in oxygen (A) and high oxygen (B). Observation of fungi recovered from Douglas surfaces with low oxygen level (C) with the light microscope

The sterile container filled with sterile water and Douglas fir, kept in the LFB, stayed sterile for the whole time of the experiment (two months). No microorganisms grew on PC-agar nor the ME-agar. Containers, which were kept outside the LFB, showed bacterial growth after 3 days on PC-agar, and fungal strains were detected on ME-agar. After 3 days the dishes that were in contact with the oxygen rich surfaces of Douglas fir specimens, showed less fungal growth compared to those that were in contact with the water submerged surface. Moreover the surface completely submerged in water showed more growth of green coloured fungi whilst the oxygen rich surface contained more black

fungal growth. From day 5 onwards, CFU on ME-agar was similar for both the oxygen rich and poor faces.

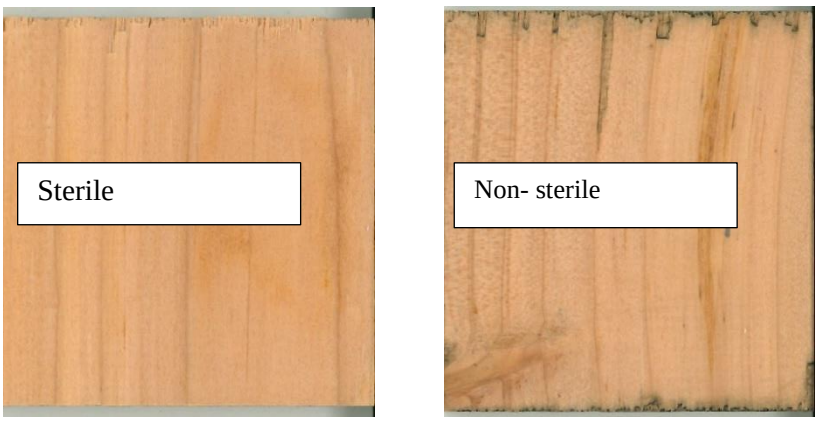


Figure 80: Scans of water exposed Douglas fir stored in sterile (left) and non-sterile (right) conditions for 2 months

The non-sterile exposed samples showed discolouration patterns starting from the transverse faces, whilst sterile samples did not show any changes visible to the naked eye.

7.2.3 Summary

- The sealed containers holding small amounts of water and wood blocks may stay sterile for at least 2 months when kept in a LFB. This indicates that the sterilisation techniques used should permit a long-term, sterile artifical weathering experiment.
- As Diano (2007) suggested, we could demonstate as well, that oxygen levels affect the morphology as well as physiology of fungal strains developing in water.
- By means of visual observation, the recovered fungal strains might belong to the genus *Aspergillus*. The fungi growing in low oxygen environment may be identified as *Aspergillus nidulans* and the strain in environment with higher oxygen level look alike *Aspergillus nigra.*
- The scans of the non-sterile wood specimens show decolouration patterns which are absent when kept in sterile environment.
- Fungal invasion started from the transverse face, where tracheid lumen are easily accessible.
- Even though this experiment demonstrates the long-term sterility inside a LFB, it needs to be verified if sterile conditions remain within the designed test device.

7.3 Sterility in the artificial weathering test device

This experiment was carried out in order to verify if conditions may remain sterile during exposure in the designed device, as shown in Figure 85. This test is therefore including spray water as well as a light source as degradation factors.

7.3.1 Procedure and results

The test chamber was cleaned with 70 % ethanol and placed in the LFB. The water circuit was sterilised with Milton's reagent, then ethanol and finally rinsed with pure sterilized water. The system was consequently filled with 4 l deionised and autoclaved[1] water. Gamma sterilized wood samples were placed on the sample grid in the test chamber.

The sterility of the system was verified by sampling water from the test chamber and plating it on PC- and ME- agar. Therefore 200 µl of the water were pipetted onto agar plates and homogeneously spread with a Drigalski spatula.

ME-agar dishes showed no fungal growth. Unfortunately, bacteria developed on PC-agar after 3 days of running the system. Consequently, a UVC sterilisation lamp (304 stainless steel ultraviolet water disinfection system, with a 16 W lamp and a flow rate of 2 GPM, from the manufacturer REALGOAL), was integrated into the water circuit, which was hoped to maintain sterility, yet again bacteria appeared in the water after 4 days.

7.3.2 Summary

- Conditions in the sterile artificial weathering device are unfavourable for fungal growth, but, allow bacterial strains to develop.
- A possible explanation for this is that the wood/water ratio is not high enough, so antimicrobial substances of the tested wood species are too diluted during the experiment. At least 4 l of H_2O are needed to circulate the water with the pump, whilst for the sealed containers were filled with only about 500 ml.
- The hypothesis is set that bacterial spores are, even after application of sterilisation methods, present, but antimicrobial properties of wood hinder the growth.
- The installation of a UVC lamp did not affect the sanity of the water in the test chamber.
- There is a high chance that the recovered bacteria may be identified as *Bacillus subtilis* (Uncertain identification through Maldi-TOF was performed).
- It was hoped that sterile conditions in the self-built weathering apparatus can be achieved without the help of chemical agents, however, this does not seem possible within this setup.

[1] Sterilised at 121 °C and 2 bars for 30 min

7.4 Impact of sodium azide on wood and water sterility

The final approach was the use of sodium azide (NaN_3), in order to maintain sterile conditions throughout the experiment. The aim of this test was to use the lowest NaN_3 concentration possible to minimize the chance of degradation effects on the wood, but at the same time maintain sterility.

NaN_3 prevents cells from using oxygen, which results in cell death if the concentration is appropriate (National Center for Environmental Health 2018). It is known to be very toxic to humans, fatal doses are ranked at approximately 10 mg/kg via oral intake (PubChem 2020). The chemical is known to increase the pH-value (Skipper and Westermann 1973; Wolf et al. 1989), however the effect of NaN_3 on wood itself is unknown, which is one reason to carry out this pre-experiment. Moreover, there is information about potential reactions of NaN_3 with metals which might lead to gas formation and a consequent explosion (National Center for Environmental Health 2018). It needs to be clarified at which concentrations these reactions take place, since view of the connection parts of the weathering device contain metal pieces. Published studies demonstrate that NaN_3 concentrations of 0.01 % were not sufficient to stop bacterial growth (Forget and Fredette 1962), whilst 0.02 % showed good inhibitory effects against *Bacillus* strains but did not hinder other types, e.g. *Salmonella* (Lichstein and Soule 1944). This experiment, monitoring the sterility of water in presence of wood, and observing effects with wood and metal, was carried out with NaN_3 concentrations of 0.02%.

7.4.1 Procedure

Figure 81: Jars filled with pure water or 0.02 % NaN_3 and wood blocks or metal piece

For the 1 molar (M) NaN_3 stock solution 6.5 g of NaN_3 powder (from Fisher scientific, CAS: 26628-22-8) were dissolved in 100 ml H_2O. Consequently 921 µl of the 1 M solution were added to 300 ml H_2O in the jars, in order to achieve a 0.02 % NaN_3 solution.

Autoclaved Douglas fir and oak wood blocks with dimensions of 50*30*10 mm^3 (Longitudinal x tangential x radial) as well as a metal piece (used as a connection element in the self-built weathering device) were placed in plastic jars filled with 300 ml sterilized water.

The presence of microorganisms in the solutions was evaluated on a weekly basis. Therefore 200 µl of the solutions were pipetted onto agar plates and homogeneously spread with a Drigalski spatula.

After 5 days incubation at 27 °C the number of CFU was assessed. Moreover, on each wood specimen 3 colour measurements were carried out after letting them dry for 2 days in a fume hood. Before and after 1 month of exposure the wood specimens were scanned, allowing visual investigation.

7.4.2 Results

The following table represents the microbiological growth in the solutions on PC- and ME- agar dishes. None of the solutions with 0.02 % NaN_3 showed any fungal or bacterial growth up to 1 month of exposure. After 1 month a few colonies started to develop on dishes where NaN_3 was present in the solutions. The jar containing pure water and oak specimen did not show bacterial growth during 1 month, whilst the jar with pure water and Douglas fir showed remarkable bacterial growth even by day 3 of the experiment. Fungi developed rapidly on both wood species in jars without NaN_3. No reactions between the NaN_3 solution and the metal part was detected.

Table 19: CFU after 1 month in water with and without 0.02 % NaN_3 in combination with wood specimens and metal parts. - : No growth, +: View colonies, ++: Remarkable growth, +++: Very strong growth

Solution	Agar	Sample	CFU
NaN_3	PC/ ME	Douglas	-
NaN_3	PC/ ME	Oak	-
NaN_3	PC/ ME	Metal	-
H_2O	PC	Douglas	+++
H_2O	PC	Oak	-
H_2O	ME	Douglas	++
H_2O	ME	Oak	++

Mean delta values of the colour coordinates (Lab*) were calculated. The colour changes over 4 weeks were plotted in Python and are shown in Figure 82. In general, oak shows a higher change in colour (ΔE) compared to Douglas fir. The difference in colour between sterile and non-sterile exposed Douglas fir is marginal. Non-sterile exposed oak wood however changes the colour drastically compared to the sterile oak. This strong change in non-sterile oak can mainly be explained by the decrease of the L* coordinate which drops considerably. The a* and b* colour-coordinates decline when oak is kept in the NaN_3 solution, while these parameters rather increase when oak was exposed to pure water only.

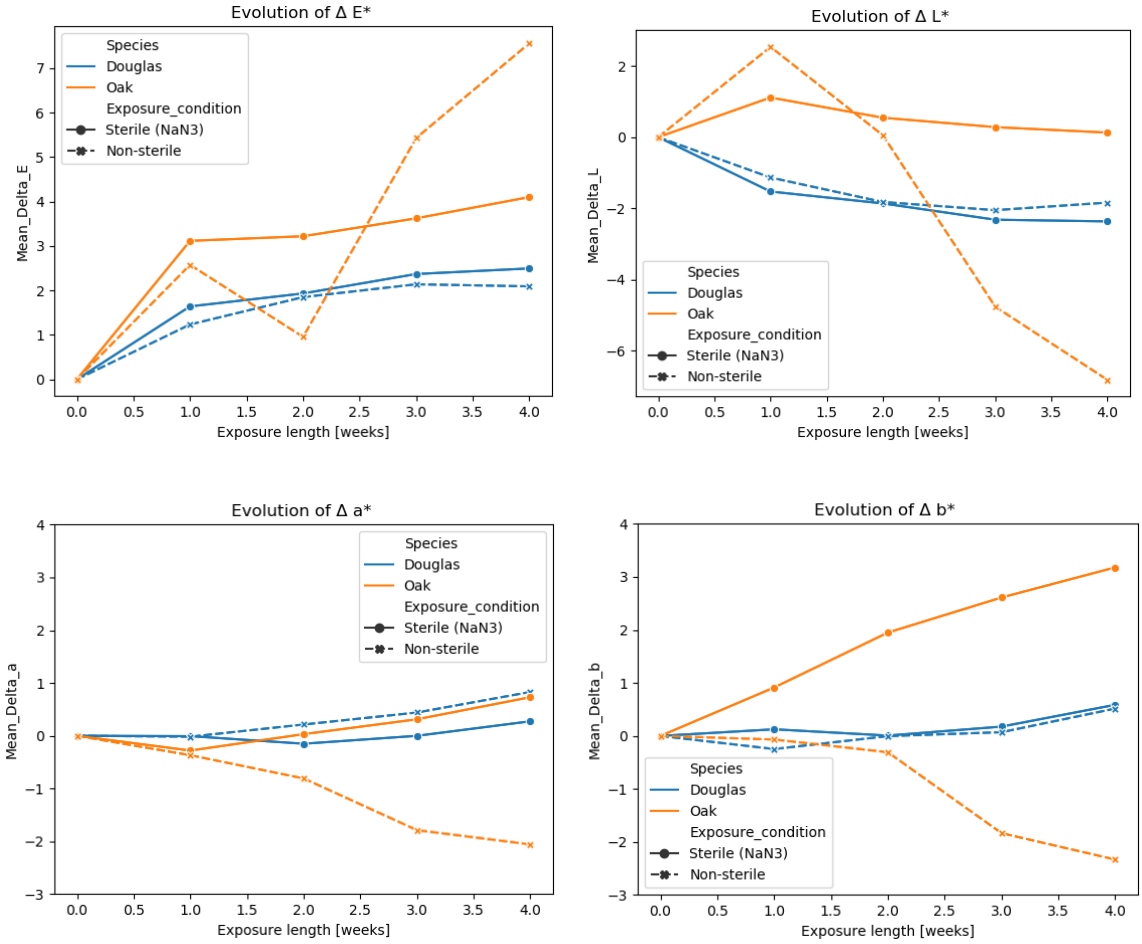


Figure 82: Evolution of the colour coordinates of wood samples exposed to pure water and 0.02 % sodium azide

Figure 83 shows scans of the wood samples before and after exposure of 1 month. The samples kept in sterile environment show only a slight lightening of the surfaces, the non-sterile exposed samples however show dark spots spread over the whole surfaces.

