Chemical composition and resistance of Italian stone pine (*Pinus pinea* L.) wood against fungal decay and wetting

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Abstract

Relevant properties of stone pine (*Pinus pinea*) wood have been only fragmentarily addressed in the past, which has been recognized as a limiting factor for its potential applications. The sorption properties, permeability to water, extractives content and durability against fungi of *Pinus pinea* sapwood and heartwood were therefore determined in the present research. A SEM analysis was also performed. The Meyer-Veltrup model for material resistance was used to test relevant data. The results showed that sapwood of *P. pinea* fits into durability class 5 (very susceptible wood), while heartwood meets the requirements of durability class 2 (durable wood), if the mass loss after fungal exposure is considered as the sole criteria for classification. Heartwood contains up to 15 % of lipophilic compounds, contributing to its hydrophobicity and influencing its sorption properties. In contrast, sapwood is very permeable and hence takes up a lot of water, while heartwood, with a higher resin content, exhibits better water performance. The higher durability of stone pine heartwood against wood decaying fungi can be linked to the presence of phenolic extractives and hydrophobic properties.

Keywords; wood; *Pinus pinea; chemical composition, durability, water performance*

1. Introduction

Wood is one of the most important building materials. Due to its positive environmental impact, sustainable character, availability and good properties, the use of wood have increased considerably in recent decades. This is the most evident in use class 2 (outside, not in ground contact, covered) and use class 3 (outside, not in ground contact, not covered) applications, as defined by EN 335 (CEN, 2013). However, sufficient durability of wood is required to meet the user's criteria for specific applications. Since a large group of classical biocides are banned due to environmental and health concerns, and in response to negative public opinion on existing biocides and exploitation of tropical timber, research philosophies are changing. Focus is in improving the durability and prolonging the service life of wood (Militz, 2015; Humar et al., 2017). Recent models clearly indicate that the service life of wood in above ground applications is a function of inherent durability (as a result of the presence of biocides and/or biologically active extractives) and water exclusion efficacy (Meyer-Veltrup et al., 2017). Industry in Europe is therefore looking for less utilized tree species that may have wood that exhibits good water exclusion efficacy, due either to the anatomical structure or chemical

structure. One of the overlooked wood species is *Pinus pinea,* with an areal throughout the Mediterranean Basin, Canaries, Madeira and reaching to Asia Minor (Nardi Berti, 2006).

P. pinea has been cultivated for many years, and the natural distribution of the tree is unknown. However, the distribution today is close to the Mediterranean Basin, extending from Portugal to Syria (Anonymus, 2017). *Pinus pinea* is often called 'Italian stone pine' and sometimes 'umbrella pine'. These names apparently come from the idea that this tree grows well in stony ground and because it has an umbrella-shaped crown (Ayrilmis et al*.,* 2009). The stone pine is a species that was brought to Italy by the Etruscans from the coasts of the Black Sea and Anatolia, and subsequently adopted by the Romans. It was planted mainly in the vicinity of ports, due to the large amount of resin (Vidrich, 1988), used to produce pitch and make a ship waterproof, because the logs were used for the construction of ships and for the production of pine nuts, food loved by the Romans. *P. pinea w*as a sacred plant linked to the goddess Cybele, the cult of the dead and was thus also planted in the vicinity of graves. In modern times, the species is considered very important because it is used to protect inland areas from salty winds coming from the sea and to fix sand dunes destroyed by erosion, together with the production of pine nuts, which are still appreciated today. The production of pine nuts has actually strongly decreased, and the role of the species is more related to the protection of coastal areas or for ornamental purposes, being one of the trees most characterizing the landscape in urban and peri-urban towns, and metropolises such as Rome (Fares et al., 2013; Gasparella et al., 2016).

Mediterranean pine forests in Italy cover an area of 226,101 ha (Anonymus, 2017) with Italian stone pine covering 46,290 ha; in the Latium Region in central Italy, there are 4790 ha of stone pine forests (Gasparella et al., 2016). The wood is nowadays used predominantly for energetic purposes or packaging. In the near future, the quantities of stone pine wood on the Italian market can be expected to increase, because of the age of the forests, increased environmental stress and the presence of forest pathogens, and landscape degradation (Scarascia Mugnozza et al., 2000). It is thus of great commercial importance to increase the number of applications of certain wood species and to select those with the highest added value. In addition, local, predominantly lesser-utilized wood species are becoming increasingly important because of the short wood supply chain.

Wood properties are to a large extent characterised by wood structure and chemistry. In addition to structural polymers, a large variety of compounds without a relevant structural role are present in woods, referred to as extractives (Fengel and Wegener, 1989). Extractives can be removed, extracted, from the woody tissue by various more or less polar solvents and are therefore classified as lipophilic (e.g., fatty acids, waxes, triglycerides and terpenoids) and hydrophilic extractives (soluble sugars, lignans, stilbenes, flavonoids, tannins) (Jansson and Nilvebrant, 2009). This extensive and heterogeneous group of wood compounds has been shown to have both an important physiological and protective function in a living tree (Holmbom, 2011; Pearce, 1996). Furthermore, it is generally considered that the structural components of wood, cellulose and lignin, contribute to the mechanical properties of wood, while extractives have a considerable impact on the natural durability of wood (Harju and Venalainen, 2006; Kai, 1991). Low molecular phenolic compounds in the wood and bark of conifers, i.e., flavonoids, stilbenes and lignans, are molecules with proven antifungal, antibacterial and antioxidant properties (Pietarinen et al., 2006; Välimaa et al., 2007). Stone pine is an economically important tree species, for which the chemical composition of bark, cones, seeds and needles has been relatively well-examined (Amri et al., 2012; Kilic and Altuntas, 2006; Nergiz and Donmez, 2004; Nunes et al., 1999; Ulukanli et al., 2014; Yesil-Celiktas et al., 2009), whereas information on the extractives of wood is very limited or almost completely lacking.

