EDUARDO MONDLANE UNIVERSITY

Faculty of Agronomy and Forestry Engineering

Master in Wood Technology

THE ROLE OF EXTRACTIVES AND LIGNIN FOR THE DURABILITY OF THREE HARDWOOD SPECIES GROWN IN MOZAMBIQUE

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DECLARATION OF AUTHORSHIP

I, Eunice Catarina Frederico Sitoe, confirm that this thesis was never presented for any degree or any other scope and is the result of my individual work and I have documented all sources used. This thesis is presented in partial compliance with the requirements to obtain the degree of Master of Science, from Eduardo Mondlane University.

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ABSTRACT

The present study is aimed at assessing separately the role of extractives and lignin content for the natural durability of three tropical hardwoods growing in Mozambique, namely: ntholo (*Pseudolachnostylis maprounaefolia* Pax), metil (*Sterculia appendiculata* K.Schum) and neem (*Azadirachta indica*). The experiment consisted of exposing wood samples to fungal attack under three treatments, namely: untreated, a set of samples from which extractives were removed and a set of samples from which lignin were removed. Thereafter, all specimens from each treatment were exposed against wood destroying fungi such as b rown rot (*Postia placenta* and *Gloeophyllum trabeum*), white rot (*Trametes versicolor* and *Pycnoporus sanguineus*) and soft rot (*Chaetomium globusum* and *Phialophora mutabilis*) under controlled environment.

In general as expressed by mass loss, all species suffered more decay after removal of extractives and lignin. The results show that **untreated** wood samples of ntholo were relatively more durable showing lower mass loss percentages: 0.26 - 2.63%. Untreated wood samples of neem: 0.74 - 15.77%. The most perishable of untreated samples was metil with higher mass loss against all fungi types: 8.44 - 29.78%.

After **removal of extractives** ntholo increased mass loss: 2.17 - 4.22%. The decay also increased for neem: 1.93 - 18.19%. Metil showed similar percentages as in untreated wood: 8.66-26.58%.

After **delignification** all species experienced severe decay as described by mass loss. Ntholo: 14.47 - 44.43%. Neem: 15.43 -62.82%. Metil: 5.57 – 51.58%

Regarding to extractives, ntholo had the highest extractives content (3.95%). Metil (1.11%) and neem (1%) had similar content. Apparently both lignin and extractives seem to play key role against fungal attack especially for ntholo and neem.

Keywords: Brown-rot, Extractives, Lignin, Metil, Natural Durability, Neem, Ntholo, Soft-rot and White-rot

RESUMO

O presente estudo teve como objectivo analisar o papel dos extractivos e da lignina na durabilidade natural de três espécies madeireiras tropicais em Moçambique, nomeadamente: ntholo, (*Pseudolachnostylis maprounaefolia* Pax), metil (*Sterculia appendiculata* K.Schum) e neem (*Azadirachta indica*). Amostras de madeira foram divididas em três tratamentos: não tratadas, após a retirada de extractivos e após a retirada da lignina. Todas amostras de cada tratamento foram submetidas ao ataque de fungos apodrecedores: podridão parda (*Postia placenta* e *Gloeophyllum trabeum*), podridão branca (*Trametes versicolor* e *Pycnoporus sanguineus*) e podridão mole (*Chaetomium globusum* e *Phialophora mutabilis*) sob condições controladas.

De acordo com os valores de perda de massa, todas as espécies apresentaram maiores perdas após a retirada de extractivos e da lignina. Os resultados mostraram que as amostras não tratadas de ntholo possuíam maior durabilidade pela sua menor percentagem de perda de massa: 0.26 - 2.63%. Amostras não tratadas de neem: 0.74 - 15.77%. As amostras de metil apresentaram baixa durabilidade com maiores percentagens de perda de massa: 8.44 - 29.78%.

Após a retirada dos extractivos a percentagem de perda de massa de ntholo aumentou: 2.17 - 4.22%. O mesmo aconteceu para neem: 1.93 - 18.19%. Nas amostras de metil a retirada de extractivos não provocou perdas de massa assinaláveis em relação as amostras não tratadas: 8.66-26.58%.

Após a retirada da lignina todas espécies sofreram ataques severos descritos pela perda de massa. Ntholo: 14.47 - 44.43%. Neem: 15.43 -62.82%. Metil: 5.57 – 51.58%.

No que concerne a quantidade de extractivos, ntholo apresentou a maior percentagem de extractivos (3.95%). Metil (1.11%) e neem (1%) apresentaram quantidades similares. De acordo com este estudo concluiu-se que a lignina e os extractivos jogam um papel importante contra o ataque de fungos apodrecedores especialmente para ntholo e neem.

Palavras-chave: Durabilidade natural, Extractivos, Lignina, Metil, Neem, Ntholo, Podridão branca, Podridão mole e Podridão parda.

Dedicated to

Liam Remane

Linka Inês

Anisha Melody

Shanyl Solé

Aécio Zandamela

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CHAPTER 1

INTRODUCTION

Wood is a biological material origin which occupies an important position on the market due to its multipurpose end-use. With the advancement of technology, wood becomes a more versatile material as a base of various products. Wood consists of organic compounds that serve as nutrient source for xylophages agents which are responsible for degradation and loss of quality (Moreschi, 2013).

Over the years, the industry acknowledged the ability of some wood species to resist against xylophages agents. This feature is variable between species and is known as natural durability of wood. Naturally durable timbers are highly appreciated on the market. In general, the natural durability of wood is attributed to the presence of chemical compounds known as extractives which are toxic or repellent to insects, fungi and other xylophages agents (FAO, 1986; Oliveira *et al*. 2005; Bossardi and Barreiros, 2011).

The overuse of the naturally durable timbers led to use of fast growth species that have moderate to low resistance against wood destroying organisms. Therefore, additional treatments to increase service life such as synthetic toxic chemicals are used instead. The majority of chemical wood preservatives are based on metals such as copper, chromium, zinc, arsenic and boron. Heavy pyrolysis oils such as creosote are also employed despite acknowledged negative impact on both the environment and human health (Machado *et al*., 2006; Bossardi and Barreiros, 2011).

There are more than 118 species in Mozambique known for their potential use in wood industry divided in precious, 1st, 2nd, 3rd and 4th classes. However, nowadays, the wood market is concentrated to only 6 species, namely: pau-preto (*Dalbergia melanoxylon*), umbila

(*Pterocarpus angolensis*), chanfuta (*Afzelia quanzensis*), mondzo (*Combretum imberbe*), jambirre (*Milletia stuhlmannii*) and muanga (*Pericopsis angolensis*). This scenario needs to be changed in order to release the pressure in these well-known timbers and expand the market with lesser known timbers (Mackenzie, 2006; EIA, 2013).