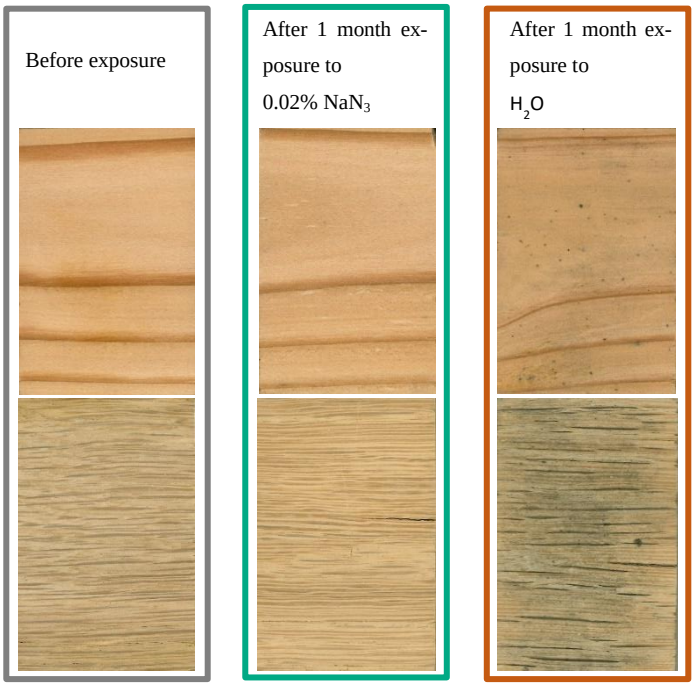


Figure 83: Scans of Douglas fir (upper row) and oak wood (lower row) before and after exposure to water and 0.02% NaN_3

7.4.3 Summary

- Solutions with 0.02 % NaN_3 kept sterile for one month in the presence of wood blocks.
- Solutions with 0.02 % NaN_3 showed bacterial growth after one month. It is assumed that in this environment the NaN_3 is degrading from week 4 onwards
- Oak has, compared to Douglas fir, stronger inhibitory effects against bacteria. The extractives as well as the acidity of oak wood are likely to play an important role.
- Incubation time of the petri dishes was 2 days longer than in previous experiments. The presence of NaN_3 in the sampled solution plated on the agars is likely to slow down microbiological growth.
- The a*- value, likely representing extractives (Gierlinger et al. 2004) decrease in oak in non-sterile environment, whilst with NaN_3 no significant changes were noticed. Therefore, the question arises: Is there a higher decomposition of extractive in oak in the presence of microorganisms as extractives are used as a nutrition source?
- The b*-value in oak, representing lignin, decreases in non-sterile conditions and increases when kept sterile. Possible explanations are reactions of NaN_3 to the acidity of oak wood.

- According to visual observation it might be possible to neglect the impact of NaN_3 on the tested wood species.
- Results of colour measurements and scans go hand in hand. However, the decolouration patterns due to microorganisms can't be easily detected when observing colour coordinates only.

7.5 Conclusion and outlook

The experiments presented in this chapter demonstate that the presence of fungi changes the appearance of wood surfaces drastically. Yet, there is not enough evidence to conclude the same for bacteria.

Oak wood showed, compared to Douglas fir, stronger inhibitory effects towards bacteria. Other studies have demonstrated good antimicrobial properties of oak wood as well (Milling et al. 2005; Pailhoriès et al. 2017). The acidity (Aviat et al. 2016) of oak, the high amount of extractives (Schönwälder et al. 2002) as well as the porosity (Gilbert and Watson 1971; Abrishami et al. 1994) have been shown to be crucial factors in amplifying bio-inhibitory properties.

It has been shown that for oak wood at water: wood ratios of ~300:40 the water remains sterile. At higher water: wood ratios, such as those in the sterile artificial weathering device, bacterial growth can develop. This phenomenon might also be a result of the antimicrobial properties of the wood itself as low concentrations of extractives might not be sufficient to inhibit bacterial growth.

The mechanisms responsible for the resistance of spores to various sterilization methods is complex and still not well understood (Setlow 2006). Contamination in the self-built apparatus might arise through the circulation of water, since the pump sits outside the LFB. Furthermore, bacterial spores may remain present in the water or wood matrix even after autoclaving or gamma sterilisation. Wet heat may inactivate core enzymes in spores but does not necessarily damage their DNA (Setlow 2006). Gamma irradiation is considered to sterilize soils, which has been verified by the absence of cultivable bacteria, however plate-culture methods are suggested to be unreliable to verify the activity of microbial communities (Buchan et al. 2012). Even though UVB and UVC light is known to have germicidal effects (Kowalski 2009), the lamps did not show sterilizing reactions. Wavelength, irradiation force and turbidity of the water are only view of the parameters influencing the penetration depth of light in water. On the one hand, light might not be traveling into the depth of the water reservoir and has therefore no sanitizing impact. On the other hand, a resistance of microorganisms to UVC inactivation may develop (Dai et al. 2012). Moreover, the water reservoir in the self-built apparatus might not allow to circulate the water homogeneously. The water might stagnate at the bottom of the reservoir where cells adhere which then leads to biofilm formation (Vert et al. 2012).

It is suggested that the recovered bacteria may be identified as *Bacillus subtilis*, which is known to be a widely adapted bacterial species, growing in many environments- terrestrial and aquatic (Earl

et al. 2008). The *Bacillus* strain is highly resilient to high temperatures and only little is known about the resistance against gamma sterilisation (Setlow 2006). Avoiding the bacteria to develop is therefore rather difficult. Active metabolites produced by strains e.g. *Bacillus* have shown antifungal activity against wood blue stain fungi (Feio et al. 2004), which might be one of the reasons for the absence of fungi in the presented experiments.

Concentrations of 0.02% NaN_3 proved to slow down bacterial growth. NaN_3 however is degrading with time and concentrations need to be topped up if conditions shall remain sterile. Especially if NaN_3 concentrations are greater than 0.02 %, side effects of NaN_3 on the visual aspect of wood and particularly oak wood are possible. Still, the use of NaN_3 is, so far, the closest setup to maintain "almost" sterile conditions. Therefore, the accelerated weathering experiment in the self-built apparatus, eliminating the impact of biotic degrading factors, was conducted with initial concentrations of 0.02% NaN_3 and is described in the following **Chapter 8**.

8. Chapter: Control of biotic and abiotic factors during weathering

Up to now, there are no standards to degrade wood in controlled and sterile environments, therefore a weathering test apparatus was built. The biggest challenge of designing the machine was to maintain sterile conditions throughout the experiment. I would like to refer to **Chapter 7**, which describes precisely the setup of the machine and how sterile conditions were tried to be achieved. The experiments presented in this chapter were carried out in order to test the self-built weathering device, to see if conditions within it remain sterile, if the abiotic degrading factors are sufficient to accelerate degradation and to observe if there are undesirable effects caused by sodium azide. Following, the abbreviations SAWE for "Sterile artificial weathering experiment" and NSAWE for "Non-sterile artificial weathering" are used to distinguish the two setups in the self-built weathering device.

8.1 Material and methods

Quercus petraea and *Pseudotsuga menziesii* specimens of 50*50*20 mm^3 (longitudinal* tangential*radial) were cut, the tangential face was planed and consequently gamma sterilized (see **Chapter 3.11.2**). 10 specimens of each species were exposed, half to SAWE and the other half to NSAWE.

On a weekly basis the solution plating technique was applied in both devices (n=3) to assess the microbiological contamination (see **Chapter 3.11.3.3**) and on each specimen 4 colour measurements were conducted (n=40 for each wood species). Wood specimens were observed visually via scans and with the ESEM (n=1). Moreover, XRF analysis was carried out as described in **Chapter 3.9**.

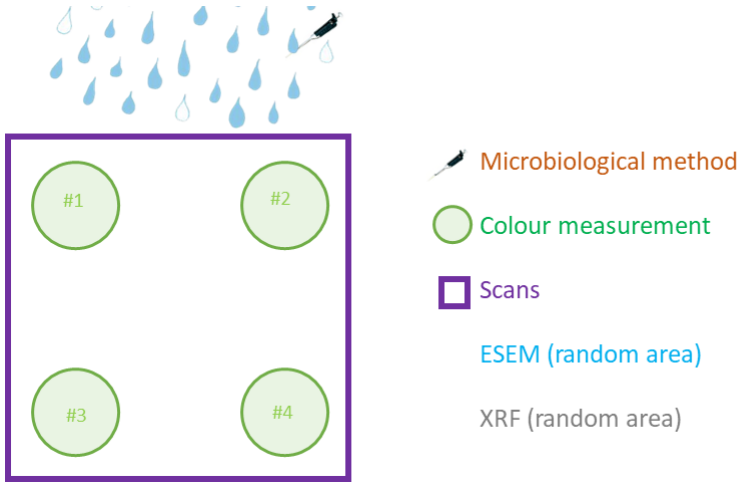


Figure 84: Scheme of a SAWE / NSAWE specimen

8.2 Weathering method

Figure 85 shows a scheme of the artificial weathering device to expose specimens to controlled conditions. A duplicate of the device was kept outside the LFB and provides a comparison to non-sterile degradation. The SAWE test chamber was cleaned with 70 % ethanol and placed in the LFB. The water circuit was sterilised with ethanol and consequently drained with pure sterilized water. The

system was filled with 4 l deionised and autoclaved water. Sterile wood samples were placed on the sample grid in the test chamber; with a slight descending angle so exceeding water can drain off; as shown on the right in the figure below.

As mentioned in the previous chapter, it was challenging to achieve and maintain sterile conditions, the utilisation of sodium azide was proposed. It was suggested (see **Chapter 7.5**), that concentration of NaN_3 degrade with time, therefore the initial 0.02 % NaN_3 solution was topped up on a regular basis, by finally adding 45 ml of 0.02 % NaN_3 to the SAWE device in total.

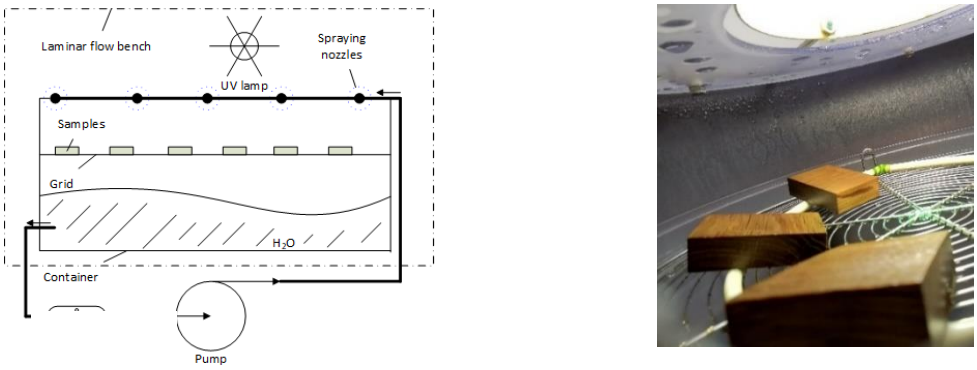


Figure 85: Scheme of the sterile artificial weathering test device (left), and exposed oak specimens (right)

The two wood species were exposed separately to SAWE and NSAWE. Weathering cycles alternating 2.5 h water spraying and 0.5 h UV irradiation, were applied for 1 month, as shown in Table 20. Temperature was measured continuously in both devices. The water temperature (H_2OT) was measured in the range of 22 °C- 34 °C and the black panel temperature (BPT) between 24 °C and 60 °C. Recorded temperatures during SAWE and NSAWE are shown in Figure 86.

Table 20: Applied conditions during SAWE and NSAWE cycles

Weathering technique	***Step***	***Function***	***Specification***	***BPT***	***H_2OT***	***Time [h]***
SAWE/ NSAWE	1	UVA/UVB	0,89 W/m²/nm	60 °C (max.)	34 °C (max.)	2.5
	2	Spray	7.5 l/h	24 °C (min.)	22 °C (min.)	0.5

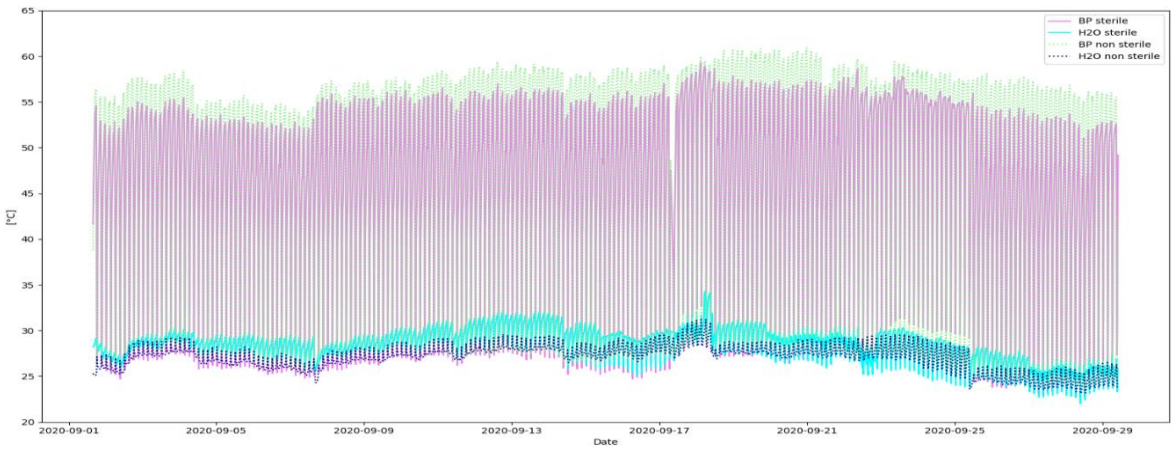


Figure 86: Recorded BPT and H_2OT during SAWE and NSAWE

8.3 Results

Following the results of the applied measurements are shown. Graphs were plotted in Python.

8.3.1 CFU

The graphs in Figure 88 and Figure 90 show mean values (n=3) of the CFU counts on PC- and on ME-agar via the solution plating technique. Only limited number of replicates were tested as the aim was to see whether or not contamination occurs. These graphs are supported by pictures of the petri dishes in Figure 87 and Figure 89.

