UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN INFORMACIJSKE TEHNOLOGIJE

MASTER'S THESIS
(MAGISTRSKO DELO)

MODIFICATION OF SCOTS PINE WOOD WITH STEAMED HARDWOOD CONDENSATES AND THE INVESTIGATION OF ITS POTENTIAL USE IN THE MARINE ENVIRONMENT

(MODIFIKACIJA BOROVEGA LESA S PARNIMI KONDENZATI IZ TRDEGA LESA IN RAZISKAVA NJEGOVE POTENCIALNE UPORABE V MORSKEM OKOLJU)

NENSI PERUŠKO

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(Modifikacija borovega lesa s parnimi kondenzati iz trdega lesa in raziskava njegove potencialne uporabe v morskem okolju)

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Izvleček:

Z ekološkega vidika je les na splošno netoksičen material, ki ga najdemo v skoraj vsakem naravnem okolju. Lokalno dostopne, manj cenjene vrste lesa, lahko za gradnjo uporabimo le v omejenem obsegu, tehnike modifikacije pa ponujajo rešitve izboljšanje lastnosti tega lesa. V tej nalogi smo za modifikacijo lokalno rastoče borovine uporabili kondenzate parjenja dražjih drevesnih vrst. Malo smole in maščobnih kislin v izpirkih vzorcev, po obdelavi s kondenzatom, kaže na nizko raven toksičnosti pridobljenega materiala, ki je imel tudi boljše elastične lastnosti in upogibno trdnost.

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Abstract:
From the ecological perspective, wood is a non-toxic material that is naturaly present in almost every environment. Because of the limited usage of local low- valuable wood species as a construction material, modification techniques offer solutions to surpass their weak points. For the purpose of this master thesis, steaming condensates of valuable tree species are used to modify localy grown pine wood. Low values of leaching of resin and fatty acids in all condensate treated samples indicated low levels of toxicity. Condensate treatment had positive effect on samples elastic properties and flexural strenght.

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LIST OF ABBREVIATIONS

ACA- Ammoniac Copper Arsenate
ACQ- Alkaline Copper Quat
ACZA-Amoniacal Copper Zinc Arsenate
ATP- Adenosine Triphosphate
CA- Citric Acid
CA-B&C- Copper Azole
CCA- Copper Chrome Arsenic
EU- European Union
G- Glycerol
GC-MS- Gas Chromatography - Mass spectometry
MOE- Modulus of Elasticity
MOR- Modulus of rupture
MTBE- tert-Butyl methyl ether
OLA- Oligomeric Lactic Acid
PAH- Polycyclic Aromatic Hydrocarbons
SA- Succinic Anhydride
SEM- Scanning Electron Microscope
TA- Tartaric acid
TMS- Trimethylsilyl
UV- Ultra Violet
WPG- Weight Percentage Gain

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1 INTRODUCTION

Wood is a natural product and it is used by humans for centuries for many different applications such as fuel, shelter, tools and furniture (Mantanis and Sahin 2017). Wood also serves as a source of material for the paper industry and industrial chemicals (Barnett and Jeronimidis 2003). This is mainly due to a fact that it is easily accessible, inexpensive, non-toxic and a renewable source of material (Sandberg et al. 2017). It is composed of cellulose, lignin, hemicelluloses and a small amount of extractives (Miller 1999). Wood originates from different tree species and comes from all over the world. Hence, we have diverse product with its own signature (physical, chemical, mechanical and aesthetical properties). It is characterized with increased strenght in relation to its weight, it is an insulating material to heat and electricity and has appealing acoustic properties. Without any doubt, it is also one of the most versatile materials. Although it has many positive characteristics, some of its characteristics are making it limited to the specific usage as a construction material in the outdoor environment. For a very firm and solid construction, construction materials need to be able to deal with different forces, momentums and impacts from its environment. As a natural product wood is susceptible to biodegradation processes (Homan and Jorissen 2004). Along with these biodegradation processes, wood is significantly affected by environmental factors: UV irradiation, moisture, insects and fungi. (Ermeydan et al. 2014). To overcome too quick deterioration of wood by the above mentioned environmental factors, wood modification becomes indispensable. It offers numerous advantages: It enhances properties of the wood, especially its dimensional stability, its biological resistance, water resistance and durability. It offers a broader spectrum of utilisation and prolongs life of wood and its products (Jirouš-Rajković and Miklečić 2018). Wood modification also prolongs the life of wood products. In this way, wood becomes a more sustainable material: the product life last longer than growing a new tree. In this master thesis, the modification of a localy grown wood species (pine) was modified, using the waste product (extractives) of comercial wood steaming processes as an environmentaly acceptable modification technique. Thereafter, the performance of this modified wood was followed in the marine environment. The toxicity of leaching products released by the modification agents in the aqeous surroundings was studied on laboratory scale. Also the effect on the elastic properties and flexural strength of the wood samples after the modification was tested.

2 WOOD MODIFICATION

2.1 INTRODUCTION

2.1.1 Development of wood modification

The article of Freeman et al. (2003) described the history of chemical wood modification: The first chemical wood modification methods started to develop in 1700s. In these times, they used mercuric chloride and copper sulphate. Afterwards, in 1815. zinc chloride was recommended. The first big development in wood preservation history was in 1836, when the coal-tar creosote was introduced. Unfortunately, the high price of oil made the creosote process expensive. Then, in 1938, one of the most popular wood modifying agents, CCA (copper chrome arsenic) was developed, followed in 1950 by ammoniac copper arsenate (ACA). CCA was inexpensive and highly effective, but toxic arsenic molecules led to a withdrawal of CCA-treated wood, which resulted in a loss of all major markets such as in the United States, Canada, EU and Japan. Wood impregnation with such biocides (containing e.g. creosote, arsenic, zinc, copper, and chromium), was until now most commonly used practice, because it prevents biological degradation. Nowadays restrictions on the number of active substances (above all arsenic) that can be used in wood preservation, and their field applications, have occured by the European directive on the use of biocides. (The European Commission Directive 2003/2/EC). Furthermore, wood treated with common preservatives is classified as hazardous waste (Ministry of the Environment 1129/2001). Aside of risks connenected with the use of such materials for treatments, there is an increasing concern over the problems appearing in the disposal of the timbers after the end of their commercial lifetime. Systems enhancing the durability of wood should be sustainable, both in production and use. „In addition to this, the treated wood products should, at the end of their life, be suitable for energy production by combustion, composting or for use as a secondary fibre source by related industries, without presenting any problems of residual chemicals arising from the treatment. Therefore, the wood industry is now oriented towards the development of new, non-toxic, sustainable ways to prolong the service life and to improve the main features of wood (Ermeydan et al. 2014).

2.2 CLASSIFICATION OF WOOD MODIFICATION

As can be seen from Figure 1, wood modification can be divided in two main categories. One is active modification, which involves changes in the chemical nature of the material. This can be done via reactions with wood polymers, cross-linking and degradation of the cell wall. The second category is passive modification, in which some change in wood properties are observed, but no chemical changes are occurring. To this category belong lumen filling and cell wall filling (Hill 2006).

- For Lumen filling, a substance, ussually a resin, is used. These treatments might increase strength properties and slow down the process of water (vapour) uptake, but they do not change the sorption behaviour of the wood over a longer periods of time.
- Bulking, to fill the cavities in the cell wall, as well as the cell lumen, is a more general approach. Bulking treatments tend to reduce the swelling and shrinkage of the wood. They might even have a beneficial effect on the long-term sorption behaviour.
- Modifying treatments are usually the most effective. The chemical structures of cell wall components (lignin, cellulose and hemi-cellulose) are altered and covalent bonds are formed. Changes in the wood structure can occur by enzymes, heat or chemical reagents

Many properties can be improved permanently, in particular durability, dimensional stability and reduced equilibrium in moisture content.

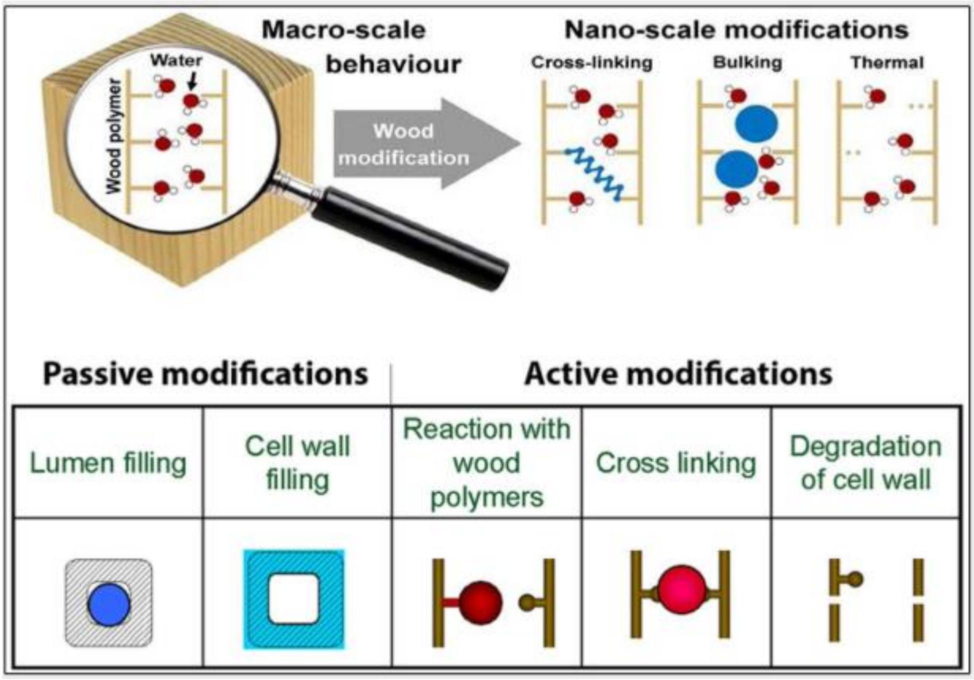


Figure 1. Schematic illustration of the effect of wood modification (Sandberg et al. 2017).

2.2.1 Chemical modification

This master thesis is focused on chemical modification of wood. Chemical modification of wood is the modification that consists of the chemical reaction of a reagent and the cell wall, creating a covalent bond. The covalent bonds are occurring between the reagent and the cell wall polymer hydroxyl groups (Hill, 2006). This reaction can produce a single chemical bond with one OH group or cross-linkage between two or more OH groups. Thanks to the occurring change in chemical nature of the cell, the cells' properties can also change (Hill, 2006). This is desirable when it comes to modification processes.

Until now, there have been many reactions studied for wood modification. Most chemical modifications are still on laboratory scale but a few methods made it also to industrial scale. Acetylation and furfurylation are the most known.

2.2.1.1 Acetylation

Acetylation is the modification of the wood cell wall with acetic anhydride. During the reaction, acetic acid is formed as a by-product, as a result of the esterification of the accessible hydroxyl groups in the cell wall with the reagent (Jirouš-Rajković and Miklečić 2018). Some of the benefits provided through this type of modification are reduced moisture absorption and improved dimensional stability of the wood. Improved dimensional stability is due to the reduction of free sites able to bind water through hydrogen bonds, and bulking the cell wall back to its green volume (Jirouš-Rajković and Miklečić 2018). One of the very important features is that it contains only non-toxic constituents. This method is commercialized by the Dutch company Accsys Technologies in Arnhem and is known under the commercial name Accoya (Sandberg et al. 2017). Researches showed that acetylated wood had an improved dimensional stability and decay resistance as well as an improved resistance to attack by termites and marine organisms (Borgers 2014).

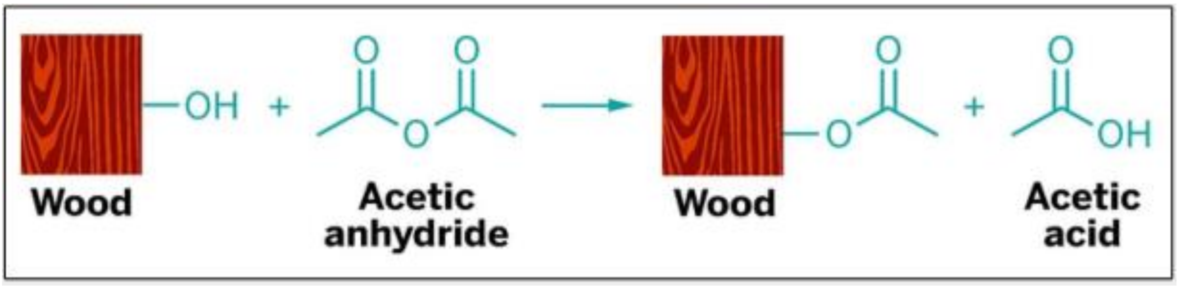


Figure 2. The main reaction of wood of wood acetylation with acetic anhydride (Sandberg et al. 2017).

2.2.1.2 Furfurylation

Another type of chemical wood modification is furfurylation. This type of wood modification uses furfuryl alcohol. In this reaction, furfuryl alcohol molecules penetrate into the wood cell walls and it conducts in situ polymerization (Jirouš-Rajković and Miklečić 2018). The goal of this method is to achieve permanent swelling of the wood cell walls. Results showed improved dimensional stability, reduced water uptake and better resistance to biological degradation. Along with these enhanced characteristics, furfurylated wood has a rich brown colour which helps to mask many blemishes and discolorations. The furfurylation method is commercialized by the Norway company Kebony AS (Sandberg et al., 2017).

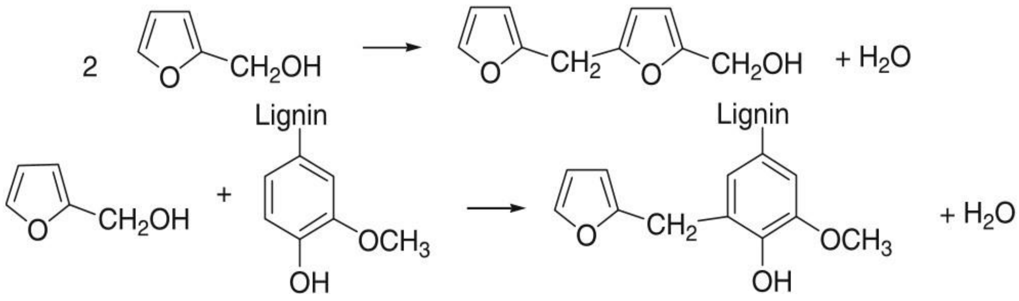


Figure 3. The main reaction ivolved in polymerisation of fufuryl alcohol (Gérardin 2015).