1.1. Problem Statement

In woods of high natural durability, extractives are the main source of resistance to biodegradation by acting as natural wood preservatives, but not all wood species extractives contain active biocide ingredients to repel degradation agents. The knowledge of the natural durability of wood is very important to recommend a more suitable use, avoid unnecessary spending on replacement of damaged parts and reduce the impact on the remaining forests (Agatha, 2006; Santos, 2010).

Mozambique is a country rich in wood species with high natural durability. The most known include *Afzelia quanzensis* (chanfuta), *Androstachys johnsonii* (cimbirre), *Berchemia zeyheri* (rosewood), *Pterocarpus angolensis* (umbila) and *Spirostachys africana* (African sandalwood) (Bunster, 2006).

Nowadays, fast grown species occupy a very important position in the market. Most of them are known for low natural durability and the service life needs to be increased with copper- based preservatives. The biocide performance of copper-based preservatives against a variety of wood destroying organisms is well known but their use has been either limited or restricted in some countries due to high level of toxicity to humans and to the environment (Bossardi and Barreiros, 2011). However, Mozambique still uses CCA and creosote as wood preservatives to treat poles, trails and fences.

EPA (1988) examined the harmful effects of metal-based preservatives, specifically chromium-copper arsenate (CCA). The main effects to humans are the risk of cancer and neurological disorders. In addition to human health risks, the effects of CCA are also found in the

environment. For example, water streams are very sensitive to these compounds and exposure can cause imbalances of ecological level as well as contamination of human food chain through the surface and groundwater system.

Therefore, the use of high natural durable timber can minimize the use of metal-based preservatives and reduce the pressure on the well-known timber species.

1.2. Objectives

General

- Assess the role of extractives and lignin on the natural durability of 3 hardwood species against fungal attack.

Specifics

- Determine the natural durability of 3 species;
- Quantify the amount of extractives of each hardwood species;
- Compare the influence of lignin and extractives against fungal attack in 3 tropical hardwood species from Mozambique;

CHAPTER 2

LITERATURE REVIEW

2.1. Wood Structure

Wood is a material formed by different tissues with distinct functions as transport fluids, transform, store and transport nutrients and support (Bom 2011).

Any wood species has two distinct zones with specific functions: sapwood and heartwood (Figure 1). Sapwood is usually light coloured wood, closest to the bark where the cells are physiologically active. The heartwood is usually darker, formed from "dead" cells of the sapwood (Wiedenhoeft and Miller, 2005).

Figure 1. Heartwood and Sapwood (b)

Bali Prefab World, 2014

2.2. Wood natural durability

Natural durability is the inherent ability of wood to resist against degradation by biological, chemical, mechanical and physical wood destroying agents. The natural durability depends on the chemical composition of wood (Jelokava and Sindler, 2001). According to FAO (1986), the reasons for this natural resistance are numerous and diverse.

Some wood species produce compounds that are toxic to biodegradation agents. Extractives toxic to biological organisms are thought to be the primary mechanism by whereby wood naturally resists attack. Lignin provides structural support to the living tree and resistance against biodegradation agents as well although some had developed the ability to attack it (Yamamoto, 1989; Scheffer and Morrell, 1988).

Natural durability can be evaluated both at laboratory and by means of field tests. Laboratory tests are made in controlled environment and the results are given in a short time (few weeks), while field tests are more realistic as the wood is exposed to the service conditions taking a long period to get the results (years) (Meyer, 2012).

2.3. Wood chemical composition

The main elements present in wood are carbon (~50%), hydrogen (~6%), oxygen (~45%) and nitrogen (~1%). Besides these elements, there are other elements present in the structure in small amounts such as calcium, potassium, magnesium and other mineral substances (Klock *et al*., 2005). Wood is composed by cellulose, hemicellulose and lignin (Figure 2).

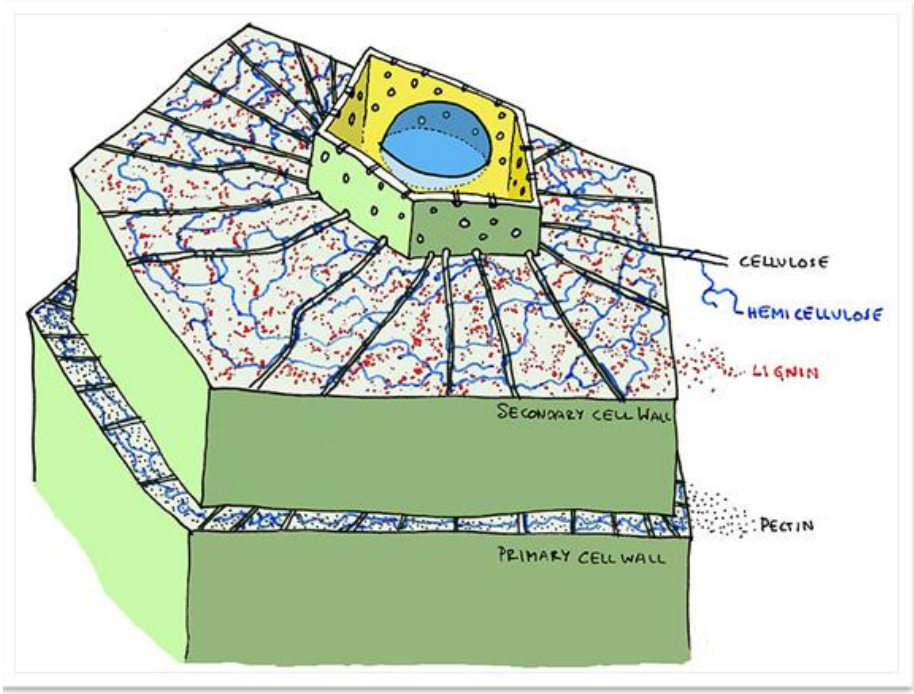


Figure 2. Wood componentes in primary and secondary cell wall

Dahl, 2011

The chemical composition of wood is summarized in the Table below:

Table 1. Wood chemical composition

Wood type	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Extractives (%)**
Hardwood	40-45	15-35	18-25	1-5
Softwood	40-45	20-32	25-35	2-10

Source: Klock *et al*., 2005 and Perez *et al*. (2002);

2.3.1. Cellulose

Cellulose is made up of long linear polymer chains of glucose forming hydrogen-bonding between hydroxyl groups. It can contain about 10 000 units of glucose. It is the major component of wood structure (40-45%) and major nutrient to biodegradation agents (fungi and insects).