The SAWE with Douglas fir showed some bacterial growth until week 2 followed by strong bacterial growth. SAWE where oak wood was exposed, showed only moderate growth throughout the duration of testing. Whereas the NSWE showed strong bacterial growth throughout the exposure.

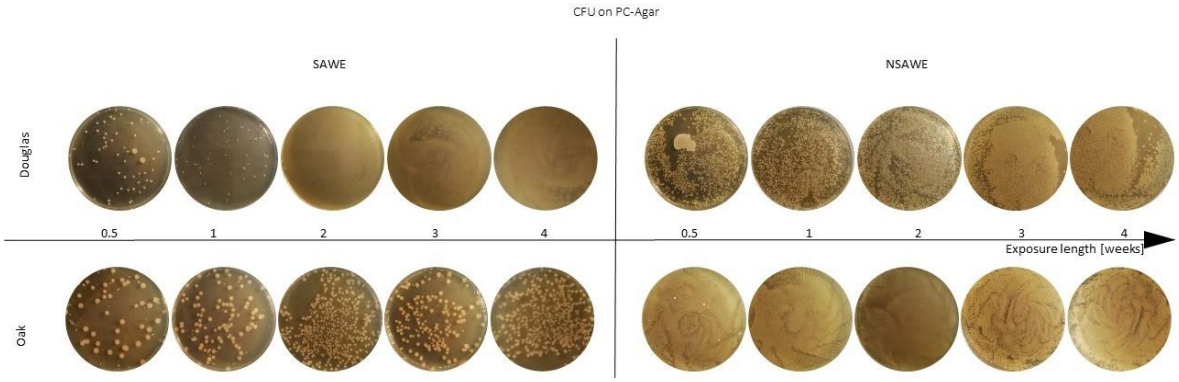


Figure 87: Pictures of PC-agar during SAWE and NSAWE

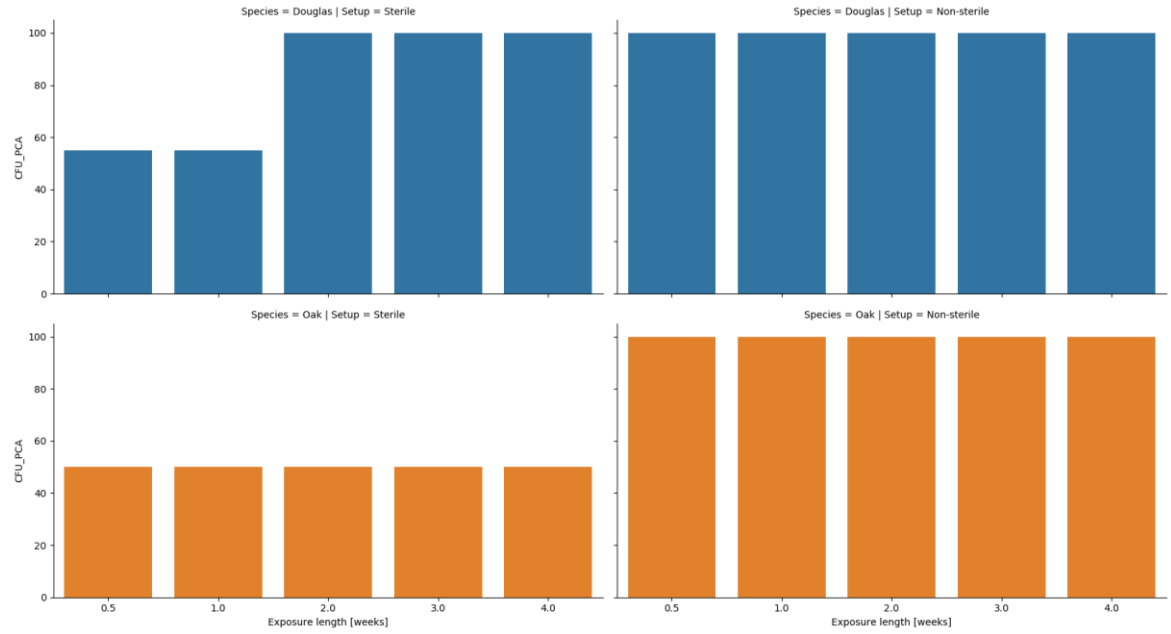


Figure 88: CFU counts (mean values n=3) on PC- agar when exposed to the self-built weathering apparatus

The SAWE with Douglas fir showed fungal growth starting from week 2 of exposure, whilst in the presence of oak wood insignificant amounts of fungal colonies developed. The NSAWE showed petri dishes covered in fungi throughout the exposure with Douglas fir as well as with oak wood.

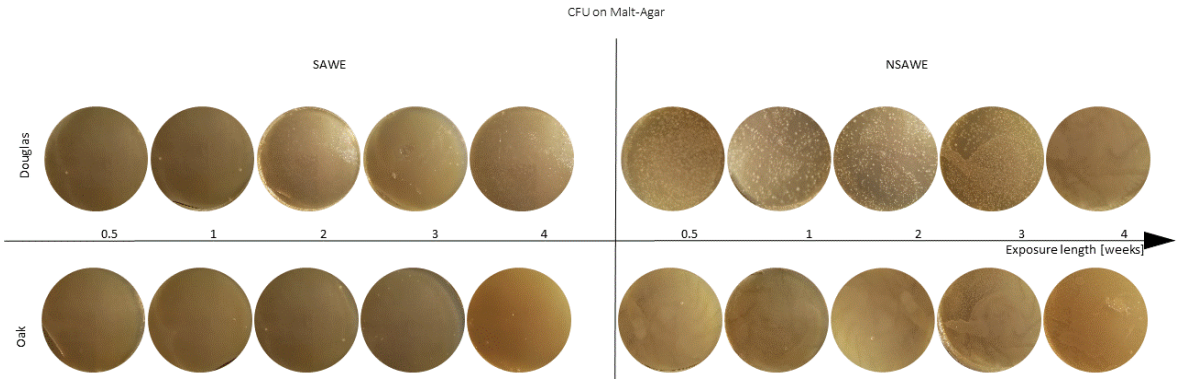


Figure 89: Pictures of ME-agar during SAWE and NSAWE

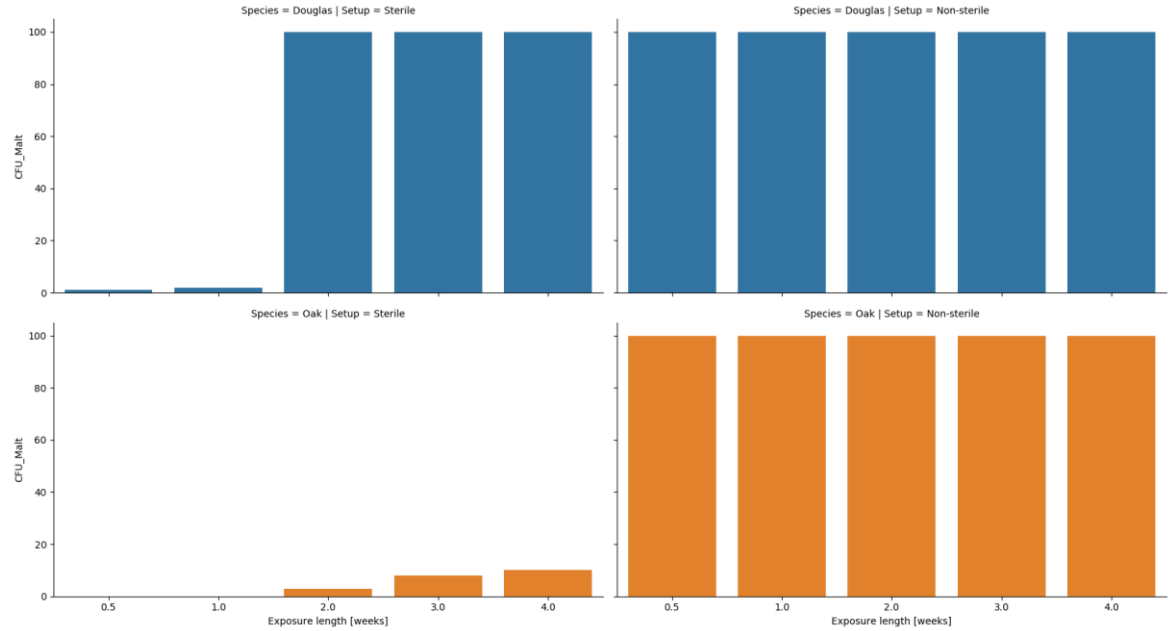


Figure 90: CFU counts (mean values n=3) on ME- agar when exposed to the self-built weathering apparatus

8.3.2 Colour

Mean values (n=40) of the Lab* colour coordinates as well as the ΔE* were calculated for each wood species and exposure scenario. The colour change after 4 weeks of exposure is shown in Figure 91. Table 21 present delta values (after exposure- before exposure) of the colour coordinates Lab. Oak shows more pronounced changes in colour (L*, b* and ΔE*) compared to Douglas fir. SAWE exposed wood changed the colour considerably compared to the non-sterile setup.

Table 21: Delta colour parameters after 1 month exposure to SAWE and NSAWE

Sample\ Delta values of colour coordinates	***ΔL****	***Δa****	***Δb****	***ΔE****
Oak NSAWE	-14.4	+1.1	+5.6	+15.6
Oak SAWE	-27.0	-1.7	-5.3	+27.8
Douglas NSAWE	-10.4	-2.5	+0.2	+11.0
Douglas SAWE	-15.3	-7.7	-7.6	+19.3

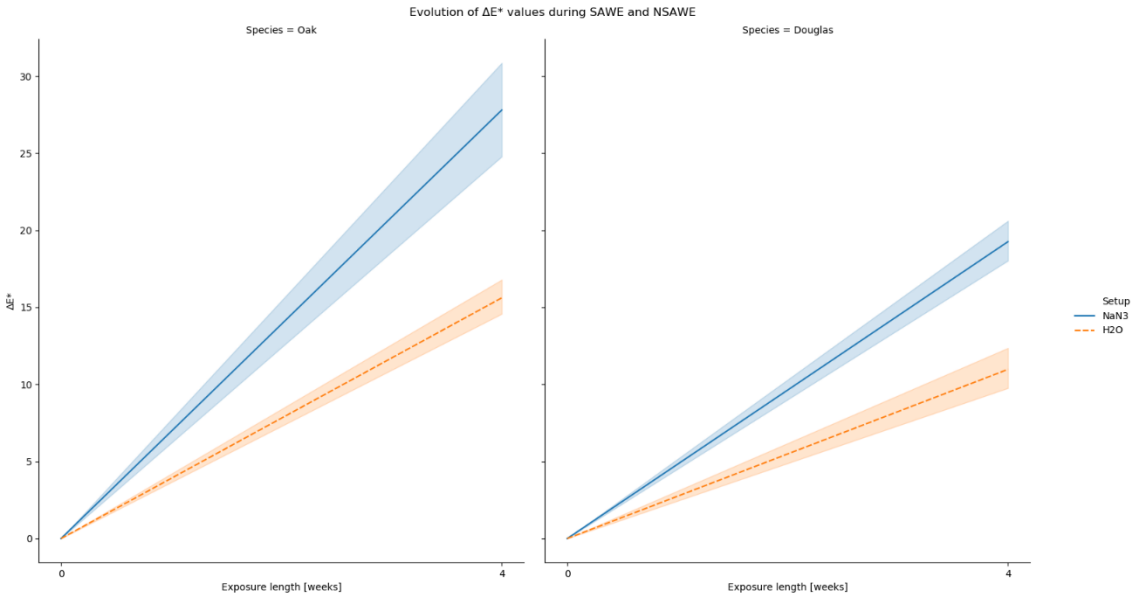


Figure 91: Evolution of ΔE during SAWE and NSAWE*

8.3.3 Scans

Samples exposed to a non-sterile environment show a more pronounced yellow colour. Douglas fir exposed to SAWE appears more silvery and latewood areas are more apparent. Oak exposed to SAWE shows very severe heterogeneous discoloration and some areas appear completely dark.

Figure 92: Scans of Douglas and oak specimens before (left), non-sterile (middle) and "sterile" (right) exposure

8.3.4 ESEM

The samples exposed to the self-built weathering machine show all degradation patterns such as defibration and cracking. Pictures taken from specimens exposed to the "sterile" device show less particles on the surfaces and appear therefore "cleaner". Douglas fir shows only minor differences comparing the non-sterile and "sterile" setup. Helical thickenings seem to degrade faster in the setup where NaN_3 was present. "Sterile" exposed oak wood however showed much stronger degradation patterns. As shown in the lower right picture in Figure 93, one can observe the brittle oak wood structure, reminding of a heavily dried –out soil.