There is also a lack of data available about the performance of stone pine wood in outdoor applications. Predominately use of wood in above ground applications is of great commercial interest.

Typical above ground applications are; decking, some claddings, sound barriers, garden furniture … The main reason for failure of wood in this kind of applications is predominately associated with fungal decay (Humar et al., 2015). Durability data vary a lot. It is predictable that the sapwood is classified as non-durable, according to EN 350 (CEN, 2016); however, there are some literature reports that the durability of stone pine sapwood is better than the durability of Scots pine (Palanti et al., 2011). Durability data for heartwood (HW) are more scattered, though. Nardi Berti (2006) classifies *P. pinea* heartwood as moderately durable (durability class 3-4), while the new version of EN 350 (CEN, 2016) downgrades *P. pinea* heartwood to non-durable wood (durability class 5). Due to the huge difference in performance classification and absence of literature data, the durability and water performance of *P. pinea* were investigated and linked to the chemical composition.

2. Material and Methods

2.1. Material

The samples for the study were from trees of Italian stone pine (*Pinus pinea* L.) from the urban area of Rome (Italy) (latitude: 41° 53' 30" N longitude: 12° 30' 40" E, altitude: 52 m). The diameter of trees was between 40 and 50 cm, and was approximately 40 to 50 years old. Planks were cut from two logs about 50 cm long and 40 cm in diameter, in order to have enough samples to perform the analysis of the relevant properties. For durability and water uptake tests, samples were 25 mm wide, 15 mm thick (in cross-cut direction) and 50 mm long. Samples were defect free, without visible signs of decay. For each test, 10 samples of sapwood and 20 samples of heartwood were used. It was decided to use a double number of samples of heartwood because a high weight loss was observed during drying in an oven, due to the excretion of resin and evaporation of resin compounds. Ten samples of heartwood were dried in an oven at 103 °C for 24 hours and the remaining ten were dried for 48 hours at a temperature of 36 °C, thus avoiding resin melt. These samples were not oven dried, so oven dry mass was assumed from parallel samples as suggested by CEN/TS 15083-1 (CEN, 2005) procedure. Beech wood (*Fagus sylvatica*), Scots pine sapwood (*Pinus sylvestris*) and Norway spruce wood (*Picea abies*) were used for comparison. The density of all materials was determined at MC 12%.

2.2. Extractive content

For the purposes of chemical analysis, different categories of wood were prepared, i.e., sapwood (SW), transition zone (TZ) and heartwood (HW). Samples of knot-wood (KW) were also taken from the trunk discs. Wood blocks were then ground with a cutting SM 2000 mill (Retsch, Haan, Germany), producing particles that passed through a 1 mm sieve. The wood meal was then stored in a cool dark place until further processing. Before extraction, all samples were freeze-dried in a Telstar LyoQuest lyophylizator (Terrassa, Spain) at 4 Pa and – 82 °C for 24 hours. Extraction was carried out in a Soxhlet apparatus, according to the protocol described by Fang et al. (2013). However, a two-step sequential extraction procedure with cyclohexane and acetone was applied (Willför et al., 2003). A quantity of 2.5 g of freeze-dried wood was first extracted with cyclohexane at 110 °C for 6 hours, in order to remove lipophilic extractives. Hydrophilic extractives were then extracted with 250 mL of an acetone/water mixture (95:5, v/v) at 110 °C for 8 hours. Several volume aliquots were taken from each pine wood extract for spectrophotometric measurements of total phenols and chromatographic evaluation of individual extractives.

Wood compounds soluble in cyclohexane and acetone, i.e., lipophilic and hydrophilic extractives, were determined gravimetrically. For this purpose, 10 mL of extract was transferred to a weighed Erlenmeyer flask and dried to a constant mass. Contents were expressed in milligrams of extracted

matter per gram of freeze-dried wood (mg/g). The remaining extracts were evaporated at 10 kPa in a vacuum chamber and properly stored at – 25 °C.

Total phenols in extracts were semi-quantitatively evaluated with a Folin-Ciocalteu reagent (Scalbert et al., 1989; Singleton and Rossi, 1965). Diluted Folin-Ciocalteu phenol reagent and sodium carbonate (aq) were added to the extracts and gallic acid solutions (aq). Reaction mixtures were incubated for 2 hours at room temperature. Absorbance was measured with a Perkin-Elmer Lambda UV-Vis spectrophotometer (Waltham, USA) at a wavelength of 765 nm. Calibration was achieved with gallic acid aqueous solutions ($R^2 \geq 0.99$) and the content of total phenols was expressed as gallic acid equivalents per gram of dry wood (mg GAE/g dw).