Cellulose has two distinct regions: amorphous and crystalline. Crystalline cellulose ensures restricted access of water and chemicals while amorphous cellulose is prone to chemical reactions becoming easily attacked by microorganisms (Perez *et al*., 2002; Klock *et al*., 2005; Nascimento *et al*., 2013).

2.3.2. Hemicellulose

Composed by different units of sugars, hexoses, pentoses and sometimes uronic acid, hemicellulose molecular chains are shorter than cellulose with low degree polymerization (100-200 sugar molecules) (Klock *et al*., 2005).

2.3.3. Lignin

Is a polyphenolic polymer that occurs in both gymnosperms and angiosperms. Lignin is an amorphous substance located in the wood cell wall and acting as adhesive between cells conferring biological resistance against xylophages agents. It is distributed through the secondary cell wall with the highest amounts in the middle lamella (Klock *et al*., 2005; Gonzaga, 2006; Jurgens, 2010 and Chen, 2014).

Lignin can be divided according to its structural elements: softwoods are mainly composed by syringyl and hardwoods are composed by guaiacyl and syringyl units. Natural durability of wood seems to be also related to the type of lignin, guaiacyl units are more resistance to fungi degradation than syringyl. (Jurgens, 2010 and Karami *et al*., 2014)

Generally, hardwoods have less lignin content than softwoods and the lignin they contain is more readily degraded making them particularly susceptible to some fungi types of attack (Klock *et al*., 2005; Perez *et al*. 2002 and Morris, 2013)

2.3.4. Wood extractives

Extractives are produced at the boundary between heartwood and sapwood. This happens when the living cells of sapwood, responsible for the synthesis and transport of molecules in the xylem, die. The deposition of these "dead" cells forms the heartwood of the tree. Several reactions happen in parenchyma cells particularly, converting the stored sugars and starch into toxic compounds which are transported to the adjacent cells through the parenchyma-vascular pits and become a constituent of the new heartwood. Usually, heartwood exhibits high natural decay resistance and sapwood of many species is susceptible to fungal and insect attack (Scheffer and Morrell, 1988; Bessa, 2009; Forest Products Laboratory, 2010).

These sets of compounds (extractives) are secondary metabolites and are not part of the plant structure. As their name implies, they can be removed or extracted from wood. Some extractives are precursors to other chemicals, others are formed as response to wounds, and some act as part of a defence mechanism. Several studies have identified various types of extractives in various trees (Wiedenhoeft and Miller, 2005).

The nature, amount and type of extractives vary among wood species and even within the same individual tree and they are predominantly deposited in cells of the heartwood. The extractives can be presented in different cells of the heartwood. The quantity decreases with increasing height of the tree and from pith to bark (Taylor *et al*., 2006).

2.3.4.1. Extractives compounds

The extractives are formed by various chemical compounds that confer toxicity to xylophages agents and each has specific function in the wood. The main groups of compounds are terpenes, phenolic compounds, aliphatic acids and alcohols (Nascimento *et al*. 2013).

a. Terpenes

Terpenes occur as special metabolites in plants and are used in the production of essential oils (due to its fragrance), insect repellents, fungicides and medicinal purposes.

b. Phenolic compounds

The most important in this group are the tannin compounds which can be divided into: hydrolyzable tannins and condensed phlobaphenes, and other substances such as stilbenes, lignans and flavonoids and their derivatives.

c. Aliphatic acids

Saturated and unsaturated fatty acids are found in wood mainly in the form of esters with glycerol (fat and oil) or alcohols (wax). Acetic acid is bound to polyoses as an ester group.

d. Alcohols

Most aliphatic alcohol in wood occurs in form of ester components, while the aromatic sterol, belonging to steroids, is found mainly as glycosides.

2.3.4.2. Extraction techniques

The extractives are made up of various components that may be isolated using polar- and non polar solvents (Jelokava and Sindler, 2001). The main substances used for extraction are acetone, hexane, ethanol, toluene, methanol and water. According to David and Nobuo (2000) there is no single solvent capable of removing all the substances called extractives. This is why the extraction procedures involve various solvents in intercalated steps.

2.4. Wood Degradation

Wood is degraded by both biotic and non-biotic agents. Non-biotic agents include physical degradation (fire, heat, humidity), chemical degradation (acids, bases).

2.4.1. Biotic agents

Biotic agents are the main cause of wood's short life in service. They consume the main wood components, as energy source, reducing the weight and the mechanical resistance. The most important biotic agents that degrade wood in service are: fungi, insects, bacteria and marine borers (Moreschi, 2013).

Biodegradation is a part of the natural life cycle of organic matter and it takes place in every natural environment (terrestrial and aquatic) when conditions are favourable. It is a process which maintains the functionality of ecosystems. For commercial purposes it can be defined as undesirable changes in wood properties caused by vital activities of organisms (Allsopp *et al*., 2004). Wood can be easily destroyed by many organisms through degradation of wood structural compounds which are solubilised both low molecular compounds and polymers (Cruz, 2005).

2.4.1.1. Fungi

Fungi are biological agents that attack wood in larger proportions because they develop quickly and occur in all types of environments where wood is used. It is a major cause of wood damage (production and use). The degradation is caused due to the action of enzymes secreted by fungal hyphae (Moreschi, 2013).

Wood decay by fungal development depends on moisture and temperature conditions and may happen due to lack of suitable protective measures when storing logs, improper seasoning, storing, or handling of the raw material produced from the log and failure to take ordinary simple precautions in using the final product (Clausen, 2010).

Biodegradation starts when the spores produced by fungal fruiting body, spread by the wind or insects, on contact with wet wood germinate and the fungal hypha penetrates through the cell

lumen (Figure 3). The fungus thrives and promotes an intense secretion of extracellular metabolites, especially enzymes, that lead to the conversion of cell wall components (cellulose, hemicellulose and lignin) into smaller molecules which can be transported across the plasma membrane and utilised by the fungal intracellular metabolism (Coleman, 2004; Carvalho *et al*., 2009 and Clausen, 2010).