2.2.1.3 Environmentaly friendly, biodegradable substances in wood protection – Research on laboratory scale

Esterification reactions, by condensation of hydroxide groups from chemical reagents and wood with carboxylic acids under moderate temperatures, is one of the most studied methods to chemically modify wood with non-toxic reagents. Citric acid, for example, is widely spread in nature and it completely satisfies strict environmental and economic requirements. Its use in wood modification methods was tested in multiple studies (Despot et al. (2008). Essoua et al. (2016) treated samples of *Pinus strobus* and *Pinus contorta* with citric acid and glycerol and cured the samples at temperatures of 180°C. L'Hostis et al. (2017) treated *Fagus sylvatica* with citric acid and glycerol and cured at temperatures ranging from 100 to 160°C. Both studies prove among other properties a better protection against fungal decay and improved mechanical properties. Berube et al. (2018) modified wood with glycerol and citric acid and found that the use of acid catalysts resulted in higher levels of polymerisation. Further a citric acid in a combination with sucrose has been used as an adhesive for particleboard production (Umemura et al. 2012, 2015; Widyorini et al. 2016). Larnoy et al. (2018) investigated an aqueous-based citric acid and sorbitol esterification method. Their study has proven that polyesterification of sorbitol and citric acid in wood results in enhanced properties of product such as increased dimensional stability, increased durability against decay fungi and less susceptibility to blue-stain fungi. Wood modification using a combination of sorbitol with citric acid was also patented by Kiljunen et al. (2011) which involved a combination of aqueous solutions of 5% citric acid and 10% of an alcohol (wt/wt) plus the addition of 2% sodium hypophosphite as a catalyst. Another promising environmentally friendly solution for wood modification is chemical modification via oligomeric lactic acid (OLA) treatment. These bio-polyesters variously provide bulking and lumen filling treatments. Grosse et al. (2019) have been impregnated wood with OLA and subsequently heat treated the wood to polymerize the OLA in situ. In this case OLA impregnation followed by heat treatment enhanced wood properties such as dimensional stability and biological resistance. L'Hostis et al (2018) investigated the combination of glycerol (G) with citric acid (CA), tartaric acid (TA) or succinic anhydride (SA). Waterborne mixture (about 40% solid ratio) were vacuum/pressure impregnated in wood, then thermal treatment was performed to induce in situ polymerization. Best results were obtained using GCA and GSA formulations, keeping leaching under 1%, reducing swelling up to 60%, ensuring high durability against white and brown rot fungi and maintaining bending mechanical performances compared to untreated wood.

Another tested procedure to bulk the cell-walls is the Maillard reaction, where amine groups are reacted with the reductive ends of sugars according to a complex mechanism. The usage of the Maillard reaction in wood modification processes is still not a very commonly spread action. But the Maillard reaction can be used in a procces of bulking of the cell wall in wood as an agent to increase the dimensional stability of wood (e.g. Hill and Jones 1996). Some of the atempts of using the Maillard reaction was performed by Hauptmann et al. (2015) that treated wood with tricine at 103°C to induce the Maillard reaction between amine of the tricine and reducing sugar of the cell wall components (cellulose and hemicellulose). Their results showed increased tensile strength and hardness of the wood, as well as decreased equilibrium moisture content. Further, Larnøy et al. (2007) treated wood with low (18 kDa) and high molecular weight (58 kDa) chitosan at 100°C, supposedly inducing the Maillard reaction to occur between amine of the chitosan (which is an amino-sugar) and reducing sugar of the wood cell components. Their method resulted in enhanced hydrophobation, and slightly reduced antifungal properties, stabile modulus of rupture and an increase of modulus of elasticity by 27% compared with untreated wood. Another attempt was evaluation of bonding properties of the Maillard-reacted chitosan in three-ply plywood by a tensile shear test preformed by Umemura et al. (2010). Use of Maillard reaction was already successfully applied as resole binders for composite wood and wood particle boards (Jackson et al. 2008; Jackson et al. 2010; Hand 2017). In the study of Peeters et al. (2018) different combinations of chemical reagents were scanned. They found that the combination of 0.1 mg L^{-1} lysine/glucose/ citric acid reacted at 120°C obtained the highest weight percentage gain, albeit with significant leaching. They have found that at 120°C mainly esterification between glucose and citric acid occurs and a reaction temperature of 160°C is necessary to achieve Maillard reaction and no leaching. They tested another reagent combination (0.1 mg L^{-1} ascorbic acid/Trizma base/citric acid) that appeared to work. However, the SEM images showed that it could damage the wood. Although the Maillard reaction classically involves primary amines, secondary amines could be used, to (Hauptmann et al., 2015).

Schwarzkopf et al. (2018) investigated utilisation of chemically modified lampante oil for wood protection. Lampante oil from the Istarska belica olive was chemically modified using two maleinisation treatments to ensure more viable for wood protection. Their results showed increased potential for the oil to react with the hydroxyl groups within the wood structure and relatively low leaching of tretment.

3 WOOD IN THE MARINE ENVIRONMENT

Despite the increasing use of artificial materials for underwater and near water constructions, wood still plays an important role in the marine infrastructure as a part of docks, wharves supporting structures, as well as other features in harbours and ports. Wood is often the material of choice because of its structural, economic, environmental and aesthetic benefits (Tarakanadha et al. 2020). As the shoreline gets more and more developed, many wooden structures are placed in shallow, nearshore waters (Weis at al. 2011). The marine environment represents harsh conditions for wood structures. Therefore, the life span of most wood species will be only a few years and wood preservation methods need to be developed. In the marine environment, wood shows suspectabilty to fungi, insects and marine borers (Bivalvia (Teredinidae and Pholadidae), Isopoda (Limnoria and Sphaeromatidae) and Amphipoda (Cheluridae)) attacks. In Europe the most aggressive woodboring bivalves belong to the Teredinidae, but species of the Pholididae are also reported. The woodboring Crustacea in Europe are Limnoridae and Cheluridae (Borges et al. 2014). Another type of negative impact of salt water exposure is the presence of biofouling organisms. Along with the biotic factors, the service life of wood and its products is affected by abiotic factors such as pH, salinity and water temperature.

Figure 4. Use of wood in boat decking (Marine News from the Great Lakes).

Figure 5. Wood as a part of docks construction (Slovenia360).

Figure 6. Effect of marine borers on wood structures (SAWPA).

3.1 WOOD PRESERVATION IN THE MARINE ENVIRONMENT

Wood is vastly present in the field of maritime construction and its biodegradation problem caused by biotic or abiotic factors need to be solved. Wood modification has been the best solution. From the variety of different chemicaly based preservative systems, only a few have been used in the marine aquatic environment. Those systems can be devided in two categories: waterborne and oil-type systems (Wood Preservation Canada).

3.1.1 Waterborn systems

Waterborne systems use water as a carrier for the preservative chemicals. The main pathway of chemicals in these systems is precipitation into the wood substrate and attachment to the wood cells that minimizes migration. As a result, this sytems leaves a dry, paintable surface and are preferably used in aquatic applications such as docks, boardwalks and bulkheads. Such systems include: CCA (Chromated Copper Arsenate), ACZA (Ammoniacal Copper Zinc Arsenate), ACQ (Alkaline Copper Quat) and CA-B & C (Copper Azole). Use of CCA may pose a hazard through leaching of toxicants. All of the present metals are known to be toxic to aquatic biota. Arsenic is carcinogenic, mutagenic and teratogenic and is mostly taken up by phytoplankton (Sanders et al. 1980). Chromium is present in the aquatic environment in a form of chromate. Chromate (IV) is known to be carcinogenic and mutagenic and also accumulates in phytoplancton (Sanders et al. 1987). Copper can be also very toxic in higher concentrations especially to algae and mollusks (Newell et al. 1986).

3.1.2 Oil-type preservatives

Oil-type systems are characterized by the presence of active preservatives or preservatives dissolved in an oil-based solvent. The presence of oil can be beneficial in such systems because it can act as a water repellant and helps to limit checkin and splitting. Oil-type preservatives are used in aquatic application for piling, timbers, bulkheads, bridges and boardwalks. The most commonly used oil-based preservatives in marine environment are Creosote, Pentachlorophenol and Copper Naphthenate. Three compounds used in preservative systems, which can create concerns in aquatic environments are: Copper, Polycyclic aromatic hydrocarbons (PAHs) and Pentachlorophenol. Copper is used in several wood modifying systems. Many preservatives use copper as a main component for biocidal activity. Fish and other aquatic organisams have been shown to be less tolerant to copper than humans and other terestrial mammals. The second toxic compound that is a byproduct of the usage of creosote preservative are PAHs. They are naturally occurring substances that are produced by fires, volacanoes, coal deposits, oil seeps and any type of combustion. PAHs are water insoluble and do not pose a treath in the water column. But on the other hand, PAHs can accumulate in the sediment to levels up to 10 to 20 parts per million, wich was associated with cancer in fish.

There is ongoing research, which is looking for new ways of modification, which are less hazardeous to the surrounding media. Researchers are searching for more environmentally friendly alternatives for wood modification methods. A few of these developed methods are still on lab-scale but are seeming to be successful, other methods are allready applied: chemical (acetylation, furfurylation, resin impregnation etc.), thermo-hydro (thermal treatment) and thermo-hydro-mechanical processing (surface densification) (Sandberg at al. 2017).

3.2 ENVIRONMENTAL CONCERNS ABOUT TREATED WOOD IN THE AQUATIC ENVIRONMENT

Nearly all materials, man-made or natural, placed in an aquatic environment will introduce chemicals which, if present in large enough concentration, will either immediately or over time pose a potential threat to plant and animal life forms, which are present in that environment. Also a certain quantity of the chemicals used to preserve wood will leach or migrate from treated wood structures built in aquatic and wetland areas into the water column and surrounding sediments. The question is how much and when will the preservatives move into the environment and under what circumstances might they represent a significant risk. Also the rate of leaching is moslty affected by the time it has been leaching (Weis at al. 2011). Marine fouling organisams are one of the most sensitive organisms to preservatives, since they are growing direclty attached to the treated wood surfaces. Chemical leaching from wood can cause potential acute or chronic toxicity. The severity of that problem goes from reduced abundance of certain species to shifts in flora and fauna. The effect of leaching diverse in different environments. In well flushed areas the effect is minored by quick dilution of the leachate. But in more stagnant waters the effect can be more severe, where concentrations of toxic chemicals can build up. While the greatest potential of environmental exposure is within-water use of treated materials, where direct contact and higher retention levels exist, the large volume of wood used in above-water structures and decking also merits risk consideration and sound chemical management. Splash and rain runoff represent potential paths for treating chemicals to move from treated wood into the environment (Wood Preservation Canada).

4 STEAMING CONDENSATES

The aim of the thesis is to use steaming condensates of more durable hardwood species to modify local grown, less valuable pine wood because of the antifungal properties. Steaming of logs or timbers is widely used in the forest industry to improve wood quality and achieve desired properties for further proccessing. Steamning proccess are summarized by Ledig (2013): Steaming takes place in steam kilns or combined steam-dry kilns at atmospheric pressure and temperatures between 80 and 100°C. Steaming is considered an autohydrolysis process because it causes several overlapping chemical reactions and decomposition reactions in the wood (Korte et al. 1991). Those reactions include breaking lignin-carbohydrate bonds and liberation of hemicelluloses (Kubinsky et al. 1973). Steaming processes produce condensates wich are composed of water-soluble hydrolysis products and other volatile substances. Present organic extractives are aliphatic hydrocarbons, alcohols, fatty acids, terpens, resin acids, sterols and waxes and water soluable extractives wich are phenol, mono- and dimeric sugar derivates, pectin and tannin (Rathke et al. 2013). The proportion of extractives ranges from 2 to 5% (Fengel and Wegener 2003). These components can be washed (or leached) out from the wood during the process of drying (Rathke et al. 2013). The obtained condensate mixture may be considered as an environmental concern (Cronn et al. 1983).

4.1 EXTRACTIVES

4.1.1 Resin acids

Resin acids are tricyclic diterpenes that are found in the resin of the tree. They have a role of protecting the tree from microbial and insect damage (Markis 2003). All resin acids have similar structure and are indentified by the red colored group, seen in Figure 7. Resin acids are very strong and stable in turns of chemical degradation (Dethlefs and Stan 1996). They are the most toxic component of wood extractives (Back and Allen 2000). When present in marine environment, resin acids can be stored in the tissues of fish. Above the concentration of 0.4 mg/L, they can even cause different kinds of liver diseases in fish (Mattsoff and Oikari 1987). Bushnell et al. (1985) showed that the treatment of red cells of fish with dehydroabietic acid caused rapid depletion in ATPs and oxygen consumption wich have resulted in cell haemolysis. Cell breakdown additionaly increases the levels of bilirubin wich cause jaundice in 2-4 days (Rigol et al. 2003). Despite the possibility of severe damages, fish crab recovered from acute resin acid intoxication within 6 days in clean water (Mattsoff at al. 1987). Other researches have shown many other toxic effects accompanied by the presece of resin asids such as erythrocytic abnormalities in adult eel

(Pacheco and Santos 1999), inhibition of K^+/Na^+ pump (Rabergh et al. 1992) and DNA damages in microorganisms (Kinae et al. 1981). More recent researches have used species such as *Dapnia magna* and *Vibrio fischeri*. Newer data said that resin acids toxicity levels are similar to those of phenol and chlorophenols (Farré et al. 2001) and equalent to some biocides (Fernandez-Alba et al. 2002).

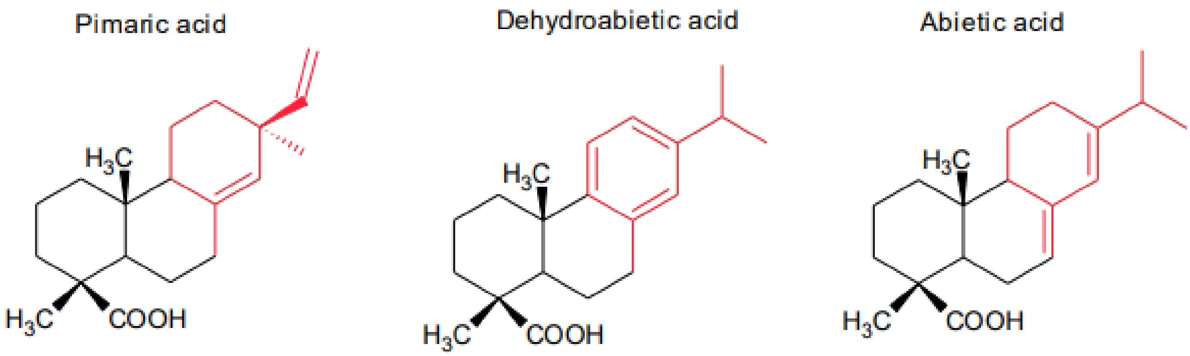


Figure 7. Three most comon examples of resin acids with similar structures having the red coloured group determining their indentity (Ismailov 2013).