Targeted compounds in the wood extracts of stone pine were investigated by a Thermo Scientific Accela HPLC-PDA system (Waltham, USA). All the extracts were evaporated to dryness, properly diluted with methanol and filtered through a 0.22 µm polyamide filter. Afterwards, 3 - 5 µL of sample was directly injected into the loop of the system and presented on the column. Separation was carried out on two Thermo Scientific's Accucore columns, viz. pentafluorophenyl (PFP) and octadecylsilyl (C18) columns, with dimensions of 2.1 mm (PFP) and 4.6 mm (C18) (i.d.) × 150 mm and 2.6 µm particle size. Sample trays and the column oven were thermostated at 4 °C and 30 °C, respectively. Water (A) and methanol (B), both containing 0.1 % formic acid (v/v), were used as the mobile phase. The elution of phenolic compounds was carried out according to a suitable gradient, i.e., from 5 % to 95 % B in 15 minutes. The presence and quantities of nortrachelogenin (NTG), pinosylvin (PS), pinocembrin (PC) and pinosylvin monomethyl ether (PSMME) were evaluated in the wood extracts (Figure 1). Reference compounds (HPLC assay, ≥ 95%) were purchased from Sigma-Aldrich (Darmstadt, Germany). The wavelength for monitoring the targeted phenolic compounds was defined as 275 nm. For peak identification, UV spectra were recorded from 200 nm to 400 nm. However, peak assignments were performed by comparison of retention times and UV spectra of separated compounds to those of analytical standards. The present chromatographic method was linear in the selected concentration range ($R^2 \geq 0.99$). The samples were measured in triplicate. The contents were expressed in milligrams of identified compound per gram of dry wood sample (mg/g dw).

Significant differences among the contents of extractives were checked by basic statistical analysis (ANOVA, LSD test at a 95.0% confidence level) in the same way as explained in one of our earlier papers (Vek et al., 2014). Measurements were performed on three parallels, only analysis of hardwood was performed on one material, due to lack of the material.

2.3. Durability test against wood-destroying basidiomycetes

The decay test was performed according to modified CEN/TS 15083-1 (CEN 2005). Conditioned samples were steam-sterilized in an autoclave before exposure to wood decay fungi; 350-mL experimental glass jars with aluminium covers and cotton wool with 50 mL of 4% potato dextrose agar (DIFCO) were prepared and inoculated with *Trametes versicolor* (L.) Lloyd (ZIM L057)) and two brown rot fungi (*Gloeophyllum trabeum* (Pers.) Murrill (ZIM L018) and *Fibroporia vaillantii* (DC.) Parmasto (ZIM L037). The fungal isolates originated from the fungal collection of the Biotechnical Faculty, University of Ljubljana and are available to research institutions on demand (Raspor et al. 1995). Information regarding the origin of the fungal isolates and details about identification are available in the relevant catalogue. One week after inoculation, two samples per jar were positioned on a plastic HDPE mesh, which was used to avoid direct contact between the samples and the medium. The assembled test glasses were then incubated at 25 °C and 80 % relative humidity (RH). After incubation, samples were cleaned of adhering fungal mycelium, and the mass loss was determined gravimetrically

after drying the samples at 103 ± 2 °C for 24 h. Fifteen sapwood and 15 heartwood samples were used in this test. Durability classes (DC) were derived from median mass loss (ML_F) according to the scheme shown in Table 1.

2.4. Short-term capillary water uptake test

Measurements were taken at room temperature of 20 °C at a relative humidity (RH) of 50 % ± 5 % on a Force Tensiometer K100MK2 device (Krüss, Germany), according to a modified EN 1609 (CEN, 1997) standard, after conditioning at 20 °C and 65 % RH until constant mass. The axial surfaces of the samples (1.5 × 2.5 × 5.0 cm^3) were positioned to be in contact with the test liquid (distillate water), and their masses were subsequently measured continuously every 2 s for 200 s. Other parameters used were velocity before contact of 6 mm/min, sensitivity of contact of 0.005 g, and a depth of immersion of 1 mm. Depending on the final weight of the immersed sample and the square surface of the axial surface of samples, the uptake of water was calculated in grams per square centimetre. Ten sapwood and 20 heartwood samples were used for this analysis.

2.5. Long-term water uptake test with drying process above freshly activated silica gel

Long-term water uptake was based on the ENV 1250-2 (CEN, 1994) leaching procedure. Before the test, samples (1.5 × 2.5 × 5.0 cm^3) were oven-dried at 103 ± 2 °C until constant mass and weighed to determine the oven-dry mass. The dry wood blocks were placed in a glass jar and positioned with weights to prevent them from floating; 100 g of distilled water (T = 23 °C) was then added per sample. The mass of the samples was determined after 1 h and 24 h, and the moisture content of the samples was calculated. In addition to wetting, outdoor performance is also influenced by drying. Wood that dries out quicker, in general performs better. After 24 h of immersion, we have positioned wet samples, above freshly activated silica gel for 24 h in a closed container and the moisture content of the samples was calculated according to the Meyer-Veltrup procedure (2017).