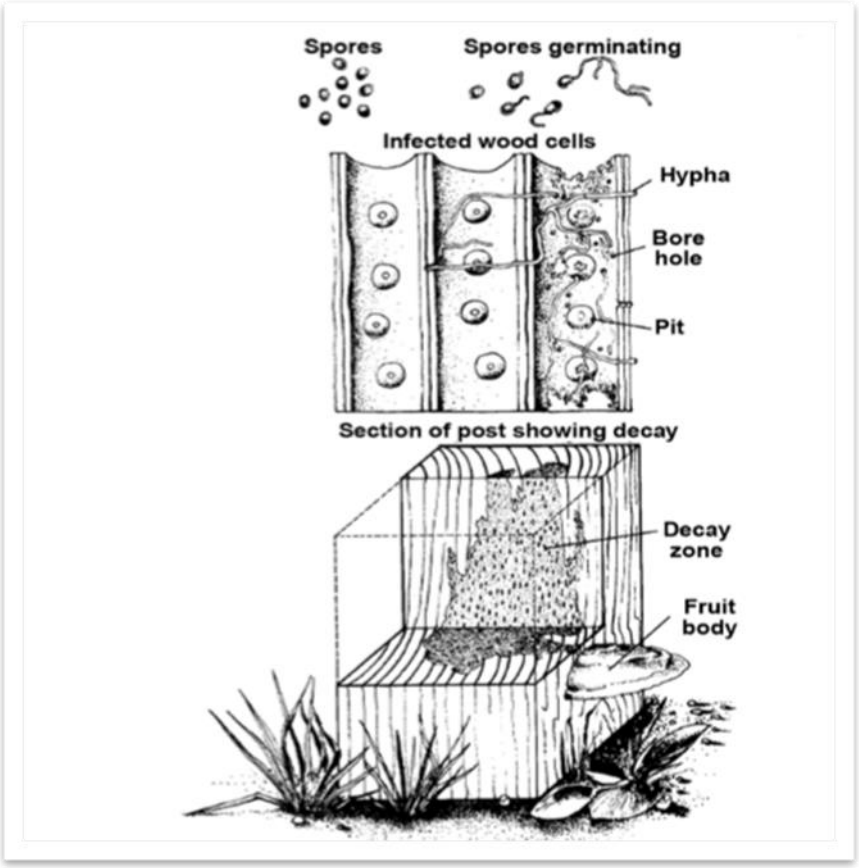


Figure 3. Fungi decay cycle

Clausen, 2010

Most of wood decay occurs when the wood has moisture content above 20% in an environment with optimal temperatures between 25º-30ºC, although some may attack in temperatures between 0º-40ºC, oxygen concentration of 20%, pH between 4.5-5.5 (Moreschi, 2013).

There are several species responsible for the degradation that can be traced by the type of damage caused. The decomposing fungi on wood can be classified in five groups: brown rot, white rot, soft rot fungi, stain or discoloration fungi and moulds (Coleman, 2004).

i) Brown rot

It is caused by fungi of *Basidiomycetes* class attacking mainly softwoods (Shupe *et al*., 2008). They are responsible for the appearance of cubic cracks and brownish colour in the wood (Figure 4). This degradation is caused by the enzymatic action of the mycelium in the cell walls. The brown rot fungi degrade primarily the cellulose and hemicellulose, turning them into nutritious, soluble and easily assimilated and digested substances, the lignin is practically intact giving a brown colour to the wood. The destruction of the structural elements causes loss of mass and mechanical strength.

Figure 4. Brown rot

Coleman, 2003

ii) White rot

This degradation is also caused by fungi of *Basidiomycetes* class, mainly in hardwoods. They leave the wood with a fibrous, coarse and whitish appearance (Figure 5). The restricted action of the enzyme system provides the formation of cracks or holes where fungi are established. During the attack these cracks are enlarged and cause a slow and complete erosion of the cell wall from the lumen towards the middle lamella. The white rot fungi can utilise mainly lignin, cellulose and hemicellulose that confers a whitish colour and has a gradual mass loss and mechanical properties (Mahajan, 2011 and Moreschi, 2013).

Figure 5. White rot

Coleman, 2003

iii) Soft rot

The microorganisms responsible for soft rot belong to *Ascomycetes* and *Deuteromycetes* (imperfect fungi) class. They penetrate the secondary cell wall and develop long helical tunnels. Within the cell wall, they can cross the middle lamella and penetrate neighbouring cell. The etched surface shows cracks as if it has been charred (Figure 6). Degradation by these fungi may be slow compared with the activity of white and brown rot fungi (Moreschi, 2013).

Figure 6. Soft rot

Moreschi, 2013

iv) Discoloration

It is caused by fungi of the *Deuteromycetes* class. They have pigmented hyphae and inhabit the parenchyma tissues (mainly in radial direction, i.e. rays) and utilise the nutrients available there. They cross the cell horizontally and make small holes in the cell wall, usually through the pits causing discolouration of the timber (Figure 7) but the mechanical properties are changed insignificantly (Moreschi, 2013).

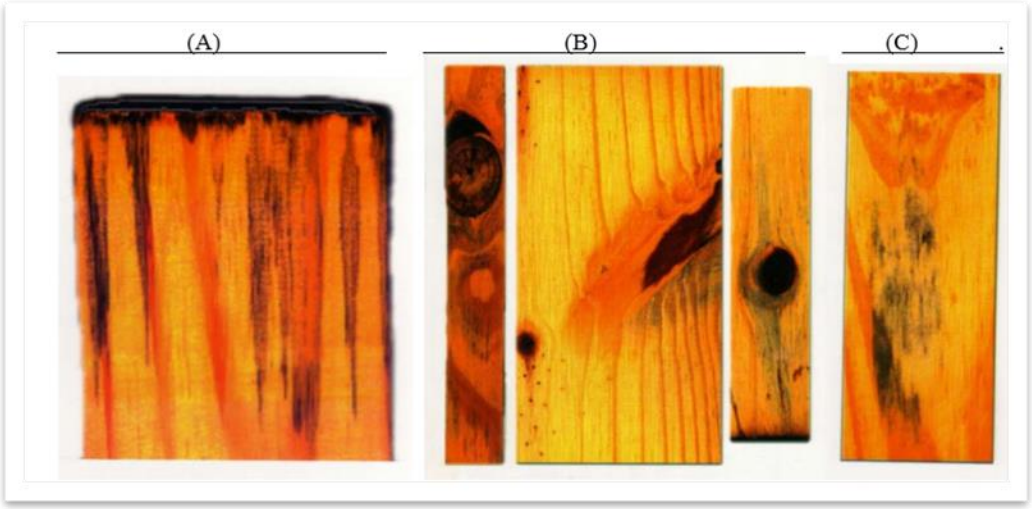


Figure 7. Discoloring fungi: A- Penetration through the top; B- Penetration via the knots and C- Radial penetration through contact with contaminated separators

Moreschi, 2013

v) Moulds

Mould fungi develop on the surface of the timber and utilize the components existing in freshly cut cells or on nutritional waste deposited on wood surface. The wood surface becomes powdery (Figure 8) and moulds are easily removed by scraping.