4.1.2 Fatty acids and triglycerides

Fatty acids are aliphatic acids, long 16 - 24 carbon molecules. They can be saturated or unsaturated and together with glycerol can form triglycerides (Ismailov 2013). Trees use fatty acids as a source of nutrients. Fatty acids are not as soluble in water in regard to resin acids. They can be toxic to but less toxic than resin acids (Back and Allen 2000).

4.1.3 Sterols and sterylesters

Wood sterols have carbohydron skeleton with five rings similar to the one of hormones found in animals. Figure 8 shows the sterol hydrocarbon skeleton with the indentifying group marked in red. The main role of sterols in trees is to act as hormones (Ismailov 2013). In contact with fatty acids, sterols forms esters what makes them more resistive to biodegradation. It has been shown that fish exposed to sterols shows signs of disturbed hormone levels and induced enzyme activity wich proves their toxicity.

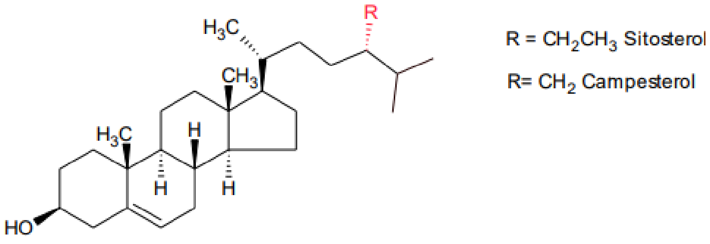


Figure 8. Sterol hydrocarbon skeleton with red R group determining the type of the sterol (Ismailov 2013).

When present in the aqueous environment, especially in the marine environment, fatty and resin acids behave different that neutral compounds like sterols. In basic conditions, such as sea water, acids are dissociating and their charged forms dissolve in water. This also depends on the temperature and metal ion concentration of the water. Since fatty- and resin acids are of amphiphilic nature, they have hydrophilic parts that are highly soluble in water and hydrophobic part with a high solubility in non-polar solvents. With their polar head groups, they tend to concentrate in the aqueous environment. on the other hand, with their aliphatic tails they concentrate toward air or hydrocarbons. These properties enable them to self-associate to form colloids like micelles (Ismailov 2013). Figure 9 shows an illustration of a resin and fatty acids mixed micelle with a solubilized sterol in the interior.

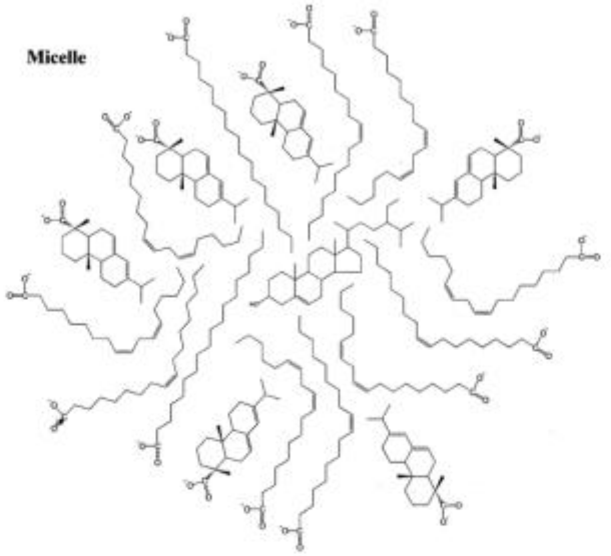


Figure 9. Cross section of a spherical resin and fatty acid mixed micelle with a solubilized sterol in the interior (Ismailov 2013).

Guided by the idea, that steaming condensates have antifouling properties, in this thesis different wood species condensates (beech, walnut, cherry) were investigated as wood preservative agents. To avoid leaching of the steaming condensate compounds in the environment, tests were done to react the reagents inside pine wood via the esterification and Maillard reaction.

4.2 CONDENSATION AND MAILLARD REACTION

As described above, in this work we want to modify pine wood with steaming condensates. Our goal is to bind the compounds, which are present in these steaming condensates, into wood, so they won't leach out over time. Therfore, lysine and citric acid were added to the condensates. By applying heat, condensation and Maillard reactions can take place between these reagents and (hemi)cellulose of wood.

The Maillard reaction in one of the most common and complex reactions that is mainly linked with food chemistry. It is induced by heating or by prolonged storage of food items. It has also been detected in mammalian organisms (Shipar, 2009). The Maillard reaction is a reaction between a reducing sugar and a primary amino acid. The reaction is complex but shortly this is what happens: loss of water in the initial stage produces an imine that is able to cyclise. Instead of cyclisation, also an Amadori rearrangement may take place. Subsequently, a range of reactions takes place, which will first form low molecular weight reaction products but in in the later stages generate melanoidins, which are predominantly high molecular weight compounds. Regadring the complexity of all the products generated in this reactions, the composition of the chemical structur of melanoidins is barely known. (Echavarria et al. 2012; Kim and Lee 2009).

A condensation reaction, such as esterifications, acetalizations and ketalizations, is a reaction between a carboxylic acid or keton, and a hydroxide or amino group, producing water as coproduct. Removal of this water from the reaction, by heat, will shift the equilibrium in favour of the desired product. These reactions can be unfortunately also reversible when water is added to the reaction product.

The advantage of these type of reactions is that it is an aqueous process and initiated by heat only, making it relatively straightforward to apply to wood in a commercial process. In addition, the reaction does not require the use of strong acids or bases, which could degrade the wood structure.

5 METHODS AND MATERIALS

5.1 MATERIALS AND INSTRUMENTS

5.1.1 Chemicals

Citric acid – CAS: 5949-29-1; purity: 99.8%; producer: Fisher scientific International INC.; origin: USA; chemical formula: $C_6H_8O_7$
L-lysine – CAS: 56-87-1; purity: ≥98.0%; producer: Sigma-Aldrich; origin: Germany; chemical formula: $C_6H_{14}N_2O_2$
Heptadecanoic acid – CAS: 506-12-7; purity: ≥98.0%; producer: Sigma-Aldrich: origin: Germany; chemical formula: $C_{17}H_{34}O_2$
Sodium hydroxide – CAS: 1310-73-2; purity: ≥98.0%; producer: Honeywell International Inc.; origin: Germany; chemical formula: NaOH
Sulfuric acid – CAS number: 7664-93-9; purity: 95.0-98.0%; producer: Honeywell International Inc.; origin: Germany; chemical formula: H_2SO_4
MTBE (tert-Butyl methyl ether) – CAS number: 1634-04-4; purity: ≥99.0%; producer: Honeywell International Inc.; origin: Germany; chemical formula: $C_5H_{12}O$
Calcium chloride – CAS number: 7774-34-7; producer: Carlo Erba Reagents; origin: France; chemical formula: $CaCl_2$
Sodium chloride – CAS number: 7647-14-5; producer: Carlo Erba Reagents; origin: France; chemical formula: NaCl
Potassium chloride – CAS number: 7447-40-7; producer: Fisher Bioreagents; origin: Spain; chemical formula: KCl
Magnesium sulfate heptahydrate – CAS number: 10034-998; producer: Fisher Bioreagents; origin: Japan; chemical formula: $MgSO_4$ X $7H_2O$
Magnesium chloride hexahydrate – CAS number: 7791-18-6; producer: Acros Organics; origin: Belgium; chemical formula: $MgCl_2$ X $6H_2O$
N,O- Bis (trimethylsilyl) trifuoroacetamide with 1% trimethylchlorosilane – CAS: 25561-30-2; producer: Sigma-Aldrich; origin: Germany; chemical formula: $CF_3C(NSi(CH_3)_3)OSi(CH_3)_3$

Chemicals were used in this thesis for a variety of different applications. Sodium chloride, potassium chloride, calcium chloride, magnesium sulfate heptahydrate and magnesium chloride hexahydrate were combined and added to deionized water to create artificial sea water. L-lysine and citric acid were both used in the modification process of wood samples. Lysine was used as a prime amine which should promote the Maillard reaction and Citric acid was applied as a crosslinking agent, with other words they were used as binders of chemical compounds, which are present in steaming condensates, to the wood cell wall. Another two chemicals from the list, Sulfuric acid and Sodium hydroxide, were incorporated in our work as adaptors of the pH of the gas chromatography mass spectroscopy (GC-MS) samples. Heptadecanoic acid was used as an internal standard solution for GC-MS. MTBE which was used as a solvent in the sample preparation prior to GC-MS measurements.

5.1.2 Instruments

An oven (Easy Oven SP-55; Kambič) was used to dry the samples prior and after the modification and leaching processes. Then, a desiccator and a chemistry pumping unit (PC 3001 basic; VARIO$^{pro)}$ was used for the removal of air from the wood lumen. For the three-point bending test, a universal testing machine (Zwick Roell Proline Z050) was operated. Wood leachates were qualitative and semi-quantitative analysed with a GC-MS apparatus (Agilent 5890 gas chromatograph coupled to the Agilent 5977B mass spectrometer). In the preparation of the GC-MS samples, a centrifuge (MIKRO 220/220R; Hettich) for liquid-liquid extraction and a Reacti-Vap 3 for solvent evaporation with N_2, have been used. Throughout the thesis smaller utensiles including digital caliper (BMI) and analytical balances (KERN&SON) were handled.

5.2 METHODS

5.2.1 Wood modification procedure

Before performing wood modification tests, an estimation of the composition of the steaming condenstates was perfomed by determining the condensate dry weight. 100 mL of steaming condensate was gradually heated until dryness. It was found that only 0.9 % of the steaming condensate was dry matter.

Small (20 × 20 × 5 mm) and defect-free sapwood specimens of pine – *Pinus sylvestris* L.) were used for the modification studies. Samples were cut such that growth rings were parallel to the tangential face, to prevent diamonding during the leaching tests. This size of sample was selected in order to ensure even penetration of reactants into the wood sample and allow for good access of the reagents to the cell wall. Samples were sanded to prevent loss of small particles during the treatment process, which could give small errors during weighing. An analytical balance (minimum reading: 1 mg) was used to weigh the wood specimens. The size of the wood samples was determined with a precision of ± 0.001 mm The size in each direction and weight was only measured once per sample. In this way, the contact with air was minimized as much as possible, which is necessary since weight increases due to the moisture present in air. Wood samples were dried in an oven and left to cool down in a desiccator. Air was removed from the wood sample during the aqueous impregnation step at ambient temperature in a desicator with vaccuum pressure. To determine the size and weight of unmodified wood samples, the wood samples were oven-dried for 16 h at 103°C to remove the moisture. Once removed from the oven, samples were transferred to a vacuum desiccator to cool to ambient temperature over silica gel, immediately thereafter the weight and volume of the wood samples were determined. Thereafter, samples were soaked in an aqueous solution of 50 mL steaming condensate of beech (*Fagus sylvatica*), cherry (*Prunus avium*) or walnut (*Juglans regia*) wood. Three categories of solutions were prepared: Each type of steaming condensate without any additives, each steaming condensate with 0.1 mg mL^{-1} citric acid, and each steaming condensate with 0.1 mg mL^{-1} citric acid and lysine (see Table 1). Citric acid and lysine were chosen to bind the chemical compounds, which are present inside the steaming condensates, with the wood. The concentrations were chosen to obtain high quantities of reagents that are completely soluble in water, while still having a solution density and viscosity that allows for easy penetration into the wood. Five replicates were made for each treatment. Control samples were treated with water only. The wood was placed into the amine-acid solution, then transferred for 1 h into a vacuum chamber to make sure all the air was removed from the wood lumen. The wood was subsequently soaked for 24 h in the solution to allow for diffusion of reagent into the cell wall. The wood samples were then removed from the solution and put into an oven for 24 h at 120°C (Figure 10), 160°C (Figure 11) or 200°C (Figure 12). After this, the wood samples were removed from the oven and cooled down to ambient temperature in a vacuum desiccator over silica gel and the volume and weight of the oven-dried wood was again determined.

The WPG was calculated according to the formula:

$$WPG(\%) = ((Wm - Wu)/Wu)x100$$

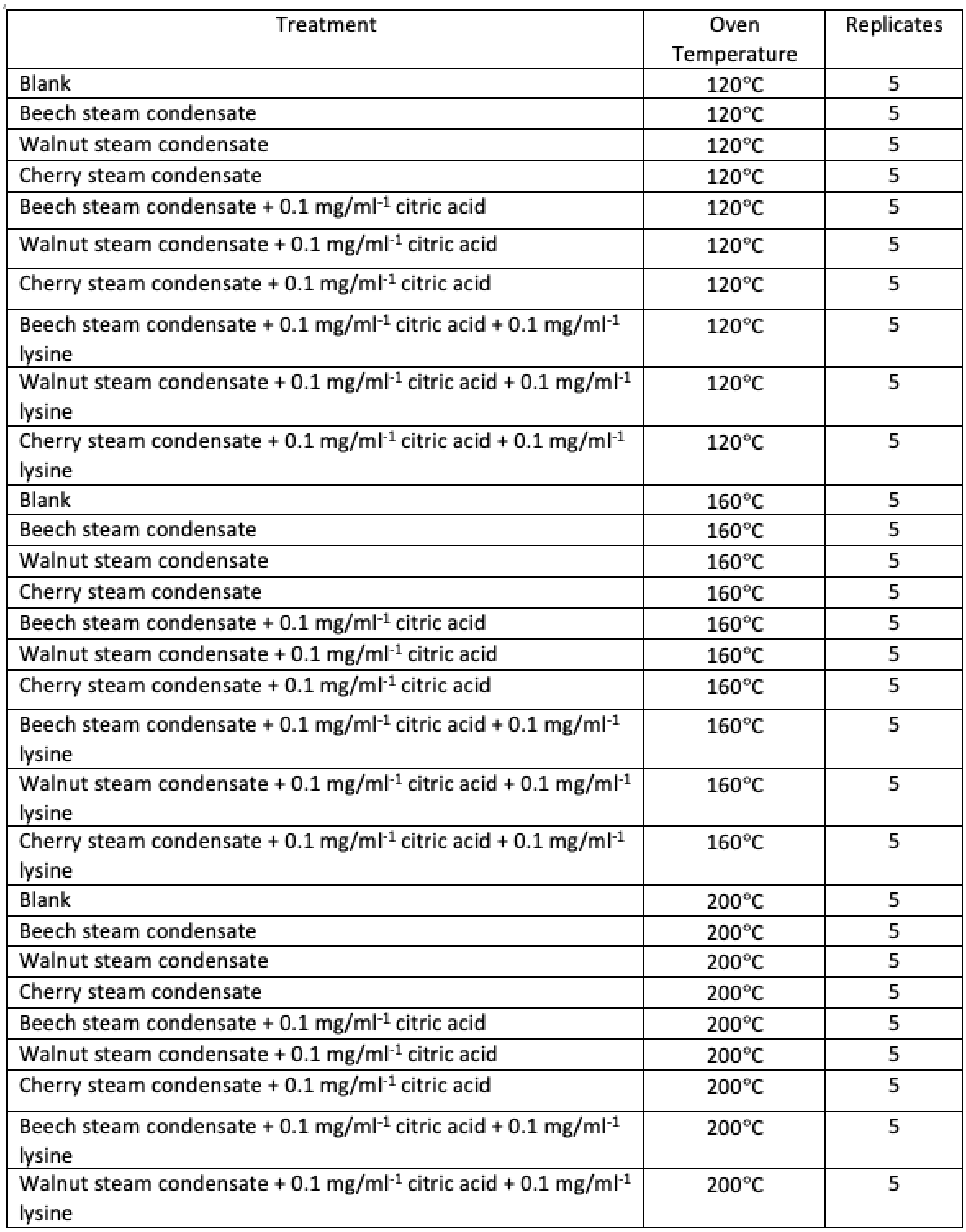
where Wm = weight of wood after modification or leaching;

Wu = weight of unmodified wood;

Leaching tests on the wood samples in water were performed in deionized water. A leaching cycle length of seven days was chosen, and the deionised water in the leaching chambers was changed three times a day. Leachates color was different between different wood sample, as can be seen in picture 13. After each leaching cycle, the wood was dried in an oven for 16 h at 103°C, cooled down to ambient temperature in a vacuum desiccator over silica gel, and the volume and weight gain of the oven-dried wood was determined.