2.6. Water vapour uptake in water saturated atmosphere

In addition to liquid water uptake, wood also absorbs water from the air. An experiment was performed to determine the performance of wood in a climate with high relative humidity. Samples were oven-dried at 103 ± 2 °C to a constant mass and weighed to determine oven-dry mass. The samples were stacked in a glass climate chamber with a ventilator, above distilled water. Samples were positioned on a plastic mesh above the water using thin spacers (Meyer-Veltrup et al., 2017). After 24 h of exposure, they were weighed again, and their moisture content was calculated.

2.7. Dynamic vapour analysis

Dynamic vapour sorption (DVS) analysis was performed on cyclohexane extracted and non-extracted sapwood and heartwood. Samples for DVS were milled on a Retsch SM 2000 cutting mill (Retsch GmbH, Haan. Germany) with a conidur perforation sieve with 1.0 mm perforations. Prior to the experiment, the wood chips were conditioned for 24 h at 20 ± 0.2 °C and 1 ± 1 % RH. Analyses of the wood samples were performed using a DVS apparatus (DVS Intrinsic, Surface Measurement Systems Ltd., London, UK). A small amount (approximately 100 mg) of pre-conditioned wood chips was placed on the sample holder, which was suspended in a microbalance within a sealed thermostatically controlled chamber, where a constant flow of dry compressed air was passed over the sample at a flow rate of 200 cm^3 s^{-1} and a temperature of 25 ± 0.2 °C. The schedule for DVS was set to 20 steps of 5 % between 0 % and 95 % RH for both the sorption and desorption steps. Two full isotherm runs were performed in order to capture the sorption behaviour of the material fully. The DVS maintained a given RH until the weight change of the sample was less than 0.002 % min^{-1} for at least 10 minutes. The running time, target RH, actual RH and sample weight were recorded every 20 s throughout the isotherm run. Sorption and desorption isotherms were produced for each material by plotting the equilibrium moisture content (EMC) change against relative humidity (RH).

2.8. SEM Analysis

Scanning Electron Microscopy (SEM) was performed to better understand the behaviour of *Pinus pinea* wood related to its microscopic structure. We first prepared smaller samples, ensured that the samples were oriented in all three anatomical directions, and cut the surface of each anatomical plane with a sliding microtome, which has been shown to be the most appropriate surface preparation procedure for SEM imaging. The SEM micrographs were then taken in low voltage (10 kV) and low vacuum (50 Pa) conditions with a large field (LFD) detector in an FEI Quanta 250 SEM microscope at a working distance of 10 mm.

2.9. Factor approach to quantify the resistance dose

A model approach was applied according to Meyer-Veltrup et al. (2017) and Isaksson et al. (2014) in order to predict the field performance of the examined wood species. The model describes the climatic exposure, on the one hand, and the resistance of the material on the other.

Acceptance of a chosen design and material is expressed as:

$$\text{Exposure} \leq \text{Resistance} \quad (1)$$

The exposure can be expressed as an exposure dose (D_{Ed}) determined by daily averages of temperature and MC. The material property is expressed as a resistance dose (D_{Rd}). The dose is expressed in days [d] with optimum moisture and temperature conditions for fungal decay (see Isaksson et al., 2013).

$$D_{Ed} \leq D_{Rd} \quad (2)$$

where D_{Ed} is the exposure dose [d] and D_{Rd} is the resistance dose [d].

The exposure dose D_{Ed} depends on the annual dose at a specific geographical location and several factors describing the effect of driving rain, local climate, sheltering, distance from the ground, and detailed design. A detailed description of the development of the corresponding exposure model is given by Isaksson et al. (2014). The present study focused on the counterpart of the exposure dose, which is the resistance, expressed as resistance dose D_{Rd}. The latter is considered to be the product of the critical dose D_{crit} and two factors taking into account the wetting ability of wood (k_{wa}) and its inherent durability (k_{inh}). This is given by the following Eq. 12, according to Isaksson et al. (2014):

$$D_{Rd} = D_{crit} \cdot k_{wa} \cdot k_{inh} \quad (3)$$

where D_{crit} is the critical dose corresponding to decay rating 1 (slight decay) according to EN 252 (CEN, 2015) [d], k_{wa} is a factor accounting for the wetting ability of the tested materials [-], relative to the reference Norway spruce, k_{inh} is a factor accounting for the inherent protective properties of the tested materials against decay [-], relative to the reference Norway spruce (Brischke et al., 2015).

Based on the results of the various moisture tests presented in this paper, the wetting ability factor k_{wa} was evaluated. The methodology for k_{wa} calculation followed the Meyer-Veltrup procedure (2017), only the size of the samples differ. Original model prescribes samples (0.5 × 1.0 × 20.0 cm_3) that are of different shape than used in respective study (1.5 × 2.5 × 5.0 cm^3). As the methodology is based on relative values, samples size has minor influence on the outcome. Results from durability tests were used to evaluate the inherent resistance factor k_{inh}, and both factors were used to determine the resistance dose D_{Rd} of the four wood materials examined in this study (Stone pine sapwood and heartwood, Scots pine, Norway spruce). Only basidiomycetes were applied to determine k_{inh} in the research. Terrestrial microcosm tests or in ground durability tests were not performed, as prescribed by original Meyer-Veltrup approach (2017).