Figure 8. Mould fungi

Kclock, 2005

2.4.1.1.1. Recognizing fungi attack

According to Moreschi (2013), the symptoms which characterize the attack in the first stages are different from those in advanced stages.

- **Color**: in the first stages of fungal attack the wood changes colour. Depending on the fungi, this color change is seen has dark bands or points or regions in wood with a lightener colour.
- **Wood softening**: the affected area will show a brittle texture.
- **Density changes**: the weight loss is characteristic of an advanced stage of damage.
- **Wood odour**: wood affected by fungi stinks similar to damp and humid places.

2.4.1.2. Insects

i) Beetles

Beetles bore the wood from the bark to the inner part making tunnels where they lay their eggs (Figure 9). After the pupa phase the adults come out by biting their way out of wood leaving the wood with "exit holes" which are seen 9 to 12 months after the larvae started the damages inside the wood. Degradation by beetles is known as the second cause of economical damages in wood after the termites (Clausen, 2010 and Morris, 2013). Some beetles attack the wood infested by fungi because they feed on them (Clausen, 2010).

Figure 9. Wood attacked by beetles

Malinoski, 2006

ii) Termites

Termites are mostly found in the tropical countries inhabiting subterranean environments (Figure 10); the termites utilise all three wood constituents and are responsible for the largest volume of degraded wood. Its control should rely on toxification or repellence of nutritious sources interesting for the termites because the temperature and humidity are difficult to control.

Figure 10. Wood attacked by termites

Source: Kraeutler, 2013

2.5.Wood preservation

The biological degradation of wood is one of the main concerns of the timber industry due to the economic losses caused (Mendes and Alves, 1988). Biodegradation is commonly delayed by use of preservatives. Wood preservative is any substance that actively causes toxification of wood as a nutrient for xylophages agents. A good preservative should theoretically have the following characteristics according Moreschi (2011):

- High toxicity: wood is used in various environments and is exposed to degradation organisms with certain characteristics and thus the preservative should be toxic to a wide variety of organisms.
- Less toxic to non-wood decay organisms: the preservative in the formulation should not contain elements that endanger the human life or the environment.
- Lasting action: It is desirable that the wood is protected for several years, and it should have high permanence without decompose or alteration.
- Good fixation: the preservative should not be easily leached and expose the wood back to xylophages agents.
- Not alter the characteristics of the wood: must neither modify the physic and mechanical properties nor the aesthetics of wood.
- Noncorrosive: wood is often used in combination with other materials and if the preservative is corrosive it can cause damage and aesthetic risks.
- Non flammable: wood is a flammable material by definition.
- Economic and easy to purchase: often the economic factor is limiting.

Preservatives used in industries belong to two classes: oil-based, such as creosote, and water based such as CCA (Galvão *et al*., 2004).

CCA (chromated copper arsenate) is a water-borne preservative formulation consisting of a mixture of compounds of arsenic pentoxide, chromium and copper used in wood protection

against fungi, insects and marine borers. Copper is used for controlling fungi and marine borers, arsenic for the control of insects and some fungi resistant to copper and chromium is used to fix the arsenic and copper in the wood (Bollin and Smith, 2013). Wood treated with CCA dominated the market for treated wood from the late 1970s until 2004 when CCA was banned in Europe and some other countries (Shupe *et al.*, 2008).

2.6. Trees description

2.6.1. Pseudolachnostylis maprounaefolia Pax

Pseudolachnostylis maprounaefolia is a member of the *Euphorbiaceae*, known also as ntholo (Figure 11). It is a small tree up to 5-10 m height that is distributed from Central to tropical East Africa (Palgrave, 1990). The leaves are alternate, simple, ovate fresh to blue green and paler green below. In Mozambique it grows north of the Save River in open forest (Zambezia, Cabo Delgado, Tete, Manica, Niassa, Nampula and Sofala) (Marzoli, 2007). It is a less known timber which is being subjected to several studies since it has potential to increase the timber industry productivity and reduce pressure of the commonly used species (Uetimane Junior *et al.*, 2009). The wood is smooth, even-grained, moderately heavy, used in toy making, turnery and handicrafts and it is very resistant to xylophagous agents (Palgrave, 1990; Bunster, 2006).

Figure 11. *Pseudolachnostylis maprounaefolia* tree (left); fruits and leaves (middle) and wood (right).

Source: Schmelzer, 2007;

2.6.2. Sterculia appendiculata K. Schum.

Sterculia appendiculata also known as metil, belongs to the *Sterculiaceae* family. It is a deciduous tree, growing up to 40 m height with straight and unbranched trunk, branching only above (Figure 12). The leaves are green, crowded towards the ends of the branches, large, 14 to 30 x 30 cm. The fruit is made up of two carpels, each up to 9 x 6 cm, covered outside with short soft brown hair. Its natural habitat is Malawi, Mozambique, Tanzania and Zimbabwe (Hyde *et al*., 2014). It is also a less known timber which showed good technology properties but has low natural durability (Uetimane Junior *et al*., 2009).

Figure 12. *Sterculia appendiculata* tree (left); fruits and leaves (middle) and wood (right).

Source: Bosch and Louppe, 2008; Barrystock, 2008; iNAturalist.org, 2014

2.6.3. Azadirachta indica A. H. L. Juss.

Azadirachta indica is a multiple use tree coming natively from India belonging to the *Meliaceae* family known as neem or margosa, native to the dry areas Indo-Pakistan. It is a small to medium-sized tree, evergreen, up to 15-30 m high, with a round large crown that is 10-20 m in diameter. The fruits are greenish-yellow, ellipsoidal, 1-2 cm long with thin exocarp (Figure 13). It grows almost anywhere in the lowland tropics in altitudes between 0-1500 m, mean annual temperature of 40ºC and mean annual rainfall of 400-1200 mm (Orwa, 2009). In Mozambique it occurs in centre and north zones. The products from this tree are known as effective pest control agents in agriculture causing antifeedant, repellent effect of egg laying, regulatory effect of growth, interference on physiological functions and effects on reproduction and in some cases death (Mossini and Kemmelmeier, 2005).

Figure 13. *Azadirachta indica* tree (left) and fruits (right)

Source: K.M. Siddiqui, 2015

2.7. Fungi Description

2.7.1. Postia placenta

Postia placenta is a member of *Coriolaceae* family (Figure 14). It is common in forest ecosystems and is largely responsible for the destructive decay of wooden structures. Softwood is the primary host of this brown rot fungus (Martinez, *et al*. 2009).