To estimate if steaming condensates have a positive or negative effect on pine wood, the procedure described above was repeated by using deionised water instead of steaming condensates, having 0.1 g mL^{-1} of citric acid, or 0.1 g mL^{-1} citric acid and lysine as additives. The samples were tested in 5 replicates.

Table 1. Experiental data for the sample modification process.



Treatment	Oven Temperature	Replicates
Blank	120°C	5
Beech steam condensate	120°C	5
Walnut steam condensate	120°C	5
Cherry steam condensate	120°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid	120°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid	120°C	5
Cherry steam condensate + 0.1 mg/ml^{-1} citric acid	120°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	120°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	120°C	5
Cherry steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	120°C	5
Blank	160°C	5
Beech steam condensate	160°C	5
Walnut steam condensate	160°C	5
Cherry steam condensate	160°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid	160°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid	160°C	5
Cherry steam condensate + 0.1 mg/ml^{-1} citric acid	160°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	160°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	160°C	5
Cherry steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	160°C	5
Blank	200°C	5
Beech steam condensate	200°C	5
Walnut steam condensate	200°C	5
Cherry steam condensate	200°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid	200°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid	200°C	5
Cherry steam condensate + 0.1 mg/ml^{-1} citric acid	200°C	5
Beech steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	200°C	5
Walnut steam condensate + 0.1 mg/ml^{-1} citric acid + 0.1 mg/ml^{-1} lysine	200°C	5

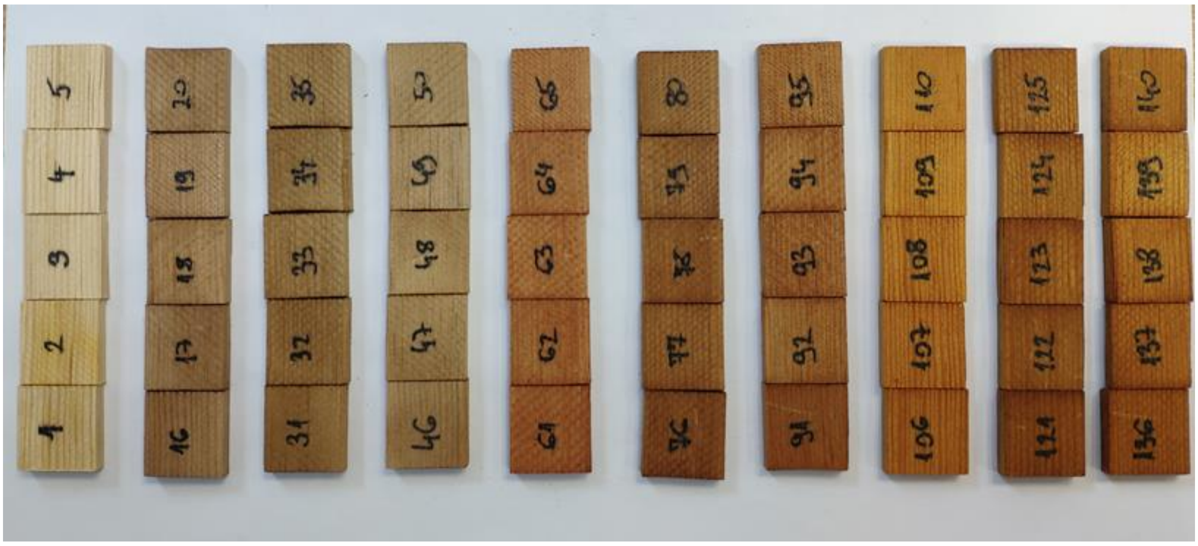


Figure 10. Wood samples treated with with steaming condensates (beech (16-20), walnut (31-35) and cherry (46-50)), steaming condensate + citric acid (beech (61-65), walnut (76-90) and cherry (91-95)) and steaming condensate + citric acid + lysine (beech (106-110), walnut (121-125) and cherry (136-140)) and control (1-5) reacted at 120°C.

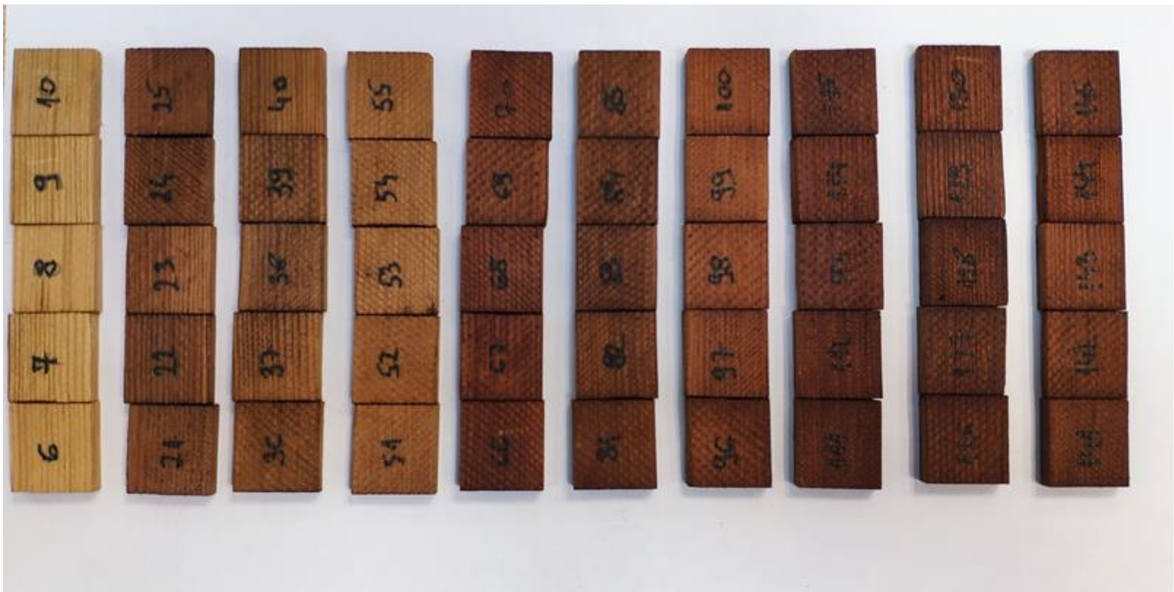


Figure 11. Wood samples treated with steaming condensates (beech (21-25), walnut (36-40) and cherry (51-55)), steaming condensate + citric acid (beech (66-70), walnut (81-85) and cherry (96-100)) and steaming condensate + citric acid + lysine (beech (111-115), walnut (126-130) and cherry (141-145)) and control (6-10) reacted at 160°C.

Figure 12. Wood samples treated with steaming condensates (beech (26-30), walnut (41-45) and cherry (56-60)), steaming condensate + citric acid (beech (71-75), walnut (86-90) and cherry (101-105)) and steaming condensate +citric acid + lysine (beech (116-120), walnut (131-135) and cherry (146-150)) and control (11-15) reacted at 200°C.

Figure 13. The compounds from the modified wood samples colour the water brightly during the leaching tests (control (1), beech (2,3,4), walnut (5,6,7) and cherry (8,9,10) leachate; from left to right).

5.2.2 Mechanical and toxicity tests

Tests were performed on laboratory scale to see the mechanical and toxic properties during water exposure of three modified pine wood samples. Both tests were performed on the same set of samples but an extra set of samples was made to do the mechanical testing before water exposure. Pine samples (180 x 10 x 10 mm) were modified with the three different steaming condensates, according to the most successful treatment procedure described in chapter 5.2.1. Shortly, 30 pine samples were treated with 300 ml of steaming condensate containing 0.1 g mL^{-1} of citric acid and lysine. A heat treatmen of 120°C for 24 hours was applied. The results of the modified samples were compared with the perfomance of untreated beech, cherry and walnut wood, steamed beech, cherry and walnut wood, and untreated pine wood. This gives a total of ten different test samples. Tests were performed on 180 x 10 x 10 mm sized wood sticks, since this is the standard size for mechanical three-point bending testing on the Zwick aparatus. For each type of sample ten replicates were made, since this is the minimum amount which is necessary to get reliable results with the Zwick test.

The toxicity of wood for the aquatic environment was determined according to the amounts of resin acids and fatty acids that leached out of these wood sticks in water. A comparison was made in salt water and deionized water.

To prepare artificial seawater (ASW), the procedure was used from Wilt and Benson 2004. Salts in different concentrations (see Table 2) were added in deionized water. The pH of the solution was adapted to pH 8 with TRIS.

Table 2. Artificial sea water component concentrations.

	Concentration (M)	g/L
$NaCl$	0.48	28.32
KCl	0.01	0.77
$MgCl_2$ ($MgCl_2 \times 6H_2O$)	0.027	5.48
$MgSO_4$ ($MgSO_4 \times 7H_2O$)	0.03	7.39
$CaCl_2$ (Anhydrid)	0.01	1.11

In summary, we had ten types of samples in ten replicates. For mechanical testing, they were tested in 3 conditions (before leaching, leached in deionized water and in salt water). The water during the leaching tests was taken for toxicity measurements.

5.2.2.1 Mechanical testing

To determine the static bending (flexural) strength of wood, a laboratory three-point bending test was carried out using Zwick Roell Z050 (see Figure 14, 15). Before testing, wood samples were oven dried at a temperature of 103°C for 24h. Bending strength (MOR) and modulus of elasticity (MOE) were calculated in N/mm^2, using following formulas:

$$MOE = \Delta F \text{ X } L^3/4 \text{ x } b \text{ x } h^3 \text{ x } \Delta f \qquad (N/mm^2)$$

where: ΔF – the load increment (N),
L – the distance between two supports (mm),
Δf – the deflection increment,
b – the sample width (mm),
h – the sample thickness (mm), (Boonstra et al. 2007).

$$MOR = 3 \text{ x } F_{max} \text{ x } L/2 \text{ x } b \text{ x } h^2 \qquad (N/mm^2)$$

where: Fmax – maximum load (N),
L – the distance between two supports (mm),
B – the sample width (mm),
H – the sample thickness (mm), (Bentsson et al. 2002).

Figure 14. Two types of samples prepared for the Zwick test.

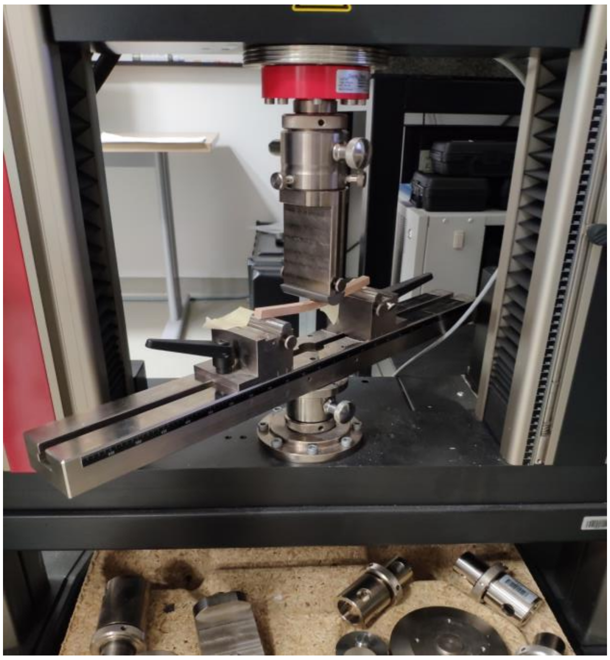


Figure 15. Zwick test procedure.

Each measurement was performed in ten replicates. The obtained data have often outliers, a high standard deviation and no normal distribution. Although these type of data normally require more advanced statistics, for a first exploration of data, descriptive and basic statistics, using F- and t-tests, were enough. A 5% uncertainty interval was used. Shortly, The F-test in a one-way analysis of variance is used to assess whether the expected values of a quantitative variable within several pre-defined groups differ from each other. The t-test is used, to determine if the means of two sets of data are significantly different from each other. If for the F-test, F critical minus the F-value is positive, the nul-hypothesis of equal variances can be rejected. For the t-test, if tcritical minus the absolute t-value is positive means that the nul- hypothesis of equal means can be rejected.

5.2.2.2 Toxicity testing

The amounts of resin and fatty acids in the wood leachates, were determined with GC-MS, according to an adapted procedure of Latorre et al. (2003). Several procedures are described in literature to measure resin and fatty acids stating that, or basic conditions (Voss and Rapsomantiotis 1985; Dethlefs and Stan 1996; Guttierez et al. 2001) or acidic conditions (Ekman and Holmborn 1989; Orsa and Holmbom 1994; Morales et al. 1992) are the most favourable to extract resin and fatty acids. Therefore, these different conditions were tested.

5 ml of wood leachate was acidified (H_2SO_4, pH3), made basic (NaOH, pH 10) or left at the same pH. The sample was put in a screw-capped glass test tube for liquid–liquid extraction. A 2-ml volume of MTBE, containing heptadecanoic acid, was added. The tube was vigorously shaken by hand for 2 min and centrifuged at 4000 rpm for 5 min. The clear MTBE layer was carefully pipetted off and the extraction was repeated twice with 2-ml volumes of MTBE. The combined MTBE extracts were evaporated under a gentle stream of nitrogen to dryness. Afterwards, the extracted resin and fatty acids were derivatized to the respective trimethylsilyl (TMS) esters. For derivatization, 100 μL of BSTFA with 1% of trimethylchlorosilane were added to the residue. The solution was kept in an oven at 70 °C for 20 min and was thereafter ready for analysis.