3. Results and discussion

3.1. Density and chemical analysis

The density of *P. pinea* sapwood (537±35 kg/m^3) was comparable to the density of *P. sylvestris* (491±16 kg/m^3) and was higher than the density of spruce wood (411±14 kg/m^3). The density of *P. pinea* heartwood (815±47 kg/m^3) was significantly higher than that of sapwood. References on the wood density of *Pinus pinea* do not distinguish sapwood from heartwood; it was usually reported as a density varying between 450 and 870 kg/m^3, with an average of 610 kg/m^3 (Giordano, 1984) or 584 kg/m^3 (CEN 2016). However, the tested samples can be considered consistent with references reported for the species in Italy (Giordano, 1984).

The results of chemical analysis of the obtained extracts and the amount of wood extractives soluble in cyclohexane and acetone for different categories of woody tissue are presented in Table 2. The highest amount of wood extractives was soluble in cyclohexane, representing lipophilic extractives. On average, heartwood of stone pine gave 322.2 mg/g of lipophilic and 13.5 mg/g of hydrophilic extractives. As presented in Table 2, statistical analysis revealed significant differences among the different types of wood, namely; sapwood (SW), transition zone (TZ), heartwood (HW) and knot-wood (KW) (ANOVA; $p < 0.05$). Samples of HW contained the highest amount of lipophilic extractives. Lower contents were characteristic of knots (KW) and TZ, and the lowest ones were in SW (Table 2) (LSD test). KW samples of stone pine were found to be relatively rich in hydrophilic extractives compared to reference data (Willför et al. 2003), containing more than forty milligrams of hydrophilic extractives

per gram of dried wood meal (Table 2). Knot-wood of trees is known to be generally a very rich source of phenolic compounds (Holmbom 2011; Willför et al. 2004). Considering the hydrophilic extractable fraction, spectrophotometric analysis revealed that HW samples of stone pine contained on average 13.8 mg/g of total phenols, while extracts of KW were characterized by the highest amounts. The content of total phenols in KW was measured to be 23.7 mg/g, representing a two to four times lower value than the literature data for knot-wood of Scots pine (Karppanen et al. 2007). Significantly lower contents were measured in TZ of stone pine (7.3 mg/g). The lowest concentrations of phenolic compounds were found in the extracts of sapwood (SW) (LSD test) (Table 2).

All the obtained extracts were analysed for characteristic phenolic compounds present in the wood of *Pinus* species (Fengel and Wegener 1989; Kai 1991; Umezawa 2000). Nortrachelogenin (NTG), pinosylvin (PS), pinocembrin (PC) and pinosylvin monomethyl ether (PSMME) were used for identification and quantitative analysis of the separated peaks of stone pine extracts. Pinobanksin, pinostilbene and pterostilbene were also used for peak assignments. Peak identification was done by comparing retention times and UV spectra of the separated compounds with references (Figure 2). Chromatographic analysis revealed the presence of stilbenes, flavonoids and lignans in the wood extracts of stone pine (Figure 2). Chromatographic analysis revealed that heartwood extracts of stone pine samples contained 0.1 mg/g of NTG, 0.04 of PS, 1.7 mg/g of PC and 0.5 mg/g of PSMME (Table 2). If compared to the wood of Scots pine, the extractives of which have been comprehensively investigated, samples of stone pine contained lower amounts of NTG, PS and PSMME, whereas concentrations of PC were slightly higher (Willför et al. 2003). While stem tissues of Scots pine contained just 0.1 % to 2 % of stilbenes, KW was considered to be a much richer source, in which the concentration of these phenolic extractives can be up to 8 % (Hovelstad et al. 2006; Willför et al. 2003). The hydrophilic extractable fraction of Scots pine KW is also characterized by a large concentration of lignans, whereas knots can hold 3% of NTG (Fang et al. 2013). In addition to pinobanksin, PC is a flavonoid that has been reported to be present in extracts of other Pine species, e.g., *P. banksina*, *P. strobus*, *P. radiata, P. resinosa, P. pinaster, P. contrata* (Conde et al. 2014; Hillis and Inoue 1968; Kai 1991; Pietarinen et al. 2006; Simard et al. 2008). In addition, the bark of stone pine has been reported to contain tannins and other flavonoids, i.e., catechin, epicatechin, catechin gallate and taxifolin (Nunes et al. 1999; Yesil-Celiktas et al. 2009).

Statistical analysis showed significant differences in the content of the identified compounds among SW, TZ, HW and KW (ANOVA; $p < 0.05$). Correlated to the results of gravimetric and spectrophotometric analysis, KW contained significantly higher amounts of the targeted compounds than normal stem wood (LSD test) (Table 2). Chromatographic analysis revealed that TZ contained higher amounts of phenolic extractives than HW. It is generally known that heartwood compounds are formed *in situ* at the location of the transition zone (TZ), i.e., a few rings thick brightly-coloured zone of wood, which is located between living sapwood and the dead central part of a stem. This physiological process is frequently explained as the genetically programmed death of parenchyma cells and, consequently, as the accumulation of extractives (Magel 2000). Differences in the content of qualitatively evaluated low-molecular compounds (NTG, PS, PC and PSMME) between SW and HW samples were not statistically significant (LSD test). The most important groups of extractives related to the durability of Scots pine heartwood are reported to be phenolic compounds and resin acids (Harju and Venalainen 2006; Harju et al. 2003). The mentioned groups of extractives can to some extent explain the higher amounts of spectrophotometrically determined total phenols in HW, since resin acids can also react with Folin-Ciocalteu reagent (Harju and Venalainen 2006; Prior et al. 2005). The higher durability of stone pine HW in comparison to SW can be attributed to the larger concentrations of lipophilic extractives and possibly also the presence of bioactive phenolic compounds, viz. lignans, stilbenes and flavonoids. In relation to the results of the present