Figure 14. *Postia placenta* in wood

Source: First Nature, 2015

2.7.2. Phialophora mutabilis

Phialophora mutabilis is a *Herpotrichiellaceae* family member, its hyphae are pink in the first stages of the development while, later, becomes blackish-brown (Schol-Schwarz, 1970).

2.7.3. Gloeophyllum trabeum

This fungus belongs to *Gloeophyllaceae* and resides normally on hardwoods timber (Figure 15). Fruit bodies may be 10 cm in dimension with the surface of the reddish-brown caps turning gray with age being lighter on the edge. They have the ability to depolymerize the cellulose and hemicelluloses leaving modified lignin (Svetlova and Zmitrivitch, 2015; Floudas *et al*., 2012).

Figure 15. *Gloeophyllum trabeum* fruiting bodies on dead tree

Svetlova and Zmitrivitch, 2015

2.7.4. Trametes versicolor

Trametes versicolor is fungal species found in temperate to subtropical forests, known also as "turkey tail" for its coloured patterns (Figure 16). It can be found in wood logs in decomposition, very often on hardwoods but occasionally on softwoods (Kuo, 2005)

Figure 16. *Trametes versicolor* on wood

Kuo, 2005

2.7.5. Pycnoporus sanguineus

Pycnoporus sanguineus is an intense orange colour fungus (Figure 17) and belongs to *Polyporaceae* which includes not only the most heavily ligninolytic fungi but is one of the most efficient wood degraders. It occurs in tropics and usually grows in trunks in natural environments and in urban tree spaces as well (Papinutti, 2013).

Figure 17. *Pycnoporus sanguineus* on wood

Source: Flickr, 2015

2.7.6. Chaetomium globosum

This fungus (Figure 18) is a member of *Chaetomiaceae* family and occurs in conditions that retard or inhibit the development of more aggressive wood destroying Basidiomycetes (Duncan and Eslyn, 1996).

Figure 18. *Chaetomium globosum* gypsum board

Source: Arx, Guarro and Figueras, 1986

CHAPTER 3

METHODOLOGY

3.1. Collection and Sample Preparation

Samples of *Pseudolachnostylis maprounaefolia* (ntholo), *Sterculia appendiculata* (metil) and *Azadirachta indica* (neem) with unknown age were collected at Pemba, Montepuez and Nicoadala respectively (Figure 19). Only healthy, defect-free wood samples were chosen.

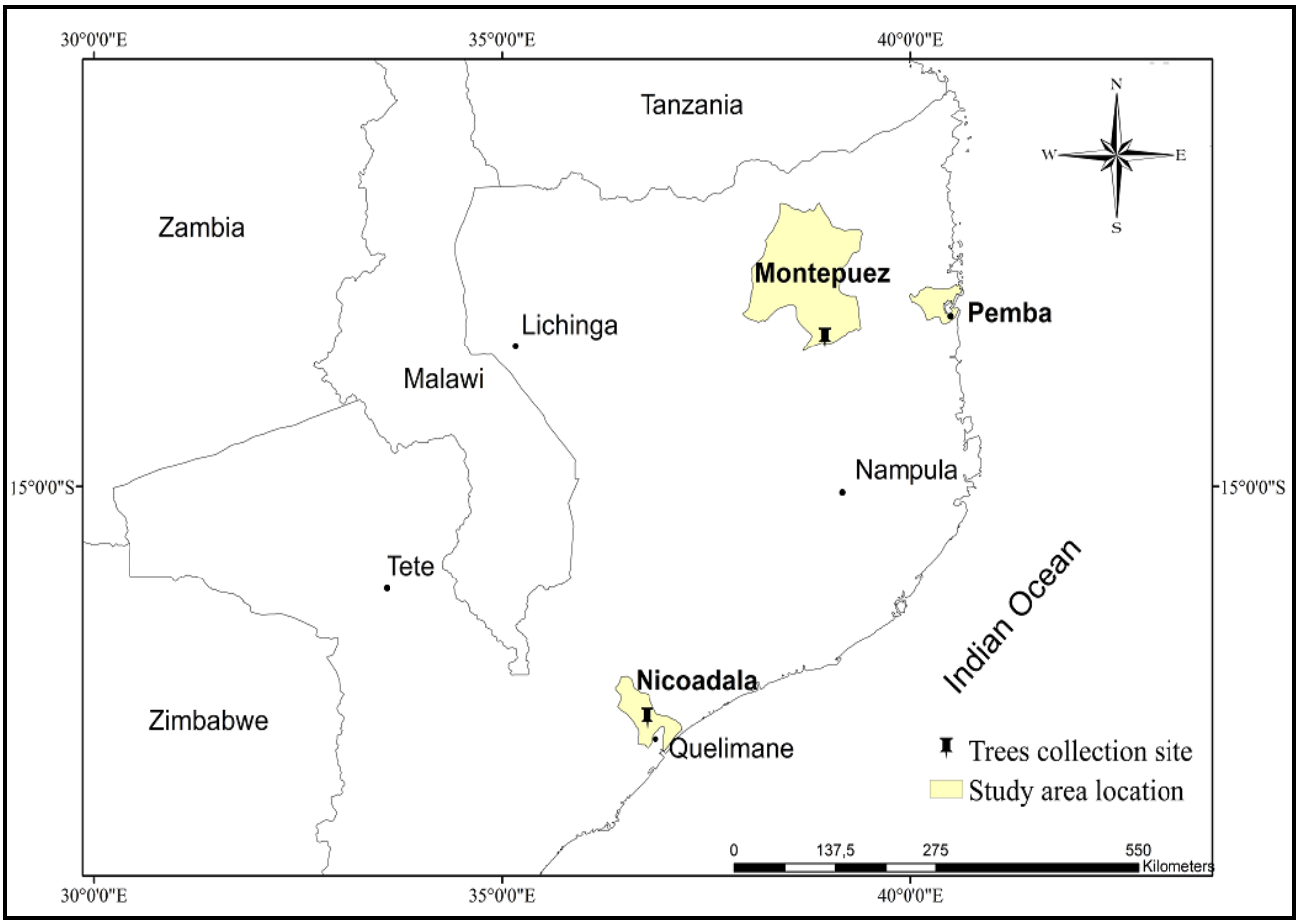


Figure 19. Tree sampling locations

Pemba

Pemba is the capital of Cabo Delgado Province. The climate is humid subtropical equatorial with dry winters (Köppen, climate classification) and the warm rainy seasons extends from December to April with March as the wettest month; the dry cold season extends from May to November with September as the driest month of the season (MAE, 2005a). Ntholo samples were collected in 2009 and were conditioned in room climate.