The same procedure was repeated with 10 mL of water sample and three times 4 mL MTBE extractions in case the resin and fatty acid concentrations were too low in the 5 mL water sample.

A GC–MS instrument with a HP-5MS column (30 mx 0.25 mm I.D. with 0.25 mm film thickness) containing 5% phenyl methyl siloxane was used. The oven temperature was held at 120 °C for 2 min and programmed to 300 °C at a rate of 4 °C min^{-1}. The final temperature was held for another 5 min. The inlet, ion source, and GC interface were 300, 230, and 310 °C, respectively. The carrier gas was 1.2 mL min^{-1} helium. The mass ionisation mode operated with an ionising energy of 70 eV. Full scan data were scanning from m/z 100 to 450 at a rate of 2 scans s^{-1}. Semi-quantitative analysis was performed in selected ion monitoring (SIM) mode using margaric acid as an internal standard (I.S.).

Once the optimized procedure was found, wood leachates from the test samples were taken after one week and one month of leaching. The water samples were measured by the procedure as described above in the basic conditions, with 10 mL of sample and 4 mL MTBE extraction volumes.

5.2.3 Testing modified wood in the marine environment

Tests are in process to determine the influence of the wood modification on fouling organisms and wood borers. The multitude of fouling organisms and wood borers will indicate if there is a biocidal effect caused by the wood modification. The experimental set-up was based on an article from Westin 1996 where they also investigated the influence of wood borers on treated wood.

Ten types of wood samples (the same as in the mechanical and toxicity tests), will be compared with each other and submerged inside the sea: steamed beech, cherry and walnut wood, unsteamed beech, cherry and walnut wood, untreated pine and pine wood treated with beech, cherry and walnut condensates (lysine and citric acid as addititives). Each type of wood will be tested in 5 replicates. The wood samples have a size of 200 x 75 x 25 mm.

All samplers were attached on a rack (160 x 60 cm), which was made from PVC. The frame tubes are 50 fi (Figure 16, indicated in blue), A hole was drilled in the wood blocks, so 12-13 pieces could be hung on each of the four 32 fi bars (see figure 16, indicated in red). The blocks were separated by 2 cm long 40 fi PVC pieces. When the Slovenian Environment Agency in conjunction with the Marjetica Koper issues the permission to perform the sea tests, the rack will be hung about 4-5 m under sea level by attaching metal chains to the rack and the pier.

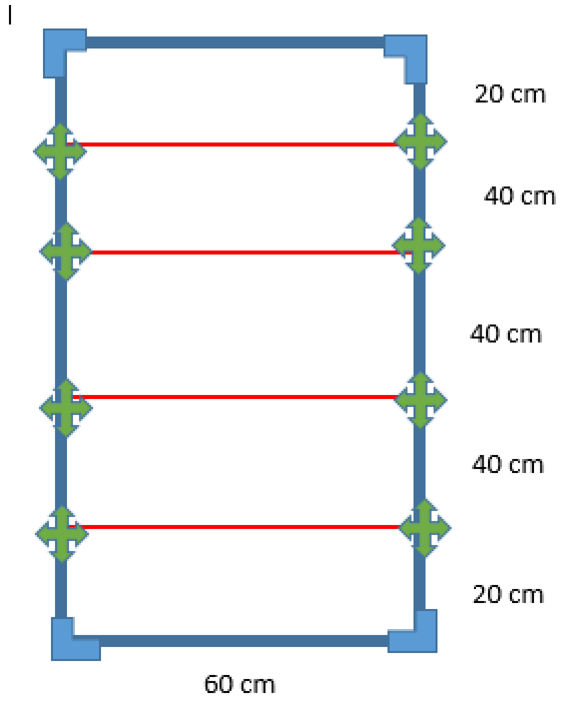


Figure 16. Schematic illustration of sample rack, which will be immersed into the sea.

Figure 17. Sample rack ready for on-site research.

6 RESULTS

6.1 WOOD MODIFICATION PROCEDURE

Small (20 × 20 × 5 mm) pine sapwood specimens of were used for the modification studies with steaming condensates (beech, cherry, pine) citric acid and lysine as described in chapter 5.1. The measurement of the WPG before and after leaching gives an indication of how much of reagents were permanently reacted inside the wood structure. The results of the measurements are presented in Figures18 and 19.

From Figure 18, it can be seen that the mean value of the WPG of wood impregnated with citric acid and lysine is greater than the mean value of wood impregnated with only citric acid. This can be expected since an extra ingredient is added, but it also means that both ingredients react with the wood and are persistent even during leaching. Wood samples reacted at 200°C showed in both cases, after leaching, a loss of weight. Weight loss can be due to the wood degradation at this high temperature. The lower weight loss, when lysine and citric acid are combined can mean that lysine is not affected by the degradation reaction or that it has a positive influence on the wood.

From the data in Figure 19 can be seen that control showed loss of mass in both after modification and after leaching period. This is expected because during treatment and leaching, the wood is added in deionized water, where its extractives are leached out. In Figure 19A, where the reaction temperature was 120°C, samples treated soly with condenstates increased in weight after modification but have lost almost 2 % of weight after the leaching period. Wood samples treated with citric acid and lysine showed a higher weight gain than the ones treated with citric acid alone. When the modified specimens were leached, the ones treated with citric acid and lysine showed a smaller difference in WPG between the modified wood and the leached modified wood. When higher reaction temperatures are used (Figure 19 B and C), wood start to degrade, which has a negative influence on the WPG and the reaction of chemicals inside the wood. Even during modification weight loss starts to appear, meaning that lots of wood components volatilize.

During the treatment also colour changes occurred (see Figures 10-12). With higher temperatures, the wood turned darker, but also more uniform on the exposed surfaces. Samples reacted at 120°C changed their colour to subtile yelow to light orange. At 160°C, the wood colour was reddish-brown. No surface cracks, warp or twist were observed in the treated wood. The color of the wood treated at 200°C was, dark brown. No surface cracks, but falling pieces and dust were observed.

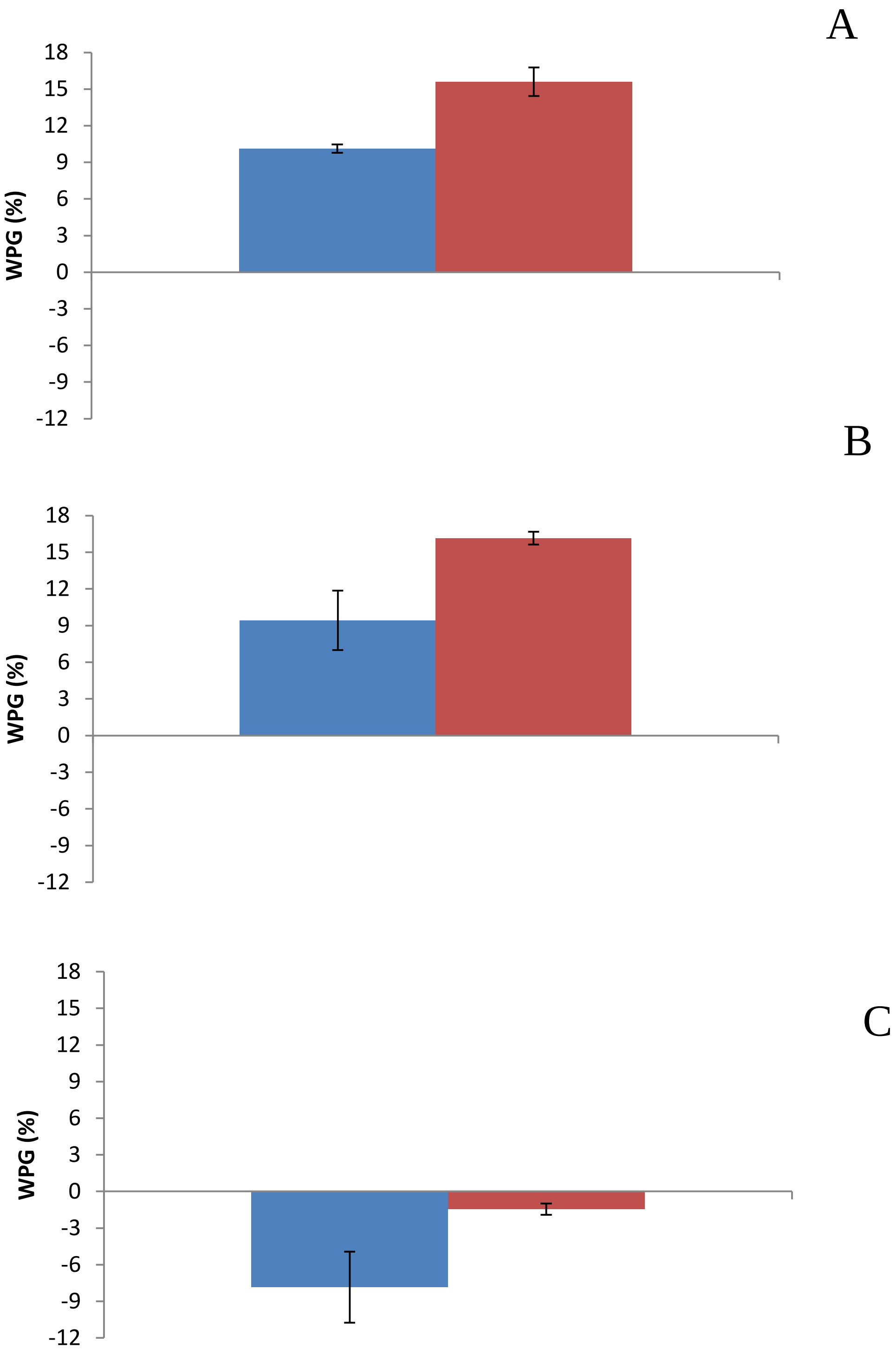


Figure 18. Change in WPG of wood samples soaked in deionized water and citric acid (blue) and deionized water, citric acid and lysine (red) reacted at (A) 120°C, (B) 160°C and (C) 200°C. The results represent the WPG after leaching the modified wood. Error bars show the standard deviation based on 10 replicate experiments.

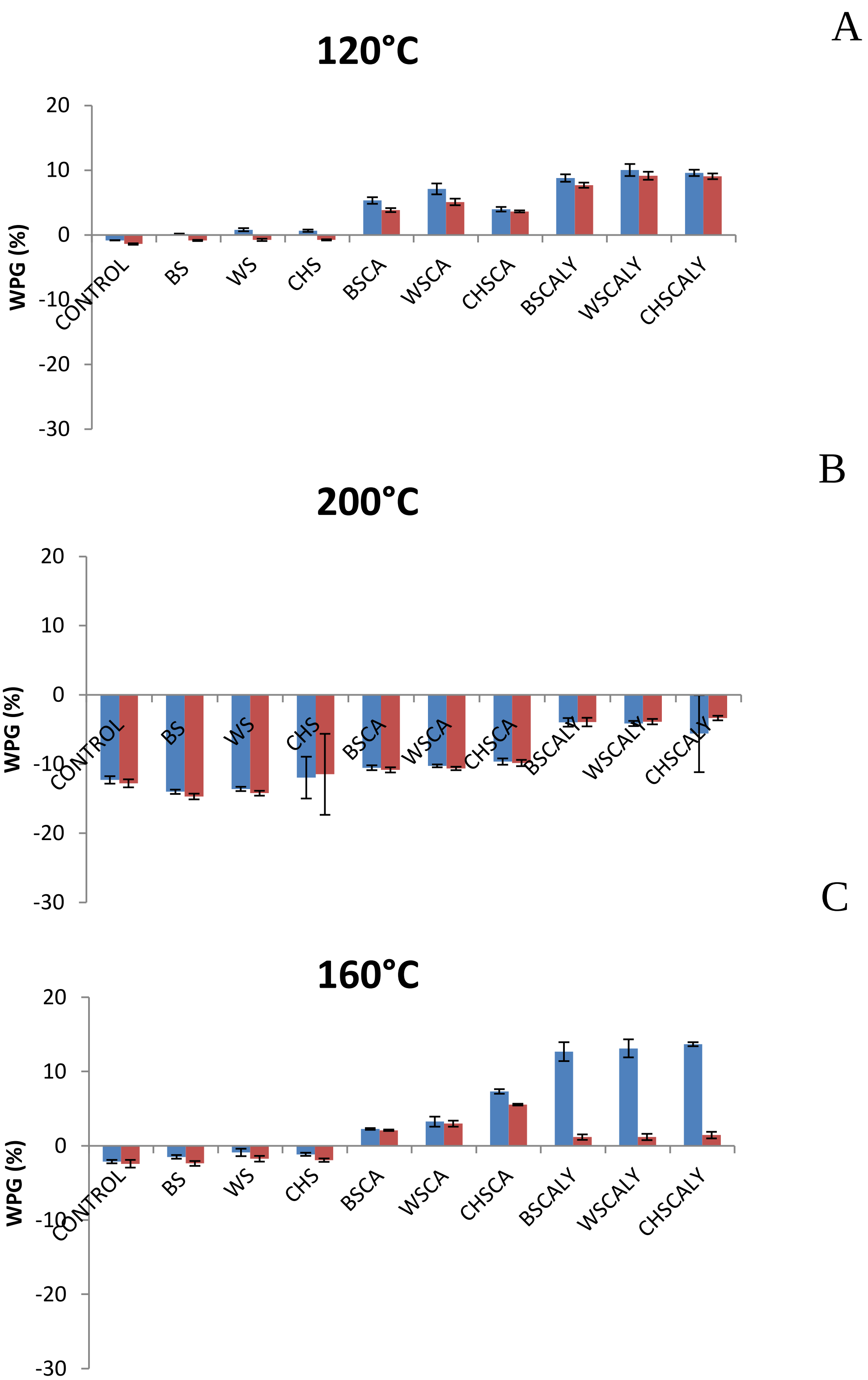


Figure 19. Change in WPG of wood samples treated with steaming condensates of Beech (B), Cherry (C) and Walnut (W), combined with citric acid (CA) and lysine (L). The different reaction temperatures were (A) 120°C, (B) 160°C and (C) 200°C. The graphs compare the WPG after modification alone (blue) and after leaching the modified wood (red). Error bars show the standard deviation based on 10 replicate experiments.

Comparing the results from Figures 18 and 19, it can be seen that the beech, walnut and cherry condensates have an influence on the reaction. Lower WPGs are obtained when citric acid and lysine are added together with the steaming condensates. Expected is that compounds present in the steaming condensates also degrade wood.