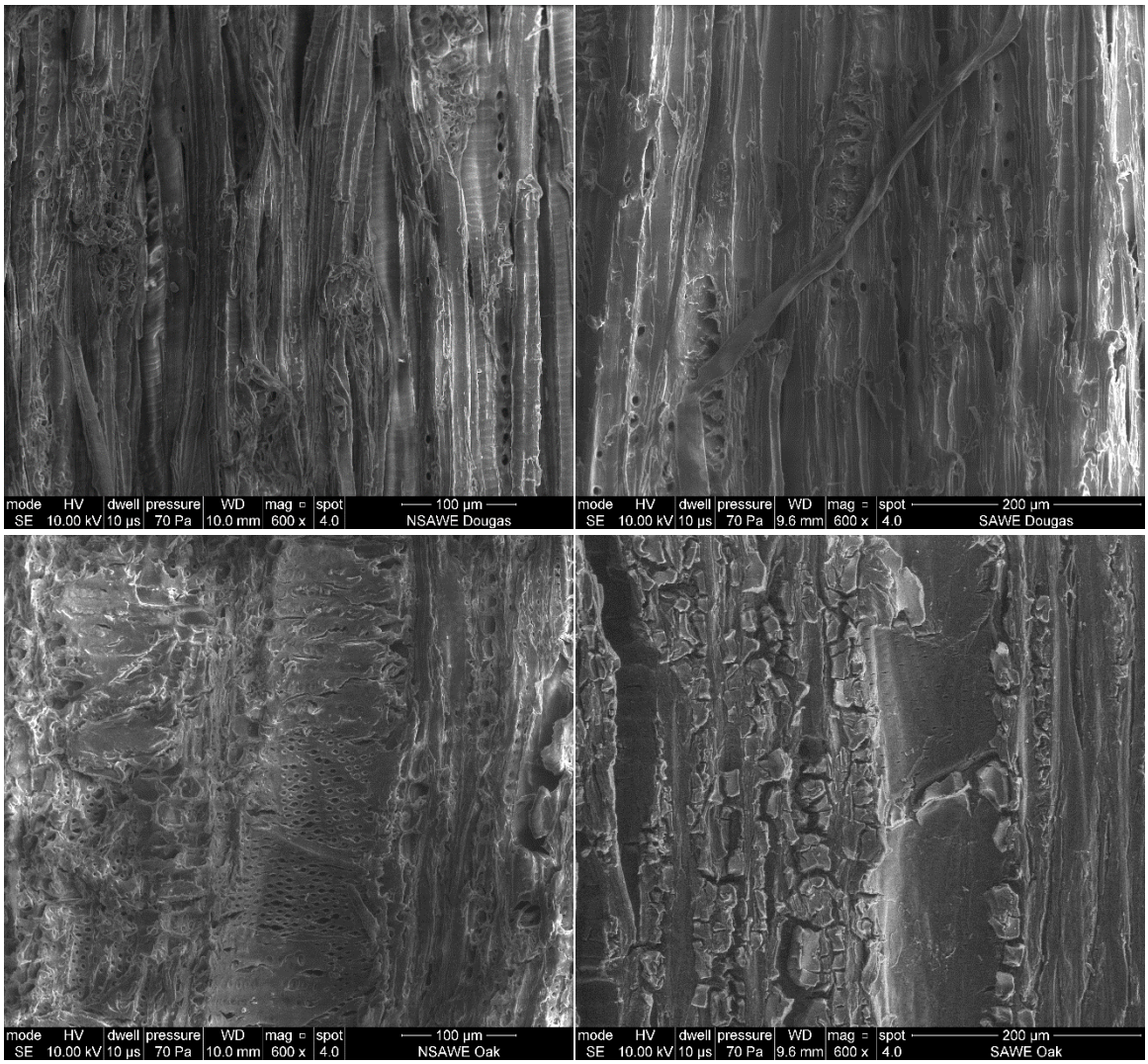


Figure 93: ESEM observation of Douglas (upper row) and Oak (lower row) exposed to NSAWE (left) and SAWE (right)

8.3.5 XRF

The XRF spectroscopy revealed the presence of copper, zinc and calcium on exposed wood surfaces. Copper (Cu) and zinc (Zn) levels are more elevated in oak wood compared to Douglas fir and the SAWE setup shows higher levels compared to NSAWE. No traces of Cu and Zn were found on the reference samples. The surfaces directly exposed to the spray shows significantly higher levels compared to the back side of the specimens (not exposed to spraying but condensation). Higher calcium (Ca) values were detected on SAWE exposed samples. Traces of manganese (Mn); mainly on Douglas, nickel (Ni); mainly on oak, selenium (Se); mainly on Douglas fir samples, strontium (Sr), antimony (Sb) and lead (Pb); only in exposed samples, were detected.

Sample\ Element [ppm]	*Cu*	*Zn*	*Ca*	*Mn*	*Ni*	*Se*	*Sr*	*Sb*	*Pb*
Douglas NSAWE	134	387		55	12	2	2	51	12
Douglas SAWE	244	185	1545	55	11	2	3	49	9
Douglas Ref.				81		2	3	51	
Oak NSAWE	463	377	1370		43	2	2	43	14
Oak SAWE	1451	1128	1523		11		3	44	35
Oak Ref.				46	10	3	2	54	

Figure 94: Elements detected on samples exposed to NSAWE and SAWE as well as samples before exposure (Ref.)

8.4 Summary and outlook of NSAWE /SAWE

Fungi developed in SAWE when Douglas fir was exposed, no fungal growth was detected in the presence of oak wood. Antimicrobial properties in oak wood may hinder fungal strains to develop. Bacterial spores still developed in the presence of both wood species. Microbiological contamination in the SAWE was nevertheless lower compared to the NSAWE setup. Bacterial strains developing in the SAWE/ NSAWE are resistant to the applied UV irradiation as well as BPT of up to 60 °C. NaN_3 may show acid effects on wood and evoke catalysis of wood. Hence low-molecular weight parts of wood may be more accessible for bacteria to feed on which encourages bacteria to develop.

Remarkable amounts of Cu and Zn were found on wood surfaces exposed to SAWE. Multiple connection parts in the self-built weathering device are made out of brass, which might explain the presence of Cu and Zn. Ca seems to be formed in the presence of NaN_3 since it was mainly detected in SAWE setup. Pb contamination of SAWE exposed oak wood reached, according to the German waste wood ordinance, the allowed limit (BMJV 2002) and all exposed samples showed very high Cu contamination (BMJV 2002; European Panel Federation 2002), recycling of the wood is prohibited.

Bacterial strains such as *Bacillus sp.* are known to be resistant to certain lead concentration and form proteins (Arifiyanto et al. 2017). The activity of some heterotrophic bacteria is governed by the availability of inorganic nutrients including Cu, Fe, Mn and Zn. These metals facilitate a variety of biological pathways in prokaryotic cells (Posacka et al. 2019) and encourage bacterial growth.

The water used in SAWE was autoclaved, a sterilisation method that does not necessarily damage bacterial DNA (Setlow 2006), thus spores might have been present in the water throughout the whole experiment.

This experiment showed that NaN_3 does have an impact on wood, especially lignin in oak seems to be affected since the b* colour coordinate decreased significantly. Elements such as Ca and Ni, known to be reactive metals forming dark oxide nitride layers when exposed to air, may play a major role in the way the wood colour changed, especially in the presence of oak wood, rich in extractives.

Scans and ESEM observations confirmed that microscopic degradation patterns are more present in SAWE compared to NSAWE samples.

The present work demonstrates that the use of 0.02 % NaN_3 in the water circuit in the self-built weathering device is not appropriate neither to maintain sterile conditions nor to investigate the phenomenon of wood weathering as the chemical itself has an impact on the wood material.

Following some suggestions in order to improve the experimental design, in order to artificially weather wood excluding biotic degradation factors, are shown, which is hoped to help future investigations.

8.4.1 Proposals to develop the experimental design of the weathering device

The established spraying system applied in the self-built weathering device, with a pump of a max. flow rate of 11.3 l/min and 5 spraying nozzles gave satisfactory wetting patterns. The UV light source may not have been powerful enough (100 W), however the desired temperature settings were achieved and kept stable.

Various abiotic and biotic degradation parameters were chosen within this experimental setup, however, minimizing abiotic degradation by the exclusion of UV irradiation and spraying cycles may simplify the experimental design by far.

Within this thesis, the self-built weathering device was not completely kept in a sterile environment, only the test chamber was placed in a LFB. The pump and electrical alimentations were placed outside the LFB, where cross contamination is not to be excluded. Placing all the equipment in a sterile environment may facilitate to test in the desired scenario. As mentioned, autoclaving water may not kill all microorganisms. Purchasing sterile deionized water may solve the contamination issue.

Also, manipulations in the LFB at the same time the experiments are running, may induce microbiological growth. In order to execute the experiment in the proposed setup, a proper microbiological laboratory with access to e.g. sterile incubators may reduce cross contamination as well.

Another experimental proposal does not necessitate a sterile environment. Selection of specific bacterial and/or fungal strains/ communities to inoculate the wood specimens with, and recover microorganisms by means of selective agars, enables investigations of e.g. survival rates, synergistic or antagonistic behaviours and might help getting a deeper insight about the impact of those selected microorganisms on wood.

8.4.2 Proposals for methods to apply

Setting and monitoring the temperature, MC and last but not least the pH-value is crucial in order to understand enzymatic degradation. Moreover, measurements of N/C- ratios may be a helpful tool.

The above proposed experimental setups demand access to microbiologic identification techniques. Identification through morphologic aspects is not reliable. DNA sequencing shall be favoured, since it allows precise identifications and gives hints about enzymatic activity. If this subject wants to be understood, sequencing is probably one of the most important and powerful tools. Databases of fungal and bacterial strains on wood and wood-product´s surfaces need to be developed on a molecular level.

9. Chapter: General discussion, implications and future directions

A lot of experimental work was carried out and plenty of data was gathered for this thesis. The performed experiments confirm the findings of many research. Exposed to weathering, the molecular structure of wood modifies. The combination of chemical, mechanical and biological induced degradation may quickly lead to very severe colour changes, increase in roughness, detachment of fibres, and results in unattractive wood surfaces (Feist 1989; Dirol and Deglise 2001; Williams 2005). The colour of lignin is naturally dark and is strongly affected when exposed to light (Volkmer et al. 2016), which results in colour changes, especially the b* colour coordinate is affected (Timar et al. 2016). The polymeric components of wood are reduced to smaller compounds through a wide range of chemical reaction, some of which are accelerated by UV-irradiation. These smaller compounds can then be leached out through water spray or rain. Furthermore, wind or other mechanical stresses such as those induced by swelling and shrinkage contribute to a breakdown of the wood as well.

Following, the weathering experiments conducted within this thesis as well as recognized artificial and natural weathering correlation are presented. Moreover, the methods to recover microorganisms are discussed and last but not least identified microorganisms are discoursed.

9.1 Outdoor weathering

At the time of writing this thesis, the samples exposed to outdoor weathering are still on the weathering racks and so the continued evolution of degradation can be followed up in the future. On outdoor exposed samples, discolouration patterns due to microorganisms were observed with the naked eye after about 4 months; the same exposure length as when severe changes of colour coordinates were found. This implies that prior chemical degradation due to abiotic factors make wood a more suitable nutrition source for microorganisms. This statement corresponds as well to finding that with increasing wood decomposition, the higher the bacterial richness tends to be (Hoppe et al. 2015).

Logically, the range of microbial spores that have landed on a given surface will increase with time. The probability that these spores will successfully germinate is likely to be very dependent on the environmental conditions at the time of germination, but also, on the nutritional value of the surface, which will increase with time as debris land on the surface.

Whereas in terms of abundancy, the OWE showed decreasing bacterial and increasing fungal invasion with increasing exposure length, supporting findings by Kielak et al. (2016b), who suggested that the decreasing wood density was the decisive factor for this phenomenon. Bacteria are known to be primary invaders of wood (De Boer and Van der Wal 2007; Schmidt 2010) and responsible for an increase in the permeability (Greaves 1969; Clausen 1996), hence may facilitate fungal invasion in later stages of wood degradation. At the same time fungal dominance is likely to be attributable to antagonistic effects, such as the release of oxalic acids and decreasing pH (De Boer and Van der Wal 2007), and therefore responsible for the exodus of bacteria in later stages of exposure.

No significant difference of bacterial abundancy between Douglas and oak wood were found. The PCA demonstrated importance of meteorological conditions on the presence of microbes rather than the wood species. Fungi and bacteria forming on wood surfaces may be considered as communities that are able to adapt to harsh conditions, such as desiccation and starvation, forming a sort of synergistic network in order to re-emerge when conditions permit. This implies that the availability of nutrition is of less importance compared to environmental conditions such as humidity, temperature and UV- irradiation.

The PCA indicates however a strong correlation between exposure length and temperature, which may indicate a stronger relationship between CFU and temperature than with exposure length. Furthermore, according to the PCA, the wind is an important parameter in terms of microbial contamination, as spores are known to be transported with wind (Tignat-Perrier et al. 2019), more and even new organisms might invade the wood surfaces in rather unpredictable cycles.

Studying the importance of each single degradation parameter during weathering experiments outdoors, where conditions are unpredictable, is rather unrealistic, especially since it has been shown that climatic conditions are the most decisive ones for the development of microorganisms. Still such outdoor experiments are indispensable in order to gain information about abundancy, richness and the phyla of strains invading wood during weathering.

9.2 Artificial weathering in non- sterile conditions

Different artificial weathering techniques were applied, two of them showing only minor differences in temperature and UV- irradiation force, still the impact on the chemical decomposition and amount of CFU recovered from the samples was significantly different. Moreover, the experiment proved, that in the absence of liquid water, growth of microorganisms is very restricted. This observation emphasizes the importance of environmental conditions, as suggested by other researches (Schönwälder et al. 2002; Milling et al. 2005; de Hoon et al. 2010; Kielak et al. 2016b).

SR measurements collected in this work show that wood surfaces increase in roughness and when exposed for longer periods significant deformations such as warping can occur. In contrary to the steady evolution of SR parameters, ATR measurements showed that the length of exposure does not necessarily result in more severe chemical changes on the surface as phenoxy radicals are produced from phenolic hydroxyl groups, oxidation processes lead to formation of carboxyls, carbonyls and quinones (Williams 2005).