Montepuez

Montepuez is located in the south part of Cabo Delgado Province at 210 km away from the capital (Pemba). The dominate climate is humid subtropical with dry winters (Köppen, climate classification). Average annual rainfall is 800-1200 mm. (MAE, 2005b and Marzoli, 2007). Two trees of metil with diameter of 53 and 60 cm were taken (coordinates S 13° 05' 31.2" and E 38° 59' 05.5") in an open dry forest.

Nicoadala

Nicoadala is located in southeast of Zambezia Province. The climate is typical for tropical savanna (Köppen, climate classification) with two distinct seasons, a pronounced dry and cold season (May to October) and a warm rainy season (November to April) (MAE, 2005c). Two trees of neem with diameter of 30 and 36 cm were taken (coordinates S 17° 35' 21.3" and E 36° 46' 11.7").

3.2. Tree sampling

Trees of metil and neem were collected in native forest and transported to a sawmill where they were converted into blocks of 60(rad) x 60(tang) x 600(long) mm according to ISO 3129-1975 standard (Figure 20). Heartwood samples were taken from breast height; sapwood sections were excluded due to relatively low extractive content.

The planks were frozen to prevent accidental drying before transportation to the laboratory.

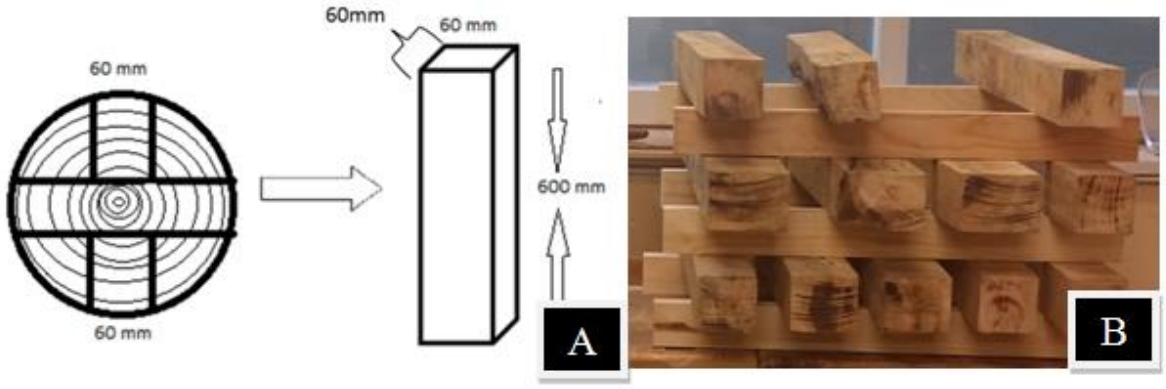


Figure 20. Tree sampling (A) and blocks (B)

Afterwards, for each wood species, the blocks were processed into specimens of 1(rad) x 0.5 (tang) x 2(long) cm (Figure 21). Two hundred specimens of each species were labelled for future identification.

Figure 21. Specimens labelled

3.3. Accelerated laboratory tests

All heartwood samples regardless the species were subjected to fungal attack under controlled conditions according to the European standard EN 113 standard. A total of 194 specimens of each species, as well as the reference species birch, were subjected to fungi to study the natural resistance of wood to fungal attack, fungal decay after extractive removal and fungal decay after lignin removal (Figure 22).

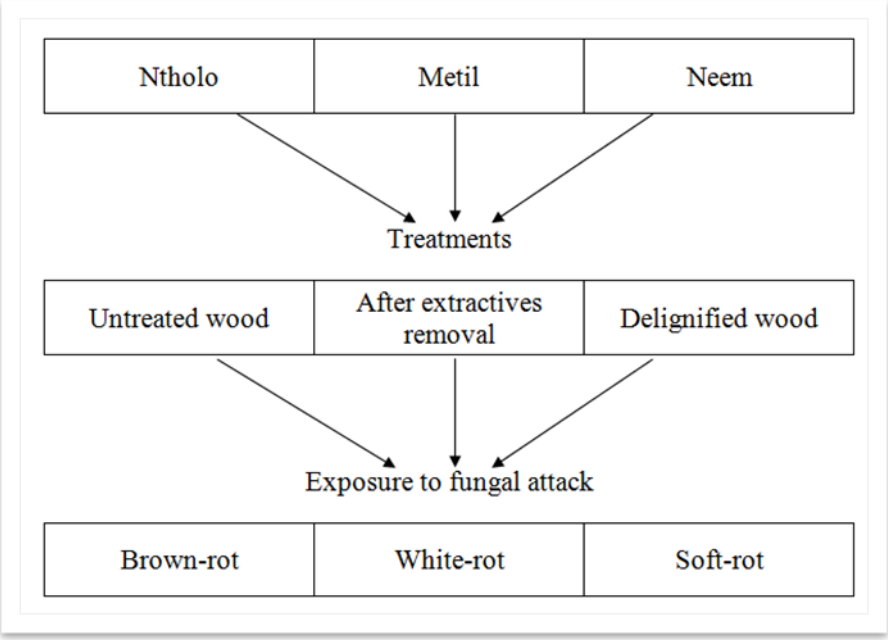


Figure 22. Acceletrated test scheme

3.2.1. Untreated wood

Sixty specimens of each species were oven-dried at 103ºC until constant weight was achieved and subsequently sterilized in appropriate bags (Figure 23) for 40 min. After sterilization the specimens were exposed to brown rot fungi (*Postia placenta* and *Gloeophyllum trabeum*), white rot fungi (*Trametes versicolor* and *Pycnoporus sanguineus*) and soft rot fungi (*Chaetomium globosum* and *Phialophora mutabilis*) with four replicates for each species (Figure 24), according to EN 113 standard.

Figure 23. Sterilization bags

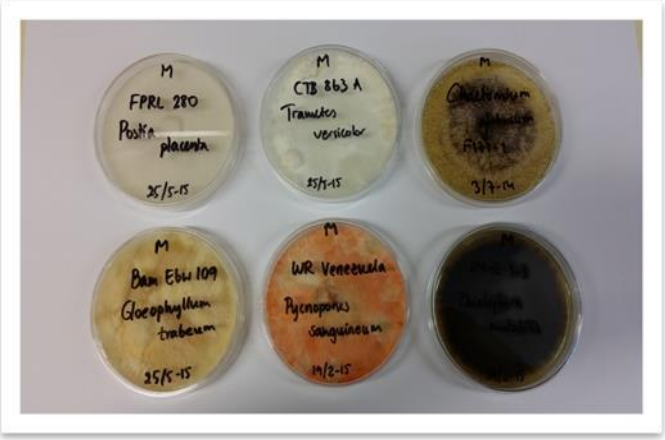


Figure 24. Tested fungi species

Four specimens of each of the wood species were placed in a Petri dishes with the fungus in the centre as shown in Figure 25, then left to decay for three months.