investigation, a similar explanation could also be accepted for KW. As has been proven, the amounts of phenolic compounds and stilbenes in the wood of pine are correlated with the natural durability of wood (Karppanen et al. 2007; Leinonen et al. 2008; Venäläinen et al. 2003), which can also be seen from our results.

3.2. Wood durability

The high concentration of extractives (Table 2) in heartwood results in fairly good durability of stone pine wood against wood decay fungi (

Table 3). Extractives in wood can be ascribed the role of bioactive compounds or they can be referred to as water repellents, inhibiting the permeability of wood for fungal hyphae. The mass loss of non-aged (leached) samples exposed to *G. trabeum* and *T. versicolor* was lower than 3 % and thus considered insignificant according to the EN 113 (CEN, 2006) standard. Only *F. vaillanti* was able to degrade 8.6 % of *P. pinea* HW. Artificial ageing (leaching) slightly decreased the durability of *P. pinea* HW against *G. trabeum* and *T. versicolor,* but this decrease did not influence the classification to durability classes based on CEN/TS 15083-1 (CEN, 2005) criteria (Table 1). Based on durability testing, *P. pinea* HW can be classified as durable wood (DC 2). Due to the hydrophobic nature of *P. pinea* HW*,* artificial ageing did not have a significant influence on the loss of extractives. This is a much higher classification than that suggested in the latest revision of EN 350 (CEN, 2016). As expected, the mass loss of *P. pinea* sapwood was much greater than that of heartwood. The highest mass loss was determined in samples exposed to *G. trabeum* with non-aged (35.9 %) and aged (27.1 %) samples. The mass loss of *P. pinea* sapwood samples exposed to the other two wood-degrading fungi was much lower. However, if mass losses of *P. pinea* sapwood are compared to other non-durable wood species, such as Scots pine sapwood or Norway spruce wood, it can be seen that mass losses of *P. pinea* sapwood are lower than determined in Scots pine, and in the same range as mass losses in Norway spruce wood. However, CEN/TS 15083-1 (CEN, 2005) classifies Scots pine sapwood, stone pine sapwood and Norway spruce wood as non-durable wood (DC 5). This classification is also in line with the literature data (Stirling et al., 2016). One of the reason for observed durability can be ascribed to hydrophobic properties of wood. However, MC of *P. pinea* heartwood exposed to the fungi was higher than 30 %, indicating that the wood moisture content increases to the level suitable for fungal decay, considering that there was a water source in the jar. MC of the wood exposed to *F. vaillanti* was 45%.

3.3. Water permeability

However, recent findings clearly indicate that the service life of wood is also determined by water exclusion efficacy, in addition to its natural durability (Meyer-Veltrup et al., 2017). A range of various sorption and capillary water uptake tests is therefore very important and they were performed as shown in Table 4 and Figure 6. Two sets of *P. pinea* HW samples were used. Half of them were oven dried (103 °C), which resulted in resin translocation to the surface. The resin thus formed a kind of a film on the surface. The other half of the HW samples were dried at 30 °C only, to avoid this effect.

After one hour of immersion in water, *P. pinea* sapwood uptakes 58.0 % of water, which is much more than determined with spruce wood (33.7 %) or Scots pine sapwood (51.1 %). The reason for the high water uptake is associated with its porous structure, as shown in the SEM micrographs (Figure 4 and Figure 5). The good permeability of stone pine sapwood can be demonstrated by short-term water uptake determined with a force tensiometer (Figure 3) and MC after 24 h (Table 4). The water uptake of *P. pinea* HW was much lower. The good water exclusion efficacy of stone pine HW can be seen from short-term and long-term uptake measurements (Table 4). Samples that were not oven dried exhibited even better water exclusion efficacy than the oven-dried ones. For example, after one hour of immersion, oven-dried samples took up 6.3 % of water, while the uptake of not oven-dried ones was only 3.9 % of water. Similar ratios were also determined after 24 h and 192 h of immersion. This is very important from a practical point of view, since the majority of the *P. pinea* wood in outdoor applications will not be oven-dried. The prime reason for the good hydrophobicity of HW is the high resin content (Table 2). As can be seen from Figure 5, resin droplets appeared on the surface during the vacuum step before SEM imaging. In addition, resin covers tracheids in the vicinity of the resin canals. It should be noted that the wood had been air dried in the laboratory for two years prior to observation and that the surface was planed one day prior to SEM analysis. The resin droplets must thus have appeared on the surface during SEM imaging, due to the low pressure in the chamber.