ATR analysis has been found to be a good tool for following the chemical decomposition of archaeological wood that was severely degraded by bacteria (Gelbrich et al. 2008). In the research presented here, however, ATR analysis did not permit to distinguish between abiotic or biotic factors decisive for decomposition. The same applies to when SR parameters were compared prior to after exposures.

Visual observation methods at various magnification levels gave insight into biotic degradation with respect to the presence of microorganisms. Although bacterial communities in heartwood might be more diverse (Zhang et al. 2008) they may be more abundant in sapwood (Jeremic et al. 2004), the latter can be confirmed due to microscopic and especially LM observations conducted within this experiment.

It is quite possible that many researchers assume that the conditions during an artificial weathering test are sterile because of the high UV doses that are often used. Experiments conducted as part of this research have shown that conditions in standardised artificial weathering devices, e.g. QUV, in fact do allow microorganisms to develop (Buchner et al. 2019). Research presenting findings about wood degradation in QUV devices, claiming to study the impact of the abiotic factors humidity and UV- irradiation (Petrillo et al. 2019), should consider that bio-deterioration is also present in artificial setups. Even though microorganisms developed on artificially exposed surfaces, it needs to be considered, that they may be very distinctive to those found in natural environments.

9.3 Artificial versus natural weathering

As contradictory findings, a decrease of fungal contamination in artificial setups and increase in outdoor exposure setups (particularly in the OWE), were observed, natural weathering cannot be replaced by artificial techniques. Bacterial strains predominant in artificial setups are likely to be very different to those found in natural environments. They may be e.g. very resistant to harsh conditions and release different sort of enzymes. Antagonistic effect may therefore be reversed in artificial setups compared to as during natural weathering and thus higher bacterial contamination (in terms of abundancy) was observed in QUVs.

9.4 Experimental work to achieve sterility

The results from experiments presented in **Chapter 7**, where an attempt at weathering in sterile conditions was made, show that oak has stronger bacterial inhibiting properties compared to Douglas fir. This is in agreement with other published experiments, where it is suggested that oak extractives are responsible for the anti-microbial behaviour (Schönwälder et al. 2002; Pailhoriès et al. 2017).

It was hoped to perform long-term wood weathering in sterile conditions with the weathering device presented in **Chapter 8**, however, sterility was never maintained throughout the experiment. Having said that, artificial weathering with limited presence of microbes was still achieved. The experimental study presented in **Chapter 7.2** was the only setup where sterile conditions were maintained throughout the experiment as the wood-water ratio was appropriate.

Antimicrobial effects of wood in small amounts of water were proven. The experiment moreover showed that microorganisms contribute to the degradation process of wood surfaces. The comparison

between sterile and non-sterile exposure of Douglas fir demonstrates that fungi changed wood´s visual aspect drastically. Decolouration started from the cross-section, where higher MC may prevail, resulting in stimulating bacterial (Gehrig et al. 2000; Milling et al. 2005) and fungal growth. The increase of MC on the transverse grain might be explained by the water entering from the open tracheids, into the lumen forward.

9.5 Artificial weathering in the presence of sodium azide

Concentrations of 0.02 % NaN_3 slowed down but did not completely stop the development of microorganisms. Even though fungi were not detected in the SAWE, bacterial strains did establish, thus the experimental setup was not suitable to study weathering in sterile conditions.

Moreover, the XRF identified remarkable amounts of inorganic elements such as Ca, Cu and Zn on the wood surfaces, especially on oak wood. The concentration of these elements was higher on the surfaces of wood samples directly exposed to the NaN_3 solution, which implies that it is corroding the equipment in some way. The high concentrations of elements on the samples surfaces might be due to complexing of metal ions with extractives which are continually circulated and sprayed on to the surface, thus potentially resulting in higher concentrations.

In turn, the presence of NaN_3 may affect the weathering process in other ways. It is known that Ca and Cu are required for several non-enzymatic decomposition processes of cellulose (Reinprecht 2016). The evolution of a darker colour on the oak specimens is contrary to that observed in natural weathering. It is possible that this is due to metal ions like Cu and Ca complexing with the extractives of oak to generate photochromic compounds. Oak is known to cause staining when in contact with various metals and this is thought to be due to the tannins complexing with metal ions since the XRF data indicates increased concentrations of metal ions.

Zn availability is crucial for heterotrophic bacteria's activity (Posacka et al. 2019). Moreover, Cu is known to have antimicrobial properties in proper concentrations and are therefore widely used in wood preservation. However, some strains tolerate elevated Cu concentrations which may even induce sporulation due to DNA repair mechanisms (Borkow 2012). Bacterial spores may have been present in the autoclaved water from the beginning on and may have created resistance against NaN_3. Nevertheless, it is unknown if the metals liberated during the SAWE contributed to preventing microbes from developing or rather emphasized sporulation.

Unfortunately, the data do not permit any conclusions on the relative impacts of biotic factors in this experiment as the abiotic factors and specifically the presence of NaN_3, are dominant. In addition, it would appear that the experiment would need to be conducted for a much longer time. In order to study the impact of biotic factors on wood, several proposals are shown in **Chapter 8.4.1**.

9.6 Recovery methods via plate counts

Through pre-experiments it has become evident that cutting homogeneous slices from weathered and deformed samples is very difficult, thus reliable LM observations are rather impractical. Consequently, microbiological methods were preferred and the quantity of microorganisms on wood surfaces was intended to be monitored with the help of plate counting. At the same time it was intended to further identify fungal and bacterial strains whilst a recovery technique is needed as a first step.

Even though plate count techniques are widely used to enumerate viable strains (Li et al. 2014), it is a time-consuming approach. Moreover, the use of plate counts due to the selection of specific agars is very selective, thus the whole spectra of organisms on the surfaces to be tested may not be captured. As doubts about these techniques already prevailed, various recovery techniques, the SCT (OWE), the scratching technique (NSWE) and the solution plating technique (SAWE/ NSAWE) were applied within this thesis. Due to this reason, a direct comparison of numbers of CFU from the different experimental setups was not possible. The CFU counts presented within this thesis may not be credible as there are many interfering parameters, however this attempt gave an overview of the practicality of the three selected techniques. Table 22 shows a comparison between the three recovery techniques.

Table 22: Comparison of the three applied plate count techniques

Recovery technique via:	***Surface contact***	***Scratching***	***Solution plating***
Techniques' characteristics:			
Fast	yes	no	yes
Non-destructive	yes	no	yes
Not only the surface layer	no	yes	
Low risk of cross contamination	yes	no	yes
Easily test equal amount of wood (particles)	no	no	
Impacting parameters:			
MC	yes	no	no
Surface properties (e.g. porosity)	yes	no	no
Bio inhibitory effects of extractives	no	yes	yes

The advantage of the recovery via surface contact is the fact that it is fast and non-destructive technique. As shown in the experiment in **Chapter 5,** CFU counts obtained by SCT are however potentially affected by the wetting history as well as porosity of the surfaces. Furthermore, through deformations of the wood specimens, it was not always possible to put the whole specimens´ surfaces in contact with the agar and is hence another disadvantage of this technique.

The scratching technique revealed some difficulties as well. As degraded wood samples tended to deform, it turned out to be rather difficult to detach the same amount of wood shavings and remove shavings to the same depth on each specimen. As multiple preparation steps are needed, this method is time consuming and shows higher risks of cross contaminations. The particles were diluted in a solution, which may result in bio inhibitory effects of extractives in the specimens' solutions. Beneficial is the fact, that particles can be removed until a certain depth of the sample, hence not only the

outer surface layer can be observed. Moreover, within this experiment it was considered that the MC as well as wood porosity do not impact the CFU counts.

The solution plating technique reveals to be a fast and practical method to verify the amount of CFU in solutions. Even though it does not permit to test the microflora on the wood itself, it allowed to observe the environment the samples were tested in.

All three techniques show some difficulties and impacting parameters that cannot be easily controlled during weathering experiments. Accordingly, for future investigations, recovery techniques need to be further developed (Keer and Birch 2003) and the impacting factors need to be investigated to a deeper extend.

The recovery of fungal and bacterial strains from surfaces is on the one hand an approach that may serve as an indicator for microbiological contamination, it remains however unclear if spores only attach to the surfaces or if they also contribute to the weathering of the wood. On the other hand, these recovery techniques allow to investigate the recovered strains subsequently. It was planned to identify strains via DNA-sequencing, which was hoped to provide a deeper insight into the present strains, their properties, their preferred nutrition sources, their possible interactions with other microorganisms and their adequate growth conditions. Unfortunately, during this work, the identification service was not possible due to the COVID health crisis. Nevertheless, strains were recovered, and several organisms were identified by means of morphology and via Maldi-TOF.

Another promising approach in order to localize bacterial strains is e.g. the use of confocal laser scanning microscopy. In combination with the application of bacteria labelling techniques such as mCherry and RedoxSensor^{TM}Green, live and dead bacterial strains can be localized on wood (Maneewan 2019).

9.7 Identified microorganisms

A database of microorganisms recovered from weathered wood surfaces was established at ESB. Further information about this databank can be seen in **Annex V.**

Identification of recovered microorganisms was planned to be conducted via Maldi-TOF analysis at a research hospital. Unfortunately, their databases were limited to microorganisms of concern to the hospital, and so only a single microorganism could be identified with this method. As an alternative, it was intended to use the sequencing service offered by Macrogen (https://dna.macrogen.com), which is based in South Korea. However, the COVID-19 health crisis prevented the analyses. So, to date no further reliable identifications have taken place.

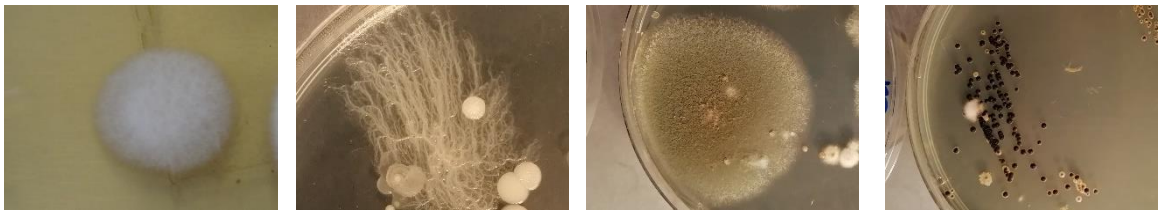


Figure 95: Strains recovered from various environments: (From left to right) Bacillus subtilis, Bacillus cereus var mycoides and Apergillus nigra and nidulans

Still, some isolated strains, shown in Figure 95, were identified: *Bacillus subtilis* was recovered from artificial environments. Identification took place via Maldi-TOF. Its appearance is fluffy white on PC- agar. The spores are known to be quite resistant to heat, radiation and chemicals including acids, bases, oxidizing agents, alkylating agents, aldehydes and organic solvents (Setlow 2006). This resilience might explain why the strain was found in artificial weathering environments e.g. QUV, where rather harsh conditions prevail. Another type of *Bacillus* was classified via morphological aspects as *Bacillus cereus var. mycoides*. It was found on various PC- agar plates and was mainly recovered from outdoor exposed specimens. *Bacillus subtilis* has shown to affect the permeability of spruce sapwood, however heartwood zones remained unchanged (Panek and Reinprecht 2011). For construction purposes as e.g. facades in use class 3, heartwood material is used only, therefore the impact of *Bacillus subtilis* on wood might be neglected. However, the strain has shown antifungal activity not only towards fungi causing diseases in crops (Kilani-Feki et al. 2016) but also towards wood inhibiting wood stain fungi and moulds such as *Aspergillus sp, Trametes versicolor* and *Penicillium sp.* (Feio et al. 2004; Sajitha et al. 2018). Through the biosynthesis of antibiotics; namely Bacillomycin, Mycobacillin, Fungistatin, Fungocin, etc.; by various members of the genus *Bacillus*, fungal enzymatic activity can disappear in few hours respectively to nutritional or genetic modifications (Katz and Demain 1977).

Aspergillus nigra and *nidulans* were recovered and grown on ME- agar. Identification took place via morphology of the strains. On ME- agar the *Aspergillus* appeared fluffy green or showed very dark, almost black and matt colonies. The widespread *Aspergillus spp*. has a varied range of industrial uses such as for applications in food, detergents, textile and pulp and paper industry (Demain et al. 2005). Production of amylolytic enzymes, pectinases and hemicellulases (Schmidt 2010) and acid pH value of 1.5 (Reiß 1998) are properties defining moulds from the *Aspergillus* species. Wood invasion by *Aspergillus* may result in severe discolouration patterns and considerable strength losses (Hamed 2013). Unsurprisingly these fungal species were found in abundance in various exposure conditions, predominantly on outdoor exposed wood samples though, again showing that exposure conditions are decisive for the occurring microbial communities on wood.

The presence of e.g. *Bacillus* slows down enzymatic activities of fungal strains such as *Aspergillus* through the release of antibiotics which may even result in damage of cell membranes and cell walls of hyphae and spores (Gong et al. 2014). Even though bacterial strains might have a minor impact

on the wood structure, their interactions with fungal strains might be beneficial, e.g. by using bacterial strains in order to prevent fungal degradation of wood.

Krüger et al. showed that between plenty of microorganisms, synergistic, antagonistic or non-competitive relationships exist, resulting in physical or chemical competitions otherwise can lead to the formation of mixed biofilms (2019). Further investigations are needed to prove existence of these phenomenon on wood surfaces as well.

Unfortunately, the above mentioned strains were the only ones to be identified within this study. In order to gain a deeper insight about wood invading microorganisms, from DNA-based identification techniques must be profited from. The 16S rRNA is probably the most important tool in order to identify microbial communities as well as to elaborate specific culture medium for specific isolates (Kielak et al. 2016a).