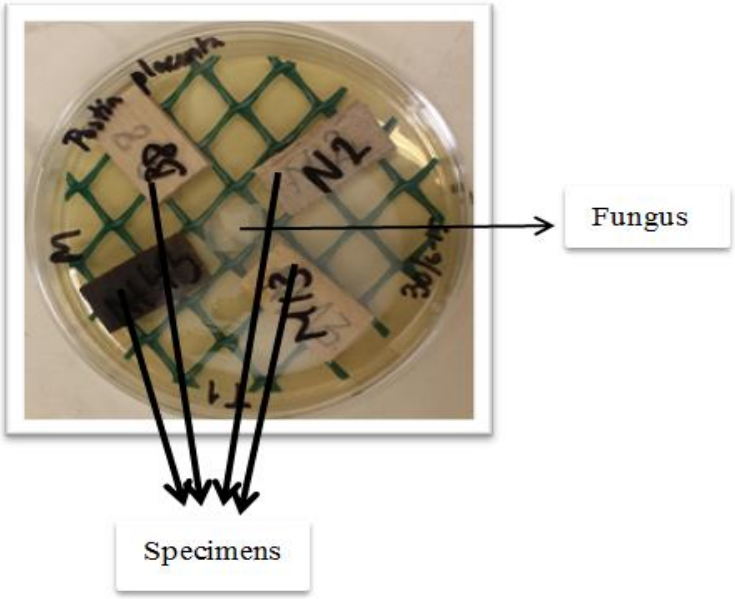


Figure 25. Specimen disposition in a Petri dish

3.2.2. Extractives removal

One hundred specimens were oven-dried at 103ºC, until constant weight was achieved and then placed in a soxhlet apparatus (Figure 26) with 150 ml of toluene, 50 ml of ethanol and 50 ml of acetone (2:1:1) for 24 h (TAPPI, 2007).

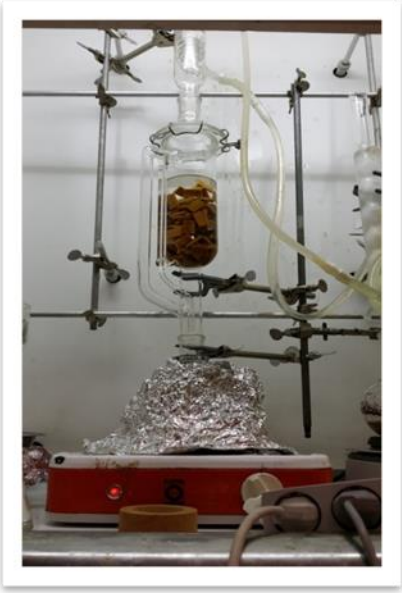


Figure 26. Soxhlet apparatus

After removal of extractives, the specimen of each wood species (four repetitions) were oven-dried again at 103ºC, until constant weight was achieved, conditioned at 20ºC and RH of 70%, and then exposed to fungi causing brown rot, white rot and soft rot (Figure 24) for 3 months.

3.2.2.1. Extractives quantification

After removing the extractives in soxhlet apparatus, the mixture of toluene, ethanol, acetone and extractives of each species were placed in rotary evaporator (Figure 27) to evaporate the solvents leaving only the extractives in a volumetric flask (Figure 28). The flask was left to dry at 103ºC in the oven and then weighed to obtain the amount of extractives for each species.

Figure 27. Separation of solvent and extractives

Figure 28. Extractives with solvents

The content of the extractives was calculated according to TAPPI standard (2007) using the formula:

$$\%\text{Extractives} = \frac{W_{f+e} - W_f}{ODW_{sample}} * 100$$

W_{f+e}: Weight of the flask plus extractives

W_f: Weight of the flask

ODW: oven dry weight of samples

3.2.2. Delignified wood

Thirty specimens were dried in oven at 103ºC until constant weight. They were placed in oven at 60ºC for 18 h in an Erlenmeyer flask (Figure 29) with 200 ml of acetone and hydrogen peroxide (1:1) (Wise and Murphy, 1946). After delignification (Figure 30) the specimens were dried, reweighed and exposed to the fungi (three replicates for each species).

Figure 29. Specimens before delignification

Figure 30. Delignified specimens

3.3. Mass loss

After the experiments, mycelia were removed from the wood samples which were dried in oven at 103ºC for 24 h. The mass loss caused by fungal attack was calculated using the formula:

$$ML(\%) = \frac{W_1 - W_2}{W_1} * 100$$

Where:

ML: is the mass loss (%)

W_1: Initial dry weight (before fungal exposure)

W_2: Final dry weight (after fungal exposure)

The durability rating was assigned based on mass loss (European standard EN 350-1) as shown in Table 2.

Table 2. Classes of wood durability (EN 350-1)

Durability class	**Results of laboratory tests expressed as x**
Very durable	$x \leq 0.15$
Durable	$x > 0.15$ but ≤ 0.30
Moderately durable	$x > 0.30$ but ≤ 0.60
Slightly durable	$x > 0.60$ but ≤ 0.90
Not durable	$x > 90$

$$x = \frac{\text{average corrected mass loss of test specimens}}{\text{average mass loss of reference specimens}}$$

3.3.2. Statistical analysis

Treatments and mass loss were evaluated using Minitab 17 with ANOVA and Fisher pair wise comparisons with confidence level of 95%.

CHAPTER 4

RESULTS AND DISCUSSION

This study consisted of accelerated laboratory decay testing of the wood for 3 months. The experiment was designed to evaluate the separate role of lignin and extractives in the natural durability of ntholo, metil and neem. Untreated wood and two treatments namely, removal of extractives and delignification were applied. Subsequently, samples from all treatments were exposed to controlled fungal attack (white, brown and soft rot) after which the mass loss was calculated as an indicator of resistance against fungal attack. Unfortunately, soft rot fungi *C. globosum* was contaminated during exposure to the result from *C. globosum* were therefore not possible for delignified wood samples as mould was growing.

4.1. Resistance against fungal attack of untreated wood (natural durability)

The performance of the untreated wood samples of all species against fungal attack is briefly represented in the graph below (Figure 31). White rot fungi were the most active, as they decayed part of the cellulose and hemicelluloses as well as lignin.