3.4. Sorption properties

The sorption properties of *P. pinea* wood can be clearly seen from Figure 6. The sorption curves are of a typical S shape, with clearly resolved hysteresis. It is clear that the adsorption and desorption curves of HW are considerably lower than those of comparable sapwood. For example, the maximum MC of sapwood was 22.69 %, while the maximum MC of non-extracted HW was considerably lower (17.14 %) (Figure 6). We presume that this can be ascribed to the presence of resins in the HW, since the maximum MC (21.66 %) of the extracted HW, as well as the shape of the sorption and desorption curves, was comparable to sapwood. Extraction did not have a major influence on the sorption properties of the sapwood (data not shown). It should be noted that the samples were milled and were not oven-dried prior to DVS analysis. We thus believe that the presence of lipophilic extractives is the predominant reasons for the obtained difference. Since the concentration of lipophilic extractives is fairly high, it can be assumed that they reduce the sorption properties through the interaction of the extractives with OH groups of cell wall polymers, filling potentially available space for water, and by the fact that increased amounts of hydrophobic resin in any case decrease the total wood MC (Table 4). Similar mechanism is evident at wood modified or impregnated with resins, where introduction of inert materials increases the mass of the wood, and thus decreases the determined percentage of water in wood (Humar et al. 2017).

3.5. Material resistance

In order to assess the relative service life of *P. pinea* according to the Meyer-Veltrup (2017) model, two factors were calculated; namely k_{wa} (wetting ability) and k_{inh} (inherent durability). Both factors are expressed as relative values to Norway spruce. As can be seen from Table 5, factors describing the wetting ability and inherent durability of Scots pine sapwood, beech wood and *P. pinea* sapwood are similar, with beech wood having the best durability among the tested materials. (

These data tend to suggest that the first signs of fungal decay on these materials will develop after 280 days (*P. sylvestris* sapwood) and 483 days (*F. sylvatica*) of favourable conditions, with a moisture content above 25 % and temperature between 4 °C and 40 °C (Isaksson *et al.,* 2013). On the other hand, the relevant model indicates that *P. pinea* HW will last much longer, up to 4648 days in favourable conditions. This is 14 times more than spruce wood (Table 5). This value indicates the great performance of stone pine HW, which is a result of good inherent durability and water exclusion efficacy. However, it should be considered, that inherent durability of wood was calculated based on the durability against basidiomycetes only, not considering soft rot fungi. Wetting ability was calculated as prescribed by Meyer-Veltrup model (2017). If the relative service life of stone pine HW ($D_{Rd\ rel}$ = 14.30) is compared to other wood species, it can clearly be seen that the relative service life of comparable wood species such as Scots pine HW ($D_{Rd\ rel}$ = 2.75), western red cedar ($D_{Rd\ rel}$ = 3.90) and European larch ($D_{Rd\ rel}$ = 5.60) is much lower (Meyer-Veltrup *et al.,* 2017). One of the open questions about *P. pinea* durability is what happens with resin during weathering. It is well known that some fungi are able to use wood resin as a food source (Paine *et al.,* 1997). If the resin in HW is degraded, there are numerous open voids present in the HW, which could decrease the water performance significantly. The diameter of the resin canals is up to 150 µm, which means fairly big voids if empty (Figure 4). Field test should be performed to confirm or reject this hypothesis.

4. Conclusions

The properties of stone pine wood were assessed in relation to its possible use in outdoor applications. Due to the high amount of extractives present, *P. pinea* heartwood exhibits superior sorption properties and performance against water and wood decay fungi, which results in rather long service

life as calculated by the Meyer-Veltrup model. The results of the model clearly indicate that the service life of *P. pinea* wood in above ground applications is up to 14 times longer than that of the reference Norway spruce. This indicates that heartwood could at least be used in above ground applications, while sapwood needs to be impregnated or modified.

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Table 1. Durability classes (DC) based on median mass loss

Durability class DC	Description	$ML_{F, med}$ [%]*
1	very durable	< 5
2	durable	5 < ML_F ≤ 10
3	moderately durable	10 < ML_F ≤ 15
4	less durable	15 < ML_F ≤ 30
5	non-durable	> 30

**Mass loss $ML_{F, med}$ according to CEN/TS 15083-1 (2005)*

Table 2. Content of soluble extractives and phenolic compounds in wood of stone pine (Pinus pinea).

	Content of extractives															
	Total solubles		**Lipophilic**		**Hydrophilic**		**total phenols**		**NTG**		**PS**		**PC**		**PSMME**	
	[%]		[mg/g]		[mg/g]		[mg/g]		[mg/g]		[mg/g]		[mg/g]		[mg/g]	
Sample	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*	*Avg*	*SD*
P. pinea SW	1.6	0.29 [a]	8.3	3.06 [a]	9.6	4.09 [a]	0.3	0.13 [a]	0.1	0.02 [a]	<0.05 [a]		0.2	0.02 [a]	0.1	0.03 [a]
P. pinea TZ	14.9	3.65 [b]	133.7	30.8 [b]	15.0	5.68 [a]	7.3	1.47 [b]	0.3	0.03 [a]	0.1	0.01 [b,c]	2.4	0.11 [b]	0.6	0.02 [b]
P. pinea HW	33.6 [d]		322.2 [d]		13.5 [a]		13.8 [c]		0.1	0.003 [a]	<0.05 [a,b]		1.7	0.04 [a,b]	0.5	0.01 [a,b]
P. pinea KW	24.4	4.74 [c]	195.6	30.0 [c]	48.8	17.4 [b]	23.7	3.87 [d]	12.0	6.36 [b]	0.2	0.21 [c]	6.3	2.01 [c]	2.3	0.78 [c]

Results are expressed as the mean value of measurements (Avg) with the corresponding standard deviations (SD).
a - d; different letters within the same column indicate statistically significant differences ata 95.0% confidence level (Fisher's least significant difference, LSD test). <0.05 mg/g; the content of qualitatively evaluated extractives was below the practical limit of detection
Different categories of woody tissue: SW - sapwood; TZ - transition zone; HW - Heartwood; KW - knotwood

Table 3: Durability of P. pinea sapwood (SW) and heartwood (HW) after exposure to wood-degrading fungi.