10. General conclusion

The hypothesis was set, that abiotic and biotic degradation factors interact during the weathering process of wood. The literature research and the experimental data presented here lead to the conclusion that biotic and abiotic induced degradation occur simultaneously and, therefore, affect one another. Unfortunately, with the executed experiments, the relative importance of each single degradation factor was not able to be established.

Figure 96 shows a scheme of degradation factors of un-treated and un- coated wood in use class 3 and their interactions. The scheme includes factors which were initially not intended to be studied. The weathering experiments resulted in the identification of 2 additional, important abiotic factors not previously considered in the experiment design, i.e. wind and pollutants.

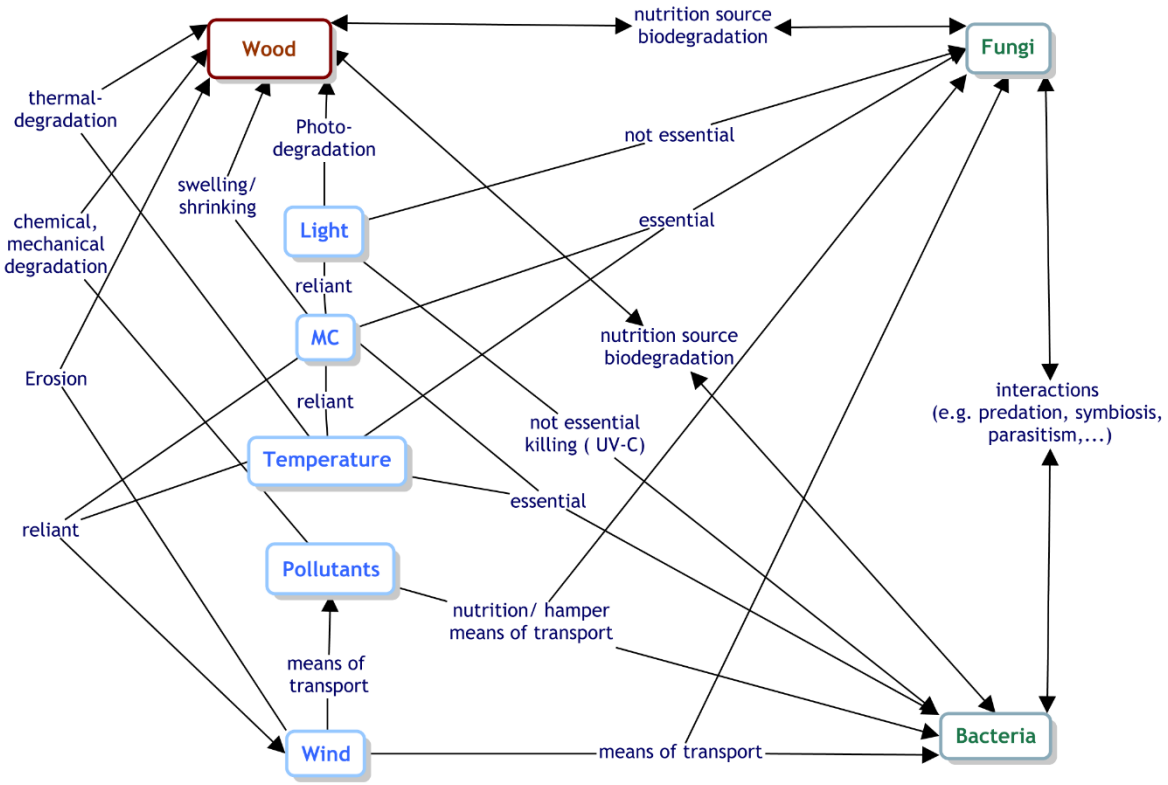


Figure 96: Scheme of degradation factors of wood in use class 3 and their interactions

Atmospheric pollution (e.g. sulphur dioxide, nitrogen dioxide) affects the degradation rate of wood (Yildiz et al. 2011). Pollen and other particles such as Cu, Zn etc. might act as a carrying host for bacteria. The motility of bacteria is depending on the strain, some have flagella to move, and others are motile only in specific conditions. Even immobile bacteria can be transported by means of wind, especially if stuck to particles.

The factors MC, fungi and bacteria show most interconnections in the proposed scheme. For example, the absence of water results in minimal microbiological growth. Furthermore, microorganism's enzymatic activities and the production of ammonia lead to interactions between them. It remains

unclear how important biotic factors are relative to abiotic factors on wood surface weathering in use class 3.

As shown in the OWE experiment, the amount of CFU throughout a year changes greatly. Even though the number of CFU recovered with the applied technique may not be certain, very dry conditions, where least amount of identified factors interfere the SCT recovery technique, showed low contamination. It became evident that bacteria are ubiquitous, and with increasing humidity the number of colonies arise. Temperature and moisture conditions affect nitrogen fixation of bacteria and determines seasonal fixation patterns (Hicks et al. 2003). However, not only seasonal changes but also in even shorter terms due to microclimatic conditions; induced by MC, wind, drying rates varying between wood species etc., microbiological contamination is strongly impacted, as shown with the PCA. Appearance of microclimates impede prediction of wood degradation modelling.

Overall, higher CFU counts were found on specimens exposed during the summer months compared to winter months. Furthermore, strong correlations between CFU counts and temperature were found, as shown in chapter 4.2.4. These observations may explain why certain buildings, with wooden elements, seem to change their visual aspect much faster than others. It seems to be of importance, when a construction is planned, to deliberate in which season wooden façades shall be mounted outdoors.

It was proven that microbes and fungi in particular, are responsible for the very dark colour changes so often seen. Very local microclimates suitable for microorganisms on wood surfaces lead to inhomogeneous patchy discoloration patterns.

A potentially important finding drawn from the experiments using standard artificial weathering techniques, which were thought to be entirely abiotic, turned out to be biotic too. The fact that strains from the genus *Bacillus spp.*, which are known to interact with fungi, have been identified in these environments, supports the hypothesis that biotic interactions take place during wood weathering.

The rest of the research focussed on trying to achieve weathering using only abiotic degradation factors. This proved impossible with the experimental apparatus designed and built for the purpose. It was possible though to perform weathering with limited biotic presence. Unfortunately, the additional abiotic factor (the presence of NaN_3) dominated the results, preventing from drawing any real conclusions.

In addition, the low UV light dosage combined with the relatively short experiment time in the purpose-built weathering device did not provide conclusive data on the relative importance of abiotic factors on wood surface degradation because no severe visual changes were observed on non-sterile specimens. In order to study the impact of biotic factors either a truly sterile apparatus needs to be created, or selective strains can be used for inoculation. It is hoped that the failings outlined in this thesis will help others create this ideal situation in the future.

The comparison of the number of CFU recovered from natural and artificial weathered wood indicate antagonistic effects between fungi and bacteria, in both senses. Environmental conditions are responsible for the microbial communities establishing on wood surfaces. During natural weathering, fungi is the predominant organisms, which can lead to very severe mechanical and chemical decomposition and thus unattractive appearance of wood surfaces. Bacteria however, the major invader of artificially weathered wood, seems to have relatively low direct impact on the wood matrix. Therefore, using bacteria, showing antagonistic effects towards wood degrading fungi, as a treatment or vaccination in order to avoid fungi to invade the surfaces, may be a promising path to prevent fungal degradation.

Annex

I Correlation between colour and humidity

During the OWE the samples are re-exposed immediately after measurements were conducted, to make sure that the degradation process is not interrupted. Thus, conditioning of the samples before e.g. performing colour measurements is unfeasible. It was noted that the colour is changing not only due to degradation but as well to the samples MC. This work intended, to discover the correlation between the MC and the colour of oak and Douglas fir.

a. Material and methods

Figure 97: Example specimen showing the colour measurement areas

Five specimens of each species, cut into 100 x 65 x 10 mm (longitudinal x tangential x radial) boards were conditioned at controlled humidity of 65 % and 20°C. On each specimen, 3 moisture content readings and 6 colour measurements were performed. The spectrophotometer X-rite Ci64 was used to measure the colour values in the L*a*b* colour space and with the Moisture Meter Logica LG6NG the humidity was measured on the surfaces.

Specific areas were marked on the tangential face of the specimens, as shown in Figure 97**Fehler! Verweisquelle konnte nicht gefunden werden.**, so the measurements would always be performed in the same area. To mark the area of colour measurements, the specimens were divided into 2 columns and 3 rows, and the area could not be closer than 1.5 cm to the edge. Traces were made so the marks don't interfere with the readings where the colorimetric spectrum equipment is placed.

The samples were wetted with deionized water with a sponge, to avoid excess of liquid water on the surfaces. Afterwards, the MC data were collected following the line order of the point 1 until 6, followed by colour analysis in the numerical order. The samples were used to collect data 5 times, totalling 150 colour measurements and 75 moisture readings.

b. Results and discussion

Data was organised in groups by MC. Every 10 % of humidity was a group, such as: until 10 %, from 10.1 % to 20 % and consequently. The oak wood was grouped into 5 MC levels from 10 to 50%, however Douglas fir´s MC increased up to 60 %. The averages of the results obtained are presented in the following tables. Moreover, the colour parameter **ΔE** was calculated based on the average of the MC groups mentioned in the tables.

Table 23: Average of colour parameters in the tangential face of oak related to the moisture content groups

Moisture content	L*	a*	b*
Until 10%	68.98	12.12	24.21
Until 50%	66.87	14.04	27.10
ΔE		4.06	

Table 24: Average of colour parameters in the tangential face of Douglas related to the moisture content groups.

Moisture content	L*	a*	b*
Until 10%	76.86	15.73	26.96
Until 60%	74.48	19.65	34.60
ΔE		8.91	

According to a visibility rating as shown in Table 25, the ΔE in oak´s colour is appreciable (between 3 and 6) and changes on Douglas fir are very appreciable (range between 6 and 12).

Table 25: Visibility ratings for ΔE of wood in accordance to Hikita et al. (2001).

Colour variation (ΔE)	Rating
0.0 – 0.5	Negligible
0.5 – 1.5	Slightly perceivable
1.5 – 3.0	Notable
3.0 – 6.0	Appreciable
6.0 – 12.0	Very appreciable

Figure 98 shows the equivalent colours for the average results of the L*a*b* analysis for each group of MC for both species. It is noticeable that the oak gets darker from the natural light brown as the MC rises, however the darkest point is in the group until 40 %. The data indicates that the values for a* and b* are higher in this group than in the group until 50 % and so it appears when transformed into visual representation.

The Douglas wood presents a uniform escalation into a darker colour as the moisture content increases. Its total colour variation is rated as “very appreciable” change, visible in the visual representation.

Figure 98: Visual representation of colours for oak and Douglas fir, for the different groups of MC

The colour variation of oak changed less compared to Douglas fir wood. Both luminosities dropped about 3 %, while the factors a* and b* increased, with 13.65% and 10.67%, respectively to oak. In comparison, the changes in Douglas were more significantly, a* raised 19.96% and b* 22.08%.

The fact that the highest MC for oak is not the same for Douglas may appear to be an uneven comparison, but even if the results are from the same moisture content group, they show that Douglas still changes more than oak.

It is remarkable, as shown in the following two graphs, that the indices a* and b* have opposite behaviour to the index L*. This indicates that when wet, both species tend to become darker and redder and more yellowish.

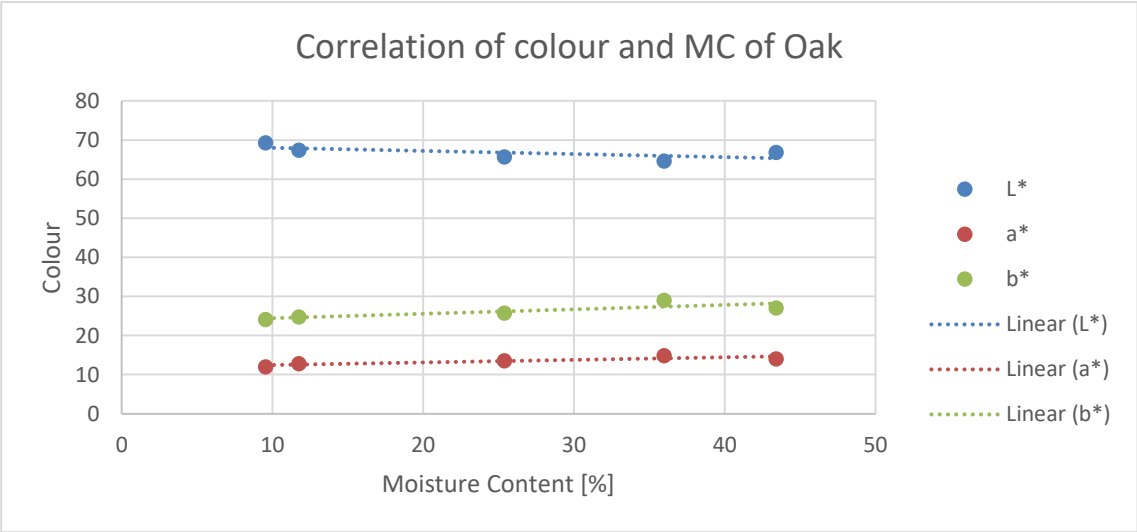


Figure 99: Correlation graphic between colour and MC for Oak measurements with linear trend curves

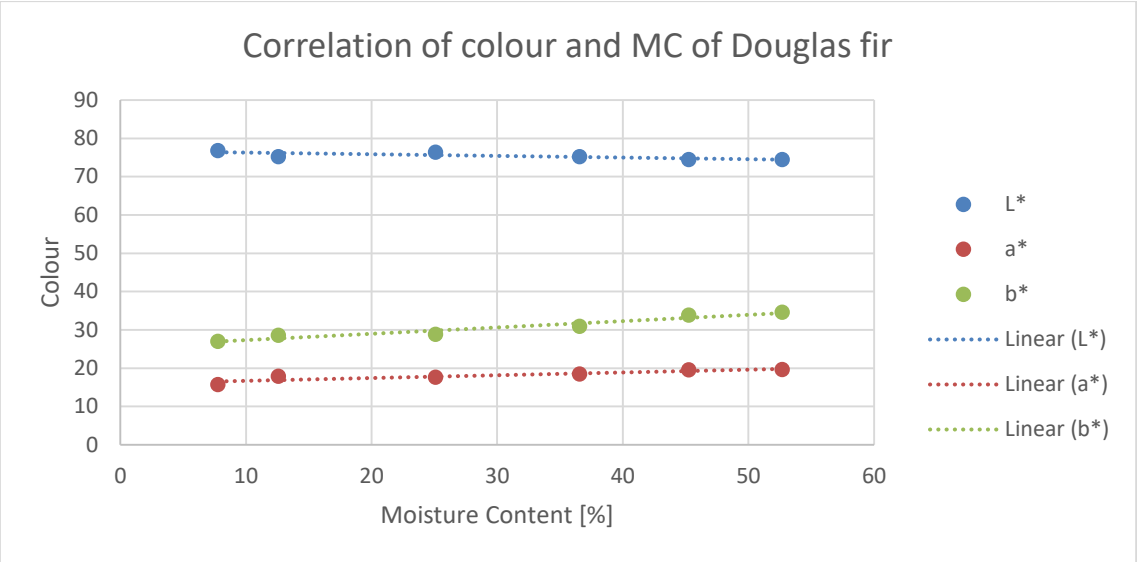


Figure 100: Correlation graphic between colour and MC for Douglas measurements with linear trend curves

It was possible to trace a linear equation that defines the colour variation according to the moisture of the wood surfaces. Their coefficient of correlation is over 70 %, except for the L* parameter. These values are shown in the following tables.