From these results it can be concluded that a combination of citric acid and lysine was the most promising treatment to have the steaming condensates reacted inside the wood. This combination at a reaction temperature of 120°C showed the highest WPG and the lowest product leaching from wood.

6.2 GC-MS DATA ANALYSIS

When wooden construction materials are used in the aquatic environment, they shouldn't only be mechanical stable over time, but also not be harmful to aquatic organisms. Therefore, the difference in toxicity was tested between steamed and unsteamed hardwood species (beech, cherry, walnut), pine, and pine modified with the steaming condensates of the researched hardwood species. The toxicity was determined by the amount of leaching of fatty and resin acids of the wood samples in deionized and salt water conditions. The measurements were performed with a GC-MS. Measurements were performed after one and three weeks of leaching. Results are normalized to the internal standard (I.S.).

Table 3. GC-MS results of different wood species after a one week leaching period in deionized water. The expected standard deviation of each measurement is expected to not be bigger than 5% of the measurement value. Ctrl- control, U- unsteamed; S- steamed; M- modified pine wood with hardwood condensates; B- beech; W- walnut; C- cherry).

	Ctrl	PINE	UB	UW	UC	SB	SW	SC	MB	MW	MC
FATTY ACIDS											
Palmitic acid	0.0014	0.0079	0.02	0.011	0.013	0.016	0.015	0.032	0.0048	0.22	0.019
Margaric acid (I.S.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Linoleic acid	<LOD	0.0011	0.000085	0.002	0.00019	0.00022	0.00024	0.0033	0.00062	0.0098	0.0024
Oleic acid	0.000008	0.0062	0.00017	0.0011	0.00025	0.00075	0.000079	0.0021	0.00054	0.0022	0.0019
Stearic acid	0.00093	0.014	0.0023	0.0031	0.0025	0.0062	0.00054	0.0065	0.0028	0.0083	0.0062
RESIN ACIDS											
Pimaric acid	<LOD	0.013	n.d	0.00007	0.000017	0.00001	0.0000046	<LOD	0.000041	<LOD	0.00011
Sandaracopimaric acid	n.d	0.0016	0.000025	0.000012	0.000022	0.00003	0.0000026	0.00017	<LOD	0.00034	0.000016
Isopimaric acid	0.000004	0.02	0.000014	0.00013	0.000034	0.00002	<LOD	<LOD	0.000071	<LOD	0.00018
Palustric acid	n.d	0.059	n.d	n.d	0.000022	<LOD	n.d	<LOD	<LOD	<LOD	0.000011
Levopimaric acid	n.d	0.024	n.d	n.d	n.d	n.d	n.d	0.000023	n.d	n.d	n.d
Dehydroabietic acid	0.000006	0.11	0.0001	0.0085	0.00068	0.00024	0.00011	0.0012	0.0053	0.00024	0.012
Abietic acid	<LOD	0.063	<LOD	0.00005	0.000046	0.000021	0.0000059	<LOD	0.000033	<LOD	0.000082
Neoabietic acid	n.d	0.026	n.d	n.d	n.d	n.d	n.d	0.0017	n.d	n.d	0.0000074

Table 4. GC-MS results of different wood species after a one week leaching period salt water. The expected standard deviation of each measurement is expected to not be bigger than 5% of the measurement value. Ctrl- control, U- unsteamed; S- steamed; M- modified pine wood with hardwood condensates; B- beech; W- walnut; C- cherry).

	Ctrl	PINE	UB	UW	UC	SB	SW	SC	MB	MW	MC
FATTY ACIDS											
Palmitic acid	0.0059	0.024	0.011	0.033	0.018	0.0099	0.0086	0.031	0.065	n.d.	0.015
Margaric acid (I.S.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Linoleic acid	<LOD	0.0010	0.00028	0.0017	0.0010	0.00063	0.00056	0.0058	0.011	0.00011	0.0058
Oleic acid	<LOD	0,0052	0.00063	0.0038	0.0011	0.0018	0.00060	0.0068	0.0081	0.00071	0.0032
Stearic acid	0.0023	0.017	0.0025	0.0061	0.0061	0.0032	0.0045	0.015	0.016	0.0040	0.0068
RESIN ACIDS											
Pimaric acid	n.d	0.0076	<LOD	<LOD	<LOD	n.d	n.d	0.00019	0.00023	n.d	0.00025
Sandaracopimaric acid	n.d	0.00095	<LOD	n.d	<LOD	n.d	<LOD	0.000037	n.d	n.d	0.000014
Isopimaric acid	<LOD	0.011	0.000019	<LOD	<LOD	n.d	<LOD	0.0003	0.00036	<LOD	0.00042
Palustric acid	n.d	0.041	n.d	<LOD	0.000025	n.d	<LOD	n.d	0.000042	<LOD	n.d
Levopimaric acid	n.d	0.015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Dehydroabietic acid	0.000094	0.19	0.00021	0.00052	0.0013	0.00012	0.000075	0.029	0.016	0.00082	0.036
Abietic acid	n.d	0.043	<LOD	0.000024	0.000073	<LOD	n.d	0.0002	0.0002	0.000043	0.00027
Neoabietic acid	n.d	0.019	0.000076	n.d	0.00020	n.d	n.d	n.d	n.d	0.00012	n.d

From the results depicted in tables 3 and 4, there can be seen that non-treated pine wood samples leached 10-100 times more fatty and resin acids than other wood specimens. Stearic acid and dehydroabiatic acid are respectively the most abundant fatty and resin acid in untreated pine, in both salt and deionized water. The fact that untreated pine shows higher concentrations than modified pine could be due to two reasons. The first reason can be explained by the fact that modified pine was already soaked before in an aqueous solution with chemicals, enabling the resin acids and fatty acids to leach into the treatment solution. The second reason could be that citric acid and lysine bind the resin and fatty acids inside the wood. The fact that modified pinewood doesn’t leach more fatty and resin acids than unmodified pine is really good news.

Steamed and unsteamed hardwood species release mainly palmitic acid and this in approximately similar concentrations. Resin acids are almost not present in the leachates of these samples.

For the leaching of resin and fatty acids, there is not a substantial difference between deionized and salt water. There is one main exception and that is the leaching of fatty acids in modified pine with walnut condensates. It is barely visible in salt water but leached in higher extent than the other treated pine samples in deionized water.

Table 5. GC-MS results of different wood species after a three week leaching period in deionized water. The expected standard deviation of each measurement is expected to not be bigger than 5% of the measurement value. Ctrl- control, U- unsteamed; S- steamed; M- modified pine wood with hardwood condensates; B- beech; W- walnut; C- cherry).

	Ctrl	PINE	UB	UW	UC	SB	SW	SC	MB	MW	MC
FATTY ACIDS											
Palmitic acid	0.016	n.d	0.08	1.21	0.21	0.14	0.43	0.16	0.99	1.027	0.1
Margaric acid (I.S.)	1.0000	1.0000	1.000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Linoleic acid	<LOD	0.0016	<LOD	0.037	0.0014	0.00044	0.021	0.0017	0.15	0.11	0.024
Oleic acid	0.00015	0.011	0.0041	0.0086	0.0032	0.0017	0.02	0.0017	0.13	0.088	0.023
Stearic acid	n.d	0.045	0.022	0.055	0.037	0.087	0.054	0.023	0.2	0.09	0.019
RESIN ACIDS											
Pimaric acid	n.d	0.029	0.00084	0.00012	<LOD	0.000081	n.d	0.000061	0.0027	0.0028	0.001
Sandaracopimaric acid	n.d	0.0037	0.00011	0.00056	0.00019	0.00027	0.00029	0.00014	0.00043	0.00052	0.00014
Isopimaric acid	n.d	0.045	0.00016	0.0002	0.000047	0.00012	0.00005	0.00019	0.0044	0.0041	0.0016
Palustric acid	n.d	0.16	0.0001	0.0001	<LOD	0.000077	n.d	<LOD	0.00027	0.00023	0.0001
Levopimaric acid	n.d	0.06	n.d	n.d	<LOD	n.d	n.d	n.d	n.d	n.d	n.d
Dehydroabietic acid	0.00029	0.7	0.0051	0.0072	0.0013	0.0027	0.0015	0.0026	0.27	0.28	0.14
Abietic acid	<LOD	0.15	0.0003	0.00046	0.00012	0.00022	0.00015	0.00016	0.0024	0.002	0.001
Neoabietic acid	n.d	0.081	0.0047	n.d	n.d	n.d	n.d	0.0029	0.000065	n.d	<LOD

Table 6. GC-MS results of different wood species after a three week leaching period in salt water. The expected standard deviation of each measurement is expected to not be bigger than 5% of the measurement value. Ctrl- control, U- unsteamed; S- steamed; M- modified pine wood with hardwood condensates; B- beech; W- walnut; C- cherry).

	Ctrl	PINE	UB	UW	UC	SB	SW	SC	MB	MW	MC
FATTY ACIDS											
Palmitic acid	0.0095	0.023	0.065	0.1	0.052	0.043	0.42	0.017	0.13	0.15	0.54
Margaric acid (I.S.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Linoleic acid	<LOD	0.0015	0.0018	0.015	0.0016	0.0037	0.063	0.0015	0.03	0.031	0.061
Oleic acid	0.00011	0.005	0.0024	0.0093	0.0077	0.0048	0.052	0.0016	0.027	0.027	0.049
Stearic acid	0.0039	0.046	0.015	0.024	0.037	0.014	0.13	0.0086	0.027	0.038	0.086
RESIN ACIDS											
Pimaric acid	n.d	0.0051	<LOD	n.d	0.000076	<LOD	<LOD	<LOD	0.00061	0.00083	0.0021
Sandaracopimaric acid	n.d	0.0076	<LOD	<LOD	<LOD	<LOD	n.d	<LOD	0.000086	0.00012	0.00033
Isopimaric acid	<LOD	0.0082	<LOD	<LOD	0.00008	<LOD	<LOD	<LOD	0.00093	0.0012	0.0032
Palustric acid	<LOD	0.031	n.d	n.d	0.000059	<LOD	<LOD	<LOD	<LOD	0.000079	0.00023
Levopimaric acid	<LOD	0.012	<LOD	n.d	<LOD	n.d	<LOD	n.d	n.d	0.000069	n.d
Dehydroabietic acid	0.00021	0.15	0.00067	0.00058	0.003	0.00048	n.d	0.0007	0.074	0.086	0.023
Abietic acid	<LOD	0.031	n.d	0.000062	0.00023	0.00019	<LOD	0.000048	0.00048	0.00079	0.0018
Neoabietic acid	n.d	0.019	n.d	0.00005	0.0017	n.d	n.d	0.00091	n.d	n.d	<LOD

The results from Tables 5 and 6 show that after 3 weeks of leaching, we have a complete other picture than after only 1 week of leaching. While after one week, modified pine leaching was negligible in comparison with unmodified pine, after 3 weeks the concentrations of leached fatty acids in modified pine increased a lot. This means that the second reason from the former paragraph was the most probable and the fatty and resin acids reacted with citric acid and lysine. The reaction is unfortunately reversible, so slowly these compounds are released into the water. In deionized water, pine wood with cherry condensates performs better than the other treatments (beech and walnut). In seawater, however, beech and walnut condensate modified pine leach less than in deionized water and perform therefore slightly better than cherry condensate modified pine. Somehow the more toxic resin acids of modified pine don’t behave differently and also barely leach out after 3 weeks.

Steamed and unsteamed hardwood species release mainly palmitic acid and this in approximately similar concentrations, but slightly higher for walnut wood. Leaching in deionized water releases higher amounts of palmitic acid than in sea water. Resin acids are almost not present in the leachates of these samples.

In general, there can be concluded that, although resin acids and fatty acids are leached from modified pine wood, the concentrations are often lower than from untreated pine wood, especially for the more toxic resin acids. Therefore, we can expect that they won’t form a major concern for the environment.

6.3 MECHANICAL PROPERTIES MEASUREMENT BY ZWICK APARATUS

The mechanical properties of the wood samples were tested by a Zwick apparatus. Two types of results were obtained. The modulus of elasticity (MOE) was used to compare the elastic limit and plastic deformation of samples. With the modulus of rupture (MOR), conclusions can be made about the strength of the samples, more precisely, the maximum force that the sample can withstand. These tests can give an answer on following questions:

- Is there a significant decrease in mechanical properties of the wood before and after soaking in water? Is there a difference in exposure to deionized water or salt water? Is there any type of samples, which are more resistant to exposure to water?
- Does modified pine wood has better mechanical properties than unmodified pine wood? Is there any treatment that is better?
- Did steaming significantly affect the properties of the hardwood species?

Each measurement was performed in ten replicates. The obtained data have often outliers, a high standard deviation and no normal distribution. Although these type of data normally require more advanced statistics, for a first exploration of data, descriptive and basic statistics, using F- and t-tests, were enough. A 5% uncertainty interval was used. Shortly, The F-test in a one-way analysis of variance is used to assess whether the expected values of a quantitative variable within several pre-defined groups differ from each other. The t-test is used, to determine if the means of two sets of data are significantly different from each other. If for the F-test, Fcritical minus the F-value is positive, the nul-hypothesis of equal variances can be rejected. For the t-test, if tcritical minus the absolute t-value is positive means that the nul-hypothesis of equal means can be rejected.

6.3.1 Modulus of elasticity (MOE)

The Zwick test was first performed on wood samples, which were not exposed to water. The results of the modulus of elasticity can be seen in table 7.

Table 7. Modulus of elasticity for steamed and unsteamed hardwood and pine. The wood samples were not exposed to water.

Sample	Mean (MPa)	Variance	Standard deviation
Unsteamed beech	9169	1088882	1043
Steamed beech	10198	1208123	1099
Unsteamed walnut	7908	1472644	1213
Steamed walnut	6527	1111539	1054
Unsteamed cherry	10213	315558	561
Steamed cherry	7376	3214202	1792
Pine	13912	1420656	1191

The F-test and t-test were performed, based on the values gathered in Table 7, to compare the elasticity between unsteamed and steamed beech, walnut and cherry samples (see Table 8).

Table 8. Statistical comparison between samples by F- and t-test.

Sample 1	Sample 2	F-test		t-test	
		F-value	$F_{critical}$	t-value	$t_{critical}$
Unsteamed beech	Steamed beech	1.10	3.17	2.14	1.73
Unsteamed walnut	Steamed walnut	1.32	3.17	2.71	1.73
Unsteamed cherry	Steamed cherry	10.18	3.17	4.77	1.79

The tested samples showed a significant difference in elasticity. In the case of unsteamed and steamed beech samples, steamed samples were more elastic than the unsteamed ones. Otherwise, walnut and cherry samples were more elastic when unsteamed. Other than that, softwood pine was more elastic than all the hardwood species.