Wood species	*Ageing*	*Wood decay fungi*											
		G. trabeum				*F. vaillantii*				*T. versicolor*			
		Avg. mass loss [%]	SD	DC	MC [%]	Avg. mass loss [%]	SD	DC	MC [%]	Avg. mass loss [%]	SD	DC	MC [%]
P. pinea SW	no	35.9	8.9	5	73	16.0	2.9	4	42	17.7	5.9	4	53
	yes	27.1	2.0	4	86	14.2	7.1	3	46	12.2	2.4	3	52
P. pinea HW	no	1.0	1.6	1	33	8.6	2.2	2	45	0.4	0.7	1	39
	yes	4.5	0.7	1	55	6.1	2.0	2	49	1.4	1.1	1	40
P. sylvestris SW	no	43.1	8.5	5	92	20.0	4.1	4	39	17.8	8.6	4	49
	yes	27.1	4.0	4	63	14.2	12.5	4	41	6.3	2.4	2	52
P. abies	no	36.8	4.3	5	57	20.1	3.7	4	42	18.0	2.8	4	53
	yes	34.2	3.5	5	62	21.7	3.3	4	39	16.5	2.5	4	55
F. sylvatica	no	36.0	8.3	5	48	15.5	2.4	5	37	30.5	4.9	5	57
	yes	35.9	3.7	5	54	13.7	8.2	3	46	20.0	2.7	4	54

Table 4: Short-term and long-term water uptake of P. pinea sapwood (SW) and heartwood (HW) and reference wood samples.

	Water uptake after 200 s [g/cm^2]		MC after 1 h [%]		MC after 24 h [%]		MC after 192 h [%]	
	Average	St. Dev.	Average	St. Dev.	Average	St. Dev.	Average	St. Dev.
P. pinea SW	0.229	0.0300	58.0	6.4	73.1	6.0	93.2	6.3
P. pinea HW 30 °C	0.006	0.0128	3.9	1.6	14.9	2.9	25.6	4.2
P. pinea HW 103 °C	0.019	0.0127	6.3	1.5	23.7	3.6	37.3	4.7
P. sylvestris SW	0.254	0.0275	51.1	6.7	62.2	4.1	83.3	5.1
P. abies	0.206	0.0152	33.7	1.3	60.4	1.8	78.5	2.2
F. sylvatica	0.102	0.0290	12.8	2.0	35.6	4.7	60.1	6.4

Table 5: Factors that determine the service life of P. pinea sapwood (SW) and heartwood (HW) and reference wood species. The explanation of the factors can be seen from Equation 3.

	k_{wa}*	k_{nh}*	D_{Rd}	$D_{Rd\ rel}$
P. pinea SW	0.9	1.1	306	0.9
P. pinea HW 30 °C	3.5	4.1	4648	14.3
P. pinea HW 103 °C	3.2	4.1	4299	13.2
P. sylvestris SW	0.9	1.0	280	0.9
P. abies	1.0	1.0	325	1.0
F. sylvatica	1.5	1.0	483	1.5

** Factors identified in table 5 are mean value of individual factors calculated according to the methodology described in details by Meyer-Veltrup et al. 2017. k_{inh} factor accounting for the inherent protective properties of the material against wood decay fungi. k_{wa} factor is describing the wetting ability. This factor is median value derived from short term water uptake, 24 h immersion and water vapour uptake and desorption test.*

Figure 1. Chemical structures of phenolic compounds present in the wood of stone pine (Pinus pinea). NTG, nortrachelogenin; PS, pinosylvin; PC pinocembrin; PSMME, pinosylvin monomethyl ether.

Figure 2. HPLC-PDA chromatograms of Pinus pinea wood extracts monitored at 275 nm. (a) Acetone extract of stone pine heartwood (HW). (b) Acetone extract of stone pine knotwood (KW). (c) The mixture of reference compounds (standards) used for identification and quantitative analysis.

Figure 3: Water uptake of P. pinea sapwood (SW) and heartwood (HW) and reference wood species in an axial direction determined with a force tensiometer

Figure 4: Cross-section of P. pinea sapwood with axial resin canals appearing in latewood.

Figure 5: Resin emerging from resin canals in P. pinea heartwood. The resin appears as drops and as a film covering the wood tissue.

Figure 6: Influence of extraction on adsorption and desorption performance of P. pinea sapwood (SW) and heartwood (HW). The second adsorption and desorption cycle is plotted. Sorption curves of extracted and non-extracted sapwood are almost identical, so only the sorption performance of non-extracted sapwood is plotted.