Table 26: Linear equations and their coefficient of determination for the experiment colour X humidity with Oak wood.

Linear equations and their coefficient of determination - Oak	
L*	y = -0.0798x + 68.82 R^2 = 0.5
a*	y = 0.066x + 11.793 R^2 = 0.8
b*	y = 0.1131x + 23.304 R^2 = 0.7

Table 27: Linear equations and their coefficient of determination for the experiment colour X humidity with Oak wood.

Linear equations and their coefficient of determination – Douglas fir	
L*	y = -0.0432x + 76.715 R^2 = 0.6
a*	y = 0.0728x + 15.973 R^2 = 0.8
b*	y = 0.1649x + 25.684 R^2 = 0.9

c. Conclusion

It was noticed that there is a correlation between the wood colour and the surface MC. Especially a* and b* colour coordinates but as well L* in Douglas fir is significantly linear correlated to the MC. Therefore, it is of importance to consider the MC on samples surfaces when colour coordinates are evaluated.

II Surface roughness parameters

Following some of the important SR parameters according to ISO 4287 (1997) and ISO 13565-2 (1998) are listed and additional information is added from Gadelmawla et al. (2002).

R_a [µm], the arithmetic mean deviation, so the mean distance of the surface to the middle line, is used to assess surface quality and gives good general description of height variations. The R_a parameter does not give information about wavelength nor is it sensitive to small changes in profiles. Since no differentiation between peaks and valleys are made it has a relatively weak information character. P_a known as the primary profile amplitude, is the arithmetical mean of the absolute ordinate values Z(x) within a sampling length and W_a of the waviness profile.

$$R_a = \frac{1}{l}\int_0^l |Z(x)|dx$$

R_q [µm] is the root mean square deviation of the assessed profile and more sensitive than R_a to large deviation from the mean line.

$$R_q = \sqrt{\frac{1}{l}\int_0^l Z^2\,(x)\,dx}$$

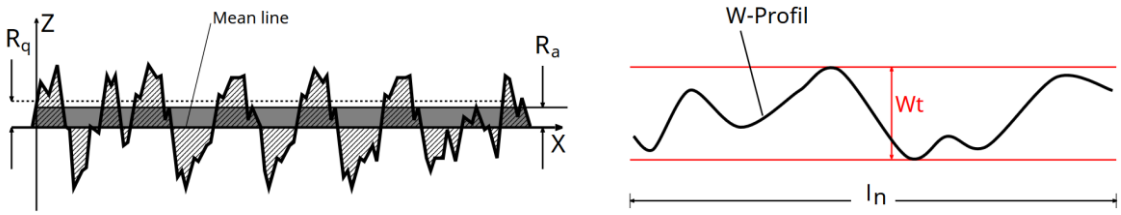


Figure 101: R_a – Mean roughness, R_q – Root mean square roughness and waviness height W_t (Mahr GmbH, fotolia.com)

P_t is the total height of the primary profile, while peaks and valleys are included. R_t is the total height of the roughness profiles and W_t the total height of the waviness profile. The sum of the height of the largest profile peak height and the largest profile valley depth within the evaluation length is calculated.

R_k [non dimensional] reflects the depth of the roughness core profile. The reduced peak height is the average height of the protruding peaks above the roughness core profile. R_{vk} is the same measure but considering valleys of the roughness core profile and R_{pk} considers peaks only. These parameters are sensitive to outlying irregularities such as fuzziness (Gurau et al. 2017). A_1 is the calculated profile peak area and A_2 the valley area.

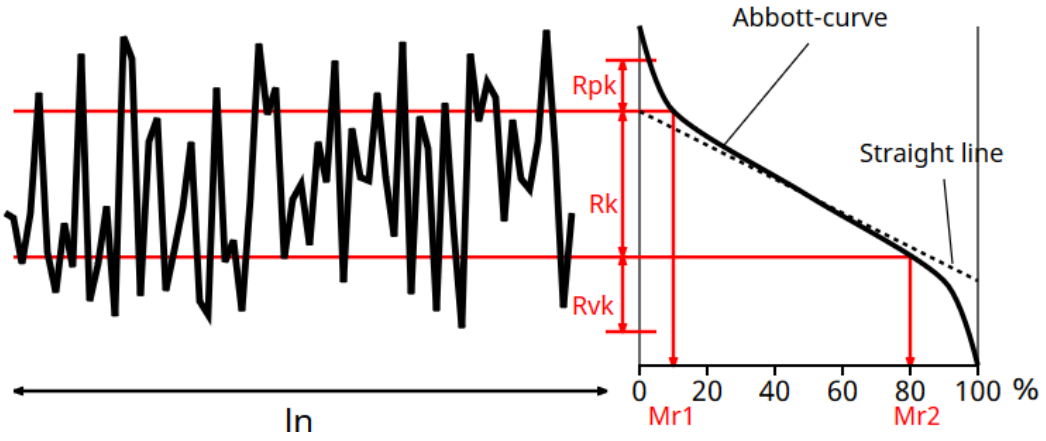


Figure 102: Surface roughness parameters R_k, R_{pk} and R_{vk} (Mahr GmbH, fotolia.com)

R_{sk} [non dimensional] is the skewness of the roughness profile. The parameter is used to measure the symmetry of the profile about the mean line and is sensitive to occasional deep valleys or high peaks. R_{sk} values below 0 indicate surfaces made up of plateaus with valleys, whereas above 0 the surface has peaks above wide valleys. The parameter can be used to distinguish between profiles having the same R_a but different shapes.

$$R_{sk} = \frac{1}{R_q^3}\left[\frac{1}{lr}\int_0^{lr} Z^3(x)dx\right]$$

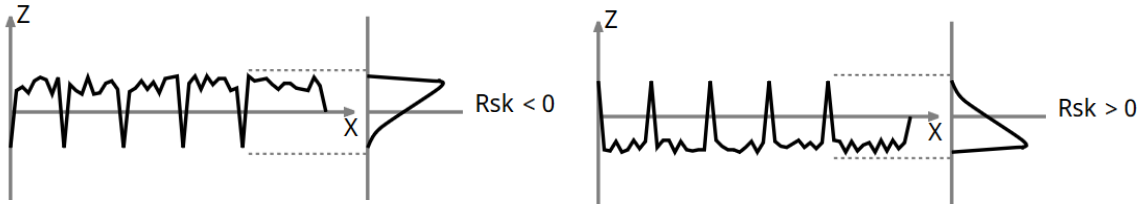


Figure 103: R_{sk}: Skewness of the roughness profile (Mahr GmbH, fotolia.com)

R_{ku} [non dimensional] refers to the kurtosis of the roughness profile. It describes the sharpness of the probability density of the profile. If $R_{ku} < 3$ the distribution curve is said to be platykurtic (narrow tips) and has relatively few high peaks and low valleys. If $R_{ku} > 3$ the curve is leptokurtic (round tip, or no tip) and has rel. many high peaks and low valleys.

$$R_{ku} = \frac{1}{R_q^4}\left[\frac{1}{lr}\int_0^{lr} Z^4(x)dx\right]$$

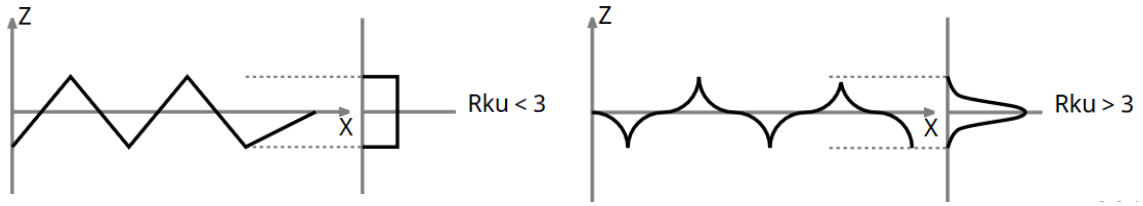


Figure 104: R_{ku}: Kurtosis of the roughness profile (Mahr GmbH, fotolia.com)

III Dyed slices under the light microscope- transition mode

Several NSWE samples were prepared to be analysed with the LM in transition mode. Most of the samples were quite deformed after weathering which made cutting slices with a microtome difficult. With a razor blade, hand cut slices were prepared, therefore the thickness of the slices was not consistent between as well as with specimens. The dyeing protocol is listed following:

- Distilled water [short rinsing]
- Bleach [5 min]
- Distilled water [short rinsing]
- 10% acetic acid [short rinsing]
- Safranin [2 min] and/or Light-green [5 min]
- Distilled water [short rinsing]
- Distilled water [5 min]
- 50% ethanol [5 min]
- 70% ethanol [5 min]
- Ethanol 94% [5 min]
- Ethanol 94% [5 min]
- Ethanol + xylene 1:1 [5 min]
- Xylene [at least 5 min]
- Embedding

The safranin and light green dies applied to the samples, did not provide information about a clear distribution of lignin and celluloses. Thicker areas of the slices were penetrated to a higher extent by the dye than thinner parts (the thicker the cell walls the more colour can be absorbed). Moreover, a difference of penetration could be observed in early- and latewood, therefore the intensity of the dye is strongly related to the density of the wood.

Trials showed that the microscopic observation allowed to observe intercellular spaces, cracks in cell walls and pits, the delamination of the S3 layer, cracks along rays (in oak and chestnut samples), deformation of cells, disappearance of spiral thickenings and the torus of pits (Douglas fir samples), diamond shaped pit degradation and the presence of hyphen. Moreover, dark spots could be located in the interior of vessels. These pollutants are likely to be microorganisms. Figure 105 shows LM pictures of Douglas fir and chestnut wood dyed with safranin and/or light-green. It was concluded that an image analysis of the coloured slices is not beneficial since the intensity of dies is related to wood density rather than exposure to weathering. Moreover, sample preparation is very time consuming. However, the use of dyes is still useful to gain contrast and facilitate observation of structural changes due to weathering.