Thereafter the same types of samples were soaked for 3 weeks in deionized or salt water. The samples were dried in an oven (24 h at 103°C) to reduce the moisture content. The MOE results of the Zwick test can be found in Table 9.

Table 9. Modulus of elasticity for steamed and unsteamed hardwood, pine and pine modified with steaming condensates. The wood samples were 3 weeks exposed to salt water or deionized water.

Sample	**Deionized water**			**Salt water**		
	Mean (in MPa)	**Variance**	**Standard deviation**	**Mean (in MPa)**	**Variance**	**Standard deviation**
Unsteamed beech	9855	4661556	2159	10402	4101623	2025
Steamed beech	11401	1112407	1054	11440	1842408	1357
Unsteamed cherry	9828	2102682	1450	9607	726932	852
Steamed cherry	8244	1478844	1216	6916	675922	822
Unsteamed walnut	5770	4345188	2084	4262	5953698	2440
Steamed walnut	3873	4851063	2202	3882	4387536	2094
Pine	13311	2344375	1531	12610	4823029	2196
Pine+ beech condensate	15860	1420656	1191	14560	805864	897
Pine + cherry condensate	15008	452108	672	14011	382705	618
Pine + walnut condensate	15018	713194	844	14759	422432	649

The results of the calculated F- and t-test values, for the comparison between steamed and unsteamed samples as well as treated and untreated pine, can be found in table 10 for deionized water and in table 11 for sea water.

Table 10. Statistical comparison between samples exposed to deionized water by F- and t-test.

Sample 1	**Sample 2**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	Steamed beech	4.19	3.17	-2.04	1.77
Unsteamed walnut	Steamed walnut	1.11	3.17	-1.97	1.73
Unsteamed cherry	Steamed cherry	1.42	3.17	2.64	1.73
Pine	Pine + beech condensate	1.65	3.17	4.15	1.73
Pine	Pine + walnut condensate	3.28	3.17	-3.09	1.76
Pine	Pine + cherry condensate	5.18	3.17	-3.20	1.78

Table 11. Statistical comparison between samples exposed to salt water by F- and t-test.

Sample 1	**Sample 2**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	Steamed beech	2.22	3.17	-1.34	1.73
Unsteamed walnut	Steamed walnut	1.35	3.17	0.37	1.73
Unsteamed cherry	Steamed cherry	1.075	3.17	7.18	1.73
Pine	Pine + beech condensate	5.98	3.17	-2.59	1.78
Pine	Pine + walnut condensate	11.41	3.17	-2.96	1.79
Pine	Pine + cherry condensate	12.60	3.17	-1.94	1.81

After soaking for three weeks in deionized water (Table 9 and 10), beech samples showed a higher elasticity in steamed samples. Walnut and cherry unsteamed samples had a significantly higher level of elasticity than the steamed ones. All treated pine samples (beech, walnut and cherry condensate) were more elastic than non-treated pine.

After soaking for three weeks in sea water (Table 9 and 11), beech and walnut samples have not shown significant difference in elasticity between steamed and unsteamed samples; while unsteamed cherry shows a higher elasticity than steamed samples. Following previous results, pine samples treated with condensate were more elastic than non-treated pine.

As can be seen in Tables 10 and 11, all condensate treated samples do not differ tremendously amog each other, both in deionized and salt water environment. It was noticed that samples, for all condesate treatments, in deionized water had better elastic properties than ones in salt water.

Next, it was compared if the choice of soaking wood in sea water in comparison with deionized water has a significant effect on the mechanical properties of wood. The results of the F-test and t-test can be found in Table 12.

Table 12. Statistical comparison between samples exposed to salt water and samples exposed to deionized water by F- and t-test.

Sample 1: destiled water	**Sample 2: salt water**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	Unsteamed beech	1.13	3.17	0.58	1.73
Unsteamed walnut	Unseamed walnut	1.37	3.17	1.48	1.73
Unsteamed cherry	Unsteamed cherry	2.89	3.17	0.41	1.73
Steamed beech	Steamed beech	1.65	3.17	0.071	1.73
Steamed walnut	Steamed walnut	1.10	3.17	0.0099	1.73
Steamed cherry	Steamed cherry	2.18	3.17	2.85	1.73
Pine + beech condensate	Pine + beech condensate	1.76	3.17	2.75	1.73
Pine +walnut condensate	Pine + walnut condesate	1.68	3.17	0.76	1.73
Pine +cherry condensate	Pine + cherry condensate	1.18	3.17	3.45	1.73
Pine	Pine	2.06	3.17	0.82	1.73

In general, it can be concluded that there is no significant difference on the elasticity between wood samples soaked in deionized water and sea water. From all tested specimens, only steamed cherry and pine treated with beach or cherry condensate showed a significant difference in elasticity between deionized and salt water exposed samples, where the salty sea water makes the wood more brittle.

In the last test, it was checked what happened with the elasticity, before and after soaking in water. The F-test and t-test results of deionized water can be found in Table 13 and of sea water in Table 14.

Table 13. Statistical comparison between samples before and after soaking period in deionized water by F- and t-test.

Before soaking	**After soaking**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	unsteamed beech	4.28	3.17	0.90	1.77
Unsteamed walnut	Unseamed walnut	2.95	3.17	-2.80	1.73
Unsteamed cherry	Unsteamed cherry	6.66	3.17	-0.78	1.78
Steamed beech	Steamed beech	1.08	3.17	-2.45	1.73
Steamed walnut	Steamed walnut	4.36	3.17	-3.43	1.77
Steamed cherry	Steamed cherry	2.17	3.17	-1.26	1.73

Table 14. Statistical comparison between samples before and after soaking period in salt water by F- and t-test.

Before soaking	**After soaking**	**F-test**		**t- test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	unsteamed beech	3.76	3.17	-1.71	1.77
Unsteamed walnut	Unsteamed walnut	4.04	3.17	-4.23	1.77
Unsteamed cherry	Unsteamed cherry	2.30	3.17	-1.87	1.73
Steamed beech	Steamed beech	1.52	3.17	2.24	1.73
Steamed walnut	Steamed walnut	3.94	3.17	-3.56	1.77
Steamed cherry	Steamed cherry	4.75	3.17	0.73	1.77

After the three weeks soaking period in deionized water, no significant difference in elasticity was seen in unsteamed beech, steamed and unsteamed cherry. The same can be said for these wood samples in sea water, but unsteamed beech and cherry are on the edge of being significantly more brittle. For both deionized and salt water, unsteamed and steamed walnut samples were characterized with a higher brittleness than before soaking. Steamed beech samples, both deionized and salt water exposed, showed better elastic properties than before being soaked.

6.3.2 Modulus of rupture (MOR)

The Zwick test was first performed on wood samples, which were not exposed to water. The results of the modulus of rupture can be seen in table 15.

Table 15. Modulus of rupture for steamed and unsteamed hardwood and pine. The wood samples were not exposed to water.

Sample	**Mean (MPa)**	**Variance**	**Standard deviation**
Unsteamed beech	101.8	58.01	7.1
Steamed beech	91.7	52.9	7.8
Unsteamed walnut	89.2	71.5	9.5
Steamed walnut	79.6	89.08	9.1
Unsteamed cherry	93.6	43.3	6.9
Steamed cherry	73.9	166.8	12.2
Pine	114.4	20.5	4.5

The F-test and t-test were performed, based on the values gathered in Table 15, to compare the flexural strength between unsteamed and steamed beech, walnut and cherry samples (see Table 16).

Table 16. Statistical comparison between samples by F- and t-test.

Sample 1	**Sample 2**	**F-test**		**t- test**	
		F-value	**$F_{critical}$**	**t-value**	**$t_{critical}$**
Unsteamed beech	Steamed beech	1.19	3.17	3.00	1.73
Unsteamed walnut	Steamed walnut	1.091	3.17	2.29	1,73
Unsteamed cherry	Steamed cherry	3.12	3.17	-4.44	1.73

No significant difference in strength was seen for steamed and usteamed walnut. In the case of beech, unsteamed samples were more stress resilient than the steamed ones.

Thereafter the same type of samples was soaked for 3 weeks in deionized or salt water. The samples were dried in an oven (24 h at 103°C) to reduce the moisture content. The MOR results of the Zwick test can be found in Table 17.

Table 17. Modulus of rupture for steamed and unsteamed hardwood, pine and pine modified with steaming condensates. The wood samples were 3 weeks exposed to salt water or deionized water.

Sample	**Deionized water**			**Salt water**		
	Mean (in MPa)	**Variance**	**Standard deviation**	**Mean (in MPa)**	**Variance**	**Standard deviation**
Unsteamed beech	109.6	149.5	12.2	104.4	176.1	13.2
Steamed beech	111.2	79.0	8.8	108.3	131.1	11.4
Unsteamed walnut	77.7	185.9	13.6	71.3	409.1	20.2
Steamed walnut	68.6	254.7	15.9	72.5	254.1	15.9
Unsteamed cherry	103.9	238.9	15.4	97.8	79.8	8.9
Steamed cherry	86.1	38.4	6.2	73.3	19.9	4.4
pine	103.7	207.0	14.3	102.4	37.8	6.1
Pine+ beech condensate	125.4	81.5	9.0	114.0	52.9	7.2
Pine + walnut condensate	117.3	44.6	6.6	113.3	47.3	6.8
Pine + cherry condensate	117.6	27.5	5.2	109.3	14.5	3.8

The results of the calculated F- and t-test values, for the comparison between steamed and unsteamed samples as well as treated and untreated pine, can be found in Table 18 for deionized water, and in Table 19 for sea water.

Table 18. Statistical comparison between samples exposed to deionized water by F- and t-test.

Sample 1	**Sample 2**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	Steamed beech	1.89	3.17	-0.34	1.73
Unsteamed walnut	Steamed walnut	1.36	3.17	-1.36	1.73
Unsteamed cherry	Steamed cherry	6.21	3.17	3.38	1.78
Pine	Pine + beech condensate	2.53	3.17	-4.03	1.73
Pine	Pine + walnut condesate	4.64	3.17	-2.72	1.77
Pine	Pine + cherry condensate	7.51	3.17	-2.88	1.79

Table 19. Statistical comparison between samples exposed to salt water by F- and t-test.

Sample 1	**Sample 2**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	Steamed beech	1.34	3.17	-0.69	1.73
Unsteamed walnut	Steamed walnut	1.60	3.17	-0.15	1.73
Unsteamed cherry	Steamed cherry	4.00	3.17	7.74	1.77
Pine	Pine + beech condensate	1.40	3.17	3.86	1.73
Pine	Pine + walnut condesate	1.25	3.17	3.74	1.73
Pine	Pine + cherry condensate	2.59	3.17	-3.02	1.73

After soaking for three weeks in deionized water (Table 17 and 18), beech and walnuts samples do not show a significant difference in strength. On the other hand, unsteamed cherry has a higher modulus of rupture than steamed cherry. All condensate treated samples showed more strength than the non-treated pine samples. The soaking in sea water (see Table 19) had similar outcome as the deionized water exposure of samples.

All condensate treatments had similar outcomes (Table 18 and 19), both in deionized and salt water. Only conspicuous reslut was noticed by beech condensate treated pine samples exposed to deionized water (Table 18) with the highest srenght level.

Next, it was compared if the choice of soaking wood in sea water in comparison with deionized water has a significant effect on the mechanical properties of wood. The results of the F-test and t-test can be found in Table 20.

Table 20. Statistical comparison between samples exposed to salt water and samples exposed to deionized water by F- and t-test.

Sample 1: deionized water	**Sample 2: salt water**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	unsteamed beech	1.17	3.17	0.90	1.73
Unsteamed walnut	Unsteamed walnut	2.19	3.17	0.83	1.73
Unsteamed cherry	Unsteamed cherry	2.99	3.17	1.09	1.73
Steamed beech	Steamed beech	1.65	3.17	0.64	1.73
Steamed walnut	Steamed walnut	1.00	3.17	0.55	1.73
Steamed cherry	Steamed cherry	3.17	1.25	5.29	1.73
Pine + beech condensate	Pine + beech condensate	1.53	3.17	3.09	1.73
Pine +walnut condensate	Pine + walnut condensate	1.06	3.17	-1.33	1.73
Pine +cherry condensate	Pine + cherry condensate	1.88	3.17	4.08	1.73
Pine	Pine	5.47	3.17	0.26	1.78

In most wood samples no significand difference was obtained between soaking in deionized water or in seawater. Only for steamed cherry samples and pine samples treated with beech or cherry condensates showed the potential of withstanding a higher maximum force load when exposed to deionized water. In the last test, it was checked what happened with the strength, before and after soaking in water. The F-test and t-test results of deionized water can be found in Table 21 and of sea water in Table 22.

Table 21. Statistical comparison between samples before and after soaking period in deionized water by F- and t-test.

Before soaking	**After soaking**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	unsteamed beech	2.89	3.17	1.73	1.73
Unsteamed walnut	Unsteamed walnut	2.03	3.17	-2.19	1.73
Unsteamed cherry	Unsteamed cherry	5.00	3.17	1.92	1.78
Steamed beech	Steamed beech	1.27	3.17	5.20	1.73
Steamed walnut	Steamed walnut	3.04	3.17	-1.90	1.73
Steamed cherry	Steamed cherry	3.88	3.17	-2.82	1.77

Table 22. Statistical comparison between samples before and after soaking period in salt water by F- and t-test.

Before soaking	**After soaking**	**F-test**		**t-test**	
		F-value	**F $_{critical}$**	**t-value**	**t $_{critical}$**
Unsteamed beech	unsteamed beech	3.41	3.17	0.55	1.76
Unsteamed walnut	Unsteamed walnut	4.46	3.17	-2.54	1.77
Unsteamed cherry	Unsteamed cherry	1.67	3.17	1.17	1.73
Steamed beech	Steamed beech	2.11	3.17	3.77	1.73
Steamed walnut	Steamed walnut	3.03	317	-1.06	1.73
Steamed cherry	Steamed cherry	7.49	3.17	0.13	1.79

After the three weeks soaking period in deionized water, unsteamed beech doesn’t show any significant differences in strength in comparison with its condition before soaking. However, steamed beech shows a higher strength after soaking (Table 21), which we are unable to explain. Walnut samples, both steamed and unsteamed, could withstand a higher maximum force load before the water exposure. While unsteamed and steamed cherry wood, showed a higher strength after soaking. For samples exposed to sea water (Table 22), there was only a significant difference in samples before and after soaking for unsteamed walnut and steamed beech. Steamed beech showed the capacity of withstanding higher levels of stress after being exposed to the salt water, while unsteamed walnut showed a decrease in withstanding these stress levels after exposure to water.