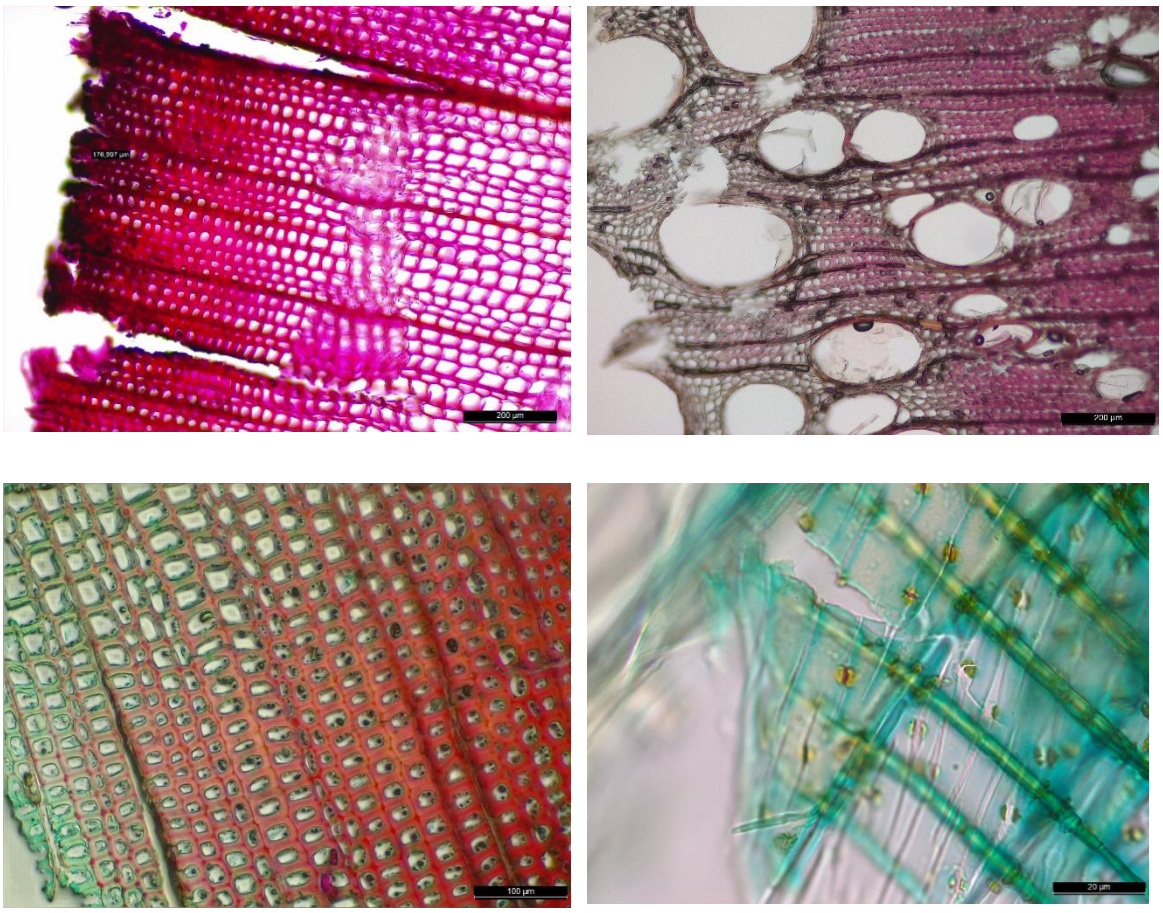


Figure 105: Microscopic observation of dyed slices from NSWE specimens: Douglas fir dyed with safranin (upper left), chestnut with safranin and light-green (upper right), Douglas fir dyed with safranin and light-green (lower left) and Douglas fir dyed with light-green (lower right)

IV Quantifying microbiological growth on petri dishes via image analysis

The amount of colony forming units (CFU) on petri dishes was established manually. Soon it became clear that it is a long process strongly depending on the operator. Therefore, trials in order to quantify the CFU on agar in an automated manner were carried out. The use of image analysing software such as OpenCFU by Quentin Geissmann, ImageJ as well as Python were tested in order to enumerate the amount of CFU on petri dishes.

Figure 106 shows an example of a PC-agar plate (left) and the resulting analysis with the OpenCFU software. Counting the CFU manually leads to the result of 1 CFU in this specific dish. The automated counting in OpenCFU shows a CFU of 161.

Pictures were then pre-treated in Python. Cropping around the dish, alpha value enhancement, contrast adaption as well as transformation into grey scale pictures was carried out, but still, the number of CFU did not show appropriate results with OpenCFU.

The agar occasionally shows imprints around the edges where the samples were pressed onto the agar. Additionally, debris or small wood particles were occasionally transferred to the plates. All these interfering factors cannot be recognized by the software and are counted as colonies as well.

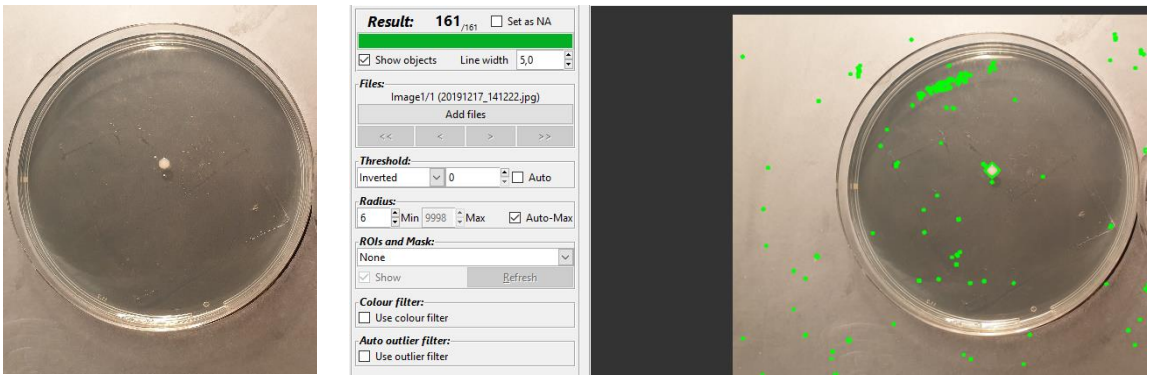


Figure 106: Image analysis with OpenCFU. Original picture of a PC-agar dish (left) and same picture after treatment (right)

Following trials with the ImageJ software were carried out. Therefore, an elliptical selection of the pictures was done and saved in a new file. Colour channels were split and only the red channel was used for analysis. Subsequently the threshold was adjusted. The Huang B&W filter proved to select colonized areas in the most suitable manner, however the threshold adjustment needed to be adapted for each picture and the process is therefore very time consuming. Figure 107 shows an example of an analysed petri dish. The number of CFU as well as the size of the area covered with microorganisms were analysed.

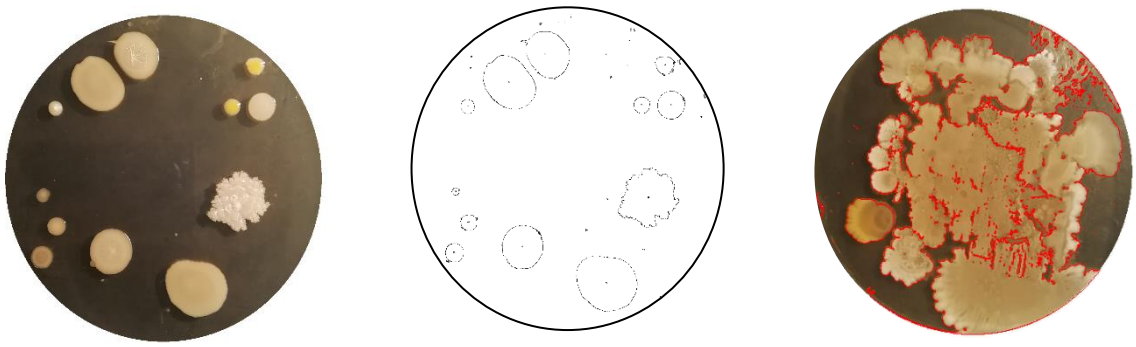


Figure 107: Image analysis with ImageJ. From left to right: Cropped original picture of a PC-agar dish, the same picture after treatment showing colony's outlines and a ME-agar dish after measurement of the colonized area

Analysis with ImageJ showed more appropriate results compared to OpenCFU, however defects of the agar such as discoloration, cracking or debris were counted as colonies as well and big colonies were often counted as multiple colonies. Therefore, it was decided that a manual counting of the colonies is the most precise method. Moreover, non-automated visual observation allows to distinguish between colours, shapes of colonies and defects of the agar.

An automation of CFU counting from pictures resulting from agar plates, where microorganisms were transferred through surface contact test failed. Still, automated analysis from "untouched" agar surfaces (where the agar is not damaged by e.g. pressing the specimens onto the nutrition) can be proposed with the use of either ImageJ or Python using the OpenCV library (Open Source Computer Vision Library).

V Microbiological database- „Mikrotheque“

About 100 bacterial and fungal strains have been isolated from natural as well as artificial weathered wood surfaces and are stored in a fridge at 6 °C. Table 28 shows an extract of strains stored in the “Mikrotheque” at ESB. Information provided in the database are:

- Consecutive number (Label on the samples)
- The type of agar the strain was developing on
- A code (Referring to the wood sample the strain was recovered from)
- Description (Such as colour, appearance and exposure conditions)
- Petri dish (A link showing a photo of the petri dish the strain was isolated from)
- Date of preparation
- Date of next re-plating (The agar tubes can be stored for 7 months in the fridge)

Table 28: Extract of the microbiological database "Mikrotheque"

No.	*Agar*	*Code*	*Description (Morphology and environment of recovery)*	
1	PC-agar	UVSCOI-02	Many white small	QUV sample, UVSC for 6 weeks
2	PC-agar	UVSCOI-02	Rose, slight pink	
3	PC-agar	UVSCOI-02	Beige, slightly rose	
4	PC-agar	UVSCOI-02	Almost neon yellow	
5	PC-agar	UVSCOI-02	Normal to dark yellow	
6	PC-agar	UVSCOI-02	Dark orange	
7	PC-agar	QUV H20	Fluffy white	Organisms found in water used in QUV
8	PC-agar	QUV H20	Slight yellow	
9	ME-agar	A	Green	OWE

VI Publications

a. Poster and oral presentations

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2016. Do biotic and abiotic factors have synergistic effects on the weathering of wood? Poster and flash talk at GDR.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2016. Optimization of crack measurements in degraded wood samples. Poster and flash talk at Journée de l´ Imagerie at Ecole Centrale, Nantes.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2017. Surface roughness of naturally and artificially weathered oak and Douglas fir wood. Poster and flash talk at GDR 2017.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2017. How do biotic and abiotic factors combine to affect the weathering of wood surfaces in end-use class 3.2? Oral presentation COST Action FP 1303.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2017. Fungal and bacterial colonies growing on wood surfaces. Oral presentation COST Action FP 1303.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2017. How do biotic and abiotic factors combine to affect the weathering of wood surfaces? Oral presentation ICWSE.

Buchner J., Irle M., Belloncle C., Michaud F., Macchioni N., 2017. Fungal and bacterial colonies growing on wood surfaces exposed to an Atlantic climate. Oral presentation at IUFRO.

Buchner J., Irle M., Belloncle C., Michaud F., 2019. Les facteurs biotique et abiotique interagissent-ils pendant le vieillissement du bois utilisé en classe d´emploi 3 ? Poster and flash talk at Xylofutur, Québec.

b. Articles

Gurau, L., Irle, M., Campean, M., Ispas, M., Buchner, J., 2017. Surface Quality of Planed Beech Wood (Fagus sylvatica L.) Thermally Treated for Different Durations of Time. BioResources 12. https://doi.org/10.15376/biores.12.2.4283-4301.

Buchner, Julia, Irle, M., Belloncle, C., Michaud, F., Macchioni, N., 2017. How do biotic and abiotic factors combine to affect the weathering of wood surfaces? ProLigno 13, 298–301

Buchner, J., Irle, M., Belloncle, C., Michaud, F., Macchioni, N., 2018. Fungal and bacterial colonies growing on weathered wood surfaces. Wood Mater. Sci. Eng. 1–9. https://doi.org/10.1080/17480272.2018.1443975.

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Titre : Les facteurs biotique et abiotique interagissent-ils pendant le vieillissement de bois utilisé en classe d'emploi 3 ?

Mots clés : altération naturelle, altération artificielle, Quercus petraea, Pseudotsuga menziesii, dégradation biotique, dégradation abiotique

Résumé : Le vieillissement du bois par des facteurs abiotiques, a fait l'objet de nombreuses études, portant surtout sur du bois traité ou modifié chimiquement. Peu d´entre elles ont été menées sur l'influence des facteurs biotiques sur du bois non traité utilisé à l'extérieur mais sans contact avec le sol, c'est-à-dire en classe d'emploi 3. L'influence de la présence de communautés bactériennes et fongiques pendant le vieillissement du bois est ainsi très peu documentée. L´hypothèse que les mécanismes d'interaction entre les champignons et les bactéries, ainsi qu'entre les microorganismes et les facteurs abiotiques, ont un impact sur la vitesse du vieillissement du bois reste donc posée.

L'objectif du projet est d'identifier les effets de synergies entre les facteurs biotiques, tels que les bactéries (Eubactéries), les champignons (*Basidiomycota* et *Ascomycota*) et les facteurs abiotiques, tels que la lumière, la température et l'humidité pendant le vieillissement de bois de chêne (*Quercus petraea*), de Douglas (*Pseudotsuga menziesii*) et partiellement de châtaigner (*Castanea sativa)* pour une utilisation en classe d'emploi 3.

Afin de mesurer le changement de l´état des surfaces en bois, différentes méthodes visuelles, chimiques, mécaniques et microbiologiques ont été utilisées.

Plusieurs méthodes de vieillissement ont été appliquées pour faire vieillir les échantillons de bois dans des conditions naturelles, ainsi que pour simuler le vieillissement tout en éliminant certains facteurs de dégradation et donc observer l'influence de facteurs spécifiques sur le processus de dégradation du bois.

Title : Do biotic and abiotic factors combine to affect the weathering of wood in use class 3 ?

Keywords : Natural weathering, artificial weathering, *Quercus petraea, Pseudotsuga menziesii*, biotic degradation, abiotic degradation

Abstract : The weathering of wood, and especially modified wood, through abiotic factors has been extensively studied. Much less research has been conducted on the influence of biotic factors on untreated wood in exterior use that is not in ground contact, i.e. use class 3. There is especially a lack of research concerning the influence of the presence of bacterial and fungal communities on the weathering process of wood. The hypothesis is set that interacting mechanisms between fungi and bacteria as well as between microorganisms and abiotic factors have an impact on the pace of the weathering of wood.

The aim of the project is to identify synergistic effects between biotic factors such as bacteria (Eubacteria) and fungi (*Basidiomycota* and *Ascomycota*) and abiotic factors such as light, temperature and moisture on the weathering of oak (*Quercus petraea*), Douglas fir wood (*Pseudotsuga menziesii*) and partially chestnut (*Castanea sativa*) in use class 3.

In order to measure the influence of weathering, different visual, chemical, mechanical and microbiological methods were used.

Multiple weathering methods were applied to degrade wood samples in natural and artificial conditions. Artificial weathering provided the opportunity of eliminating certain degrading factors and therefore observe the influence of specific factors on the degradation process of wood.