7 DISCUSSION

7.1 WOOD MODIFICATION IN THE LIGHT OF NATURAL CONSERVATION SCIENCE

The entire planet is a network of integrated ecosystems. Ecosystem services are the base for fulfilling the needs to sustain the life on Earth. This occurs even on very fundamental levels like creating the air we breath and the supply and distribution of the water we drink. Ecosystems, especially forests, are also successful mitiagators for climate change. Tropical forests act as a heat and humidity pumps (Sodhi et al. 2007). Extensive tropical deforestation is expected to lead to higer temperatures, reduced precipitation and increased incidences of droughts and fires. Because of that, sustainable and reparative use of forest products should become indispensible. As well, use of locally grown tree species should become more frequent practice to mellow the pressure on the already dramatically degraded forest habitats. In our work we used locally grown pine to implement in the modification procedure. Furthermore, oceans and seas, which covers 70% of Earth`s surface, take up a large portion in the ecosystem network. Oceans are crucial for human existence because the ocean can accumulate carbon dioxide surplus from the atmosphere and act as a buffer. It may seem that oceans are very balanced systems, but external sources of organic and inorganic matter inputs can throw these ecosystems out of their steady state. Excessive organic and inorganic matter introduced in marine ecosystems can be persistent in water column and be spread at large distances, where it can pose a threat to different organism present in the water column. It can accumulate also at the sea bottom where it can pose a threat to various bottom dwellings and boring organisms. External input of organic and inorganic matter can also cause a change in the nitrogen and phosphorus cycling system, which can lead to oxygen depletion, which could cause the death of aerobic organisms. Therefore, we have used steaming condensates of hardwood species (beech, walnut and cherry), which consist of compounds which are already naturally present in wood. For the binding agents naturally present compounds, citric acid and lysine were used. To promote a desired reaction, the Maillard reaction was used, that is induced with heat only. Our toxicity results show low concentrations of resin acids which is a great news. Resin acids, when present in aquatic environment can be accumulated in the sediment and pose acute and chronic toxicity to fish such as rainbow trout and salmon (Michniewicz et al. 2000).

7.2 THE IMPORTANCE OF THE OBTAINED RESULTS

Research on wood modification is mostly concentrated on the elimination or reduction of limitations of wood as a raw material. Modified wood is expected to reach certain criteria such as: better dimensionally stability, intacked mechanical properties, enhanced stability and resistance to weathering and fungal decay when compered with unmodified wood. Until now, biocides were most commonly used agents for wood preservation. In contact with water, these preservatives could potentially be leached from the wood, contaminating the environment (Hingston et al. 2001). Because of technological and environmental pressures, finding the alternatives to use preservative treated wood is desirable and indispensable. Hill (2006) stated that an effective impregnation modification must be non-toxic under service conditions and, furthermore, there should be no release of any toxic substances during its service life, or after disposal or recycling of the modified wood, requiring that the polymeric network formed in the cell wall should react with the wood polymers or become entangled with them. Chemical modification has been recognized as an efficient protecting strategy for wood as it permanently alters wood cell wall polymers and/or deposits chemicals in cell voids. Main reactions in chemical modification implies on etherification, esterification or acetylation between some chemical and OH groups of wood (Despot et al. 2008).

The topic of this thesis was to find way to modify wood, so it can be used as a construction material in the marine environment, where it is exposed to several harsh conditions like sun, wood borers and salt water. The method was focused to comply with several problematics described in literature:

- Pine was the construction material of interest because using local low-valuable wood can reduce the use of less readily available expensive hardwood species.
- Hardwood steaming condensates were used. Reuse of waste materials starts to be an important point on the political agenda. It would save lots of costs and energy, which are now necessary to clean the condensate before release in the environment.
- Chemicals where used to bind these condensates inside wood. Care was taken that these chemicals were non-toxic and renewable.
- The reaction of these chemicals inside wood was initiated by heat, avoiding the use of catalysts, or strong acids and bases.
- Leaching studies were performed to confirm that the treatment method is not harmful for the environment.

Therefore, a good outcome of these experiments would have a positive effect on our society.

The performed experiments lead to following results:

- It was seen that reaction of citric acid and lysine performed best at a temperature of 120°C. The fact that such a low temperature is the most optimal for the reaction to occur is good news, because it means that the modification can take place in drying kills which are commercially used.
- Leaching tests showed also promising results. Although after three weeks there were higher concentrations of fatty acids in modified wood than in treated pine, the concentrations were not that extremely different that we would expect environmental concerns. The more toxic resin acids, which are of higher concern, barely leach out.
- Although variations occur in elasticity and strength of the wood samples before and after soaking in water, there are after 3 weeks of exposure to water no indications that the construction would fail.

With these results it can be concluded that the tested method of modifying wood has some potential for further research. Our actions should not be short-sighted. We depend on our environment for the food we eat, the air we breathe and the water we drink. We also should not forget about the esthetical value of our environment and satisfaction and fulfillment we get when surrounded by nature, which makes wood a more preferred choice for construction than concrete.

8 CONCLUSION

Wood is, no doubt, one of the most versatile materials. The use fo different modification methods can enhance its characteristics, both mechanical and chemical, and prolonge its life span. The majority of those methods had shown negative environmental footprint. With the increasing urge for finding environmentaly acceptable modification techniques, this thesis has investigated the use of steaming condensates of valuable tree species (beech, walnut and cherry), to modify localy grown low-valuable pine wood to be able to apply it in the marine environment. This method was also tested in the sea to see the influence of biofouling and marine wood borers. As a result of our work we had concluded further:

- To bind the compounds of steaming condensates inside wood, the addition of lysine and citric acid seems to be the most effective. A reaction temperature of 120°C is optimal, since higher temperatures already degrade wood.
- Heat treatment has affected the color change of wood proportional to the exposed temperature.
- Although resin acids and fatty acids are leached from modified pine wood, the concentrations are often lower than from untreated pine wood, especially for the more toxic resin acids. Therefore, we can expect that they won’t form a major concern for the environment.
- Water exposure has a negative impact on the elastic properties of all tested specimens.
- All condensate treated pine samples have a higher elastic limit than non-treated pine, both in salt and deionized water.
- All condensate treated pine samples have a better flexural strength than the non-treated pine samples, both in salt and deionized water.

This thesis shows that condensates of hardwood species, such as beech, walnut and cherry, are suitable for enhancement of pine wood mechanical characteristics, especially for it`s use in the aquatic environment. Further research should be concentrated on providing information about condensate toxicity towards coastal marine communities to ensure safe implementation of condensate treated wood in the marine environment. Using wood condensate, a byproduct from wood steaming facilities, as a mean of wood modification offers a promising environmentaly friendly solution for wood modification.

9 POVZETEK V SLOVENSKEM JEZIKU

Les je priljubljen gradbeni material, saj je vsestranski, naraven in obnovljiv, vendar pomanjkljivosti, kot na primer občutljivost na vodo, nizka dimenzijska stabilnost in trdota, nizka odpornost proti biotskem propadanju zaradi delovanja gliv, žuželk, morskih lesnih škodljivcev (MLŠ) in nizka odpornost na UV-sevanje vplivajo na lastnosti in funkcionalnost ter življenjsko dobo lesa in lesnih izdelkov. Za izboljšanje lastnosti lesnega materiala lahko les obdelamo na različne načine in mu spremenimo mehanske lastnosti, toplotno in zvočno izolativnost, odpornost proti vplivom vode in vlage, znižamo gorljivosti in podaljšamo življenjsko dobo. Poznamo štiri glavne vrste postopkov: (1) kemična obdelava; (2) termo-hidro in termo-hidro-mehanska obdelava; (3) modifikacije, ki temeljijo na bioloških procesih. V magistrski nalogi smo se osredotočili na raziskovanje lesa kot gradbenega materiala, za uporabo v morskem okolju. Zaradi zahtevnih razmer v morskem okolju so raziskave za izboljšanje obstojnosti lesnih materialov zelo pomembne. Lesene konstrukcije so običajno zasnovane z željo po življenjski dobi od 40 do 50 let, vendar je ta zaradi abiotskih in biotskih dejavnikov za nezaščitene lesene konstrukcije v Evropskih morjih precej krajša. Zato je uporaba lesenih materialov zahtevna, zlasti ker so podkonstrukcije pomolov lahko zelo drage, tudi nekaj milijonov evrov. Najbolj uničujoči škodljivci, ki jih najdemo v Evropi so predstavniki iz družin *Teredinidae, Pholadidae, Limnoriidae, Sphaeroma* in *Chelura,* druge skupine pa povzročajo le površinsko škodo potopljenemu lesu in ne predstavljajo večje nevarnosti. Ker so nekatere vrste toplovodne, se lahko njihova aktivnost v prihodnosti poveča tudi zaradi globalnega segrevanja. Drugi trend, ki ga je mogoče opaziti, je, da se ozemlje tistih MLŠ povečuje. MLŠ imajo raje običajno okolje z višjo koncentracijo soli, vendar se v zadnjih desetletjih zdi, da uspevajo tudi pri nižjih koncentracijah, kar omogoča preživetje tudi v morjih, kot je Baltsko. Različne vrste ovojev (fizične ovire) in kemičnih biocidov (zaščitna sredstva za les) so že uporabili za odvračanje napadov MLŠ. Les lahko zaščitimo s sredstvi, ki omejijo dostop kisika, preprečijo naselitev škodljivcev ali povrnejo strukturno celovitost poškodovanim stebrom. Ti postopki do sedaj niso bili popolnoma uspešni, saj jih je zahtevno načrtovati/oblikovati, so učinkoviti le za nekatere vrste lesa, predvsem pa, če so premazni sloji nepoškodovani. Moramo tudi vedeti, da se razmere v morskih okoljih med morji razlikujejo. Izbor razpoložljivih zaščitnih sredstev je zaradi direktive EU omejen in preverjenih učinkovin, kot sta kreozot in baker-krom-arzen v lesu, namenjenem za uporabo v gradnji v morskem okolju, ni dovoljeno več uporabljati. V zadnjem času se uveljavljajo učinkovite, okolju prijazne metode za kemično modifikacijo, (furfurilacija), ki je za konstrukcije v morjih vse bolj razširjena (t. i. les Kebony).

Naše delo je bilo osredotočeno na razvoj alternativne metode za obdelavo lesa, ki je učinkovita, poceni in okolju sprejemljiva. Modificiran les ne sme vsebovati toksičnih spojin, prav tako ne sme prihajati do sproščanja toksičnih spojin med odstranjevanjem ali recikliranjem modificiranega lesa. Ker so modifikacije namenjene boljši odpornosti lesa proti biološki razgradnji, povzročijo tudi nastanek biocidnih lastnosti. V diplomski nalogi smo preizkusili odpornost borovine, modificirane s kondenzati parjenja trpežnejših vrst listavcev, proti napadu z MLŠ. Glavni cilji raziskave so bili:

- preizkusiti različne vrste modifikacij manj cenjene borovine s kondenzati parjenja listavcev, da bi dosegli boljšo trajnost tega lesa v morskem okolju
- preizkusiti, če se pri tovrstni obdelavi (potencialno v okolje) sproščajo toksične spojine
- primerjati trdnost / krhkost modificiranega lesa pred in po stiku z vodo ter rezultate primerjati z nemodificirano borovino ter parjeno in neparjeno bukovino, orehovino in češnjevino.
- vzpostaviti poskus za testiranje modificirane borovine v morskem okolju za primerjavo z nemodificirano borovino ter parjeno in neparjeno bukovino, orehovino in češnjevino.

Poskus smo zasnovali po pregledu znanstvene literature s tega področja. Eksperimentalni del smo razdelili na tri sklope. V prvem sklopu smo optimizirali različne parametre za modifikacijo borovine. Uporabili smo kondenzate parjenja vrhunskega lesa treh vrst (bukovino, orehovino in češnjevino). Vezavo spojin, prisotnih v različnih kondenzatih, v les (z esterifikacijo) smo proučevali pri različnih temperaturah in z uporabo različnih naravnih vezavnih reagentov (npr. citronske kisline, lizina). Izvedli smo preizkuse izpiranja, da smo preverili, kakšna množina reagentov se učinkovito veže na/v les. V drugem sklopu smo izvajali dve vrsti preizkusov: (1) primerjava trdnosti / krhkosti lesa z upogibnimi testi pred in po izpostavljanju vodi ter (2) določevanje vrst toksičnih spojin in njihove količine (polkvantitativno, z GC-MS), izlužene v vodo. Rezultate teh testov z modificirano borovino smo primerjali z nemodificirano borovino ter parjeno in neparjeno bukovino, orehovino in češnjevino. Za izpiranje smo uporabili dve vrsti vode: sintetično morsko in destilirano voda. Zadnji sklop raziskav pa je bil namenjen preizkušanju modificirane in nemodificirane borovine ter parjene in neparjene bukovine, orehovine in češnjevine v morju za proučevanje biocidnega delovanja modifikacij oz. preprečitve naselitve morskih organizmov na testne vzorce.

Glavne ugotovitve opravljene raziskave so:

- Za vezavo spojin v kondenzatih na les se je kot najučinkovitejša izkazala citronska kislina z dodatkom lizina. Najprimernejša reakcijska temperatura za vezavo je bila 120°C, medtem ko uporaba višjih temperatur povzroča razgradnjo lesa.
- Toplotna obdelava je vplivala na proces razgradnje vzorca in spreminjanje barve, sorazmerno s temperaturo.
- Čeprav se iz modificirane borovine izperejo smolne in maščobne kisline, so koncentracije pogosto nižje kot iz nemodificirane borovine; zlasti to velja za bolj toksične smolne kisline, zato modifikacija celo zmanjša vpliv na okolje
- Izpostavljenost vodi je negativno vplivala na elastične lastnosti vseh preizkušenih vzorcev.
- Vsi vzorci bora, obdelani s kondenzatom, so bili bolj elastični v primerjavi z neobdelano borovino, tako v slani kot v deionizirani vodi.
- Vsi vzorci borovine, obdelani s kondenzatom, so imeli po izpostavitvi v slani in deionizirani vodi boljšo upogibno trdnost kot neobdelani vzorci.

V okviru magistrskega dela smo ugotovili, da so kondenzati lesa listavcev, kot so bukovina, orehovina in češnjevina, primerni za izboljšanje mehanskih lastnosti borovine za uporabo v vodnem okolju. Nadaljnje raziskave bi morale biti osredotočene na proučevanje vpliva kondenzata na biotske združbe morskega obalnega pasu, da bi potrdili varnost uporabe lesa, obdelanega s kondenzati, v morskem okolju. To bi omogočilo proučevanje uporabe tehnik modifikacije lesa s kondenzati parjenja obstojnejših lesnih vrst v komercialne namene in na industrijski ravni. Uporaba lesnega kondenzata, stranskega proizvoda iz obratov za parjenje lesa, kot sredstva za obdelavo lesa, predstavlja obetavno okolju prijazno rešitev.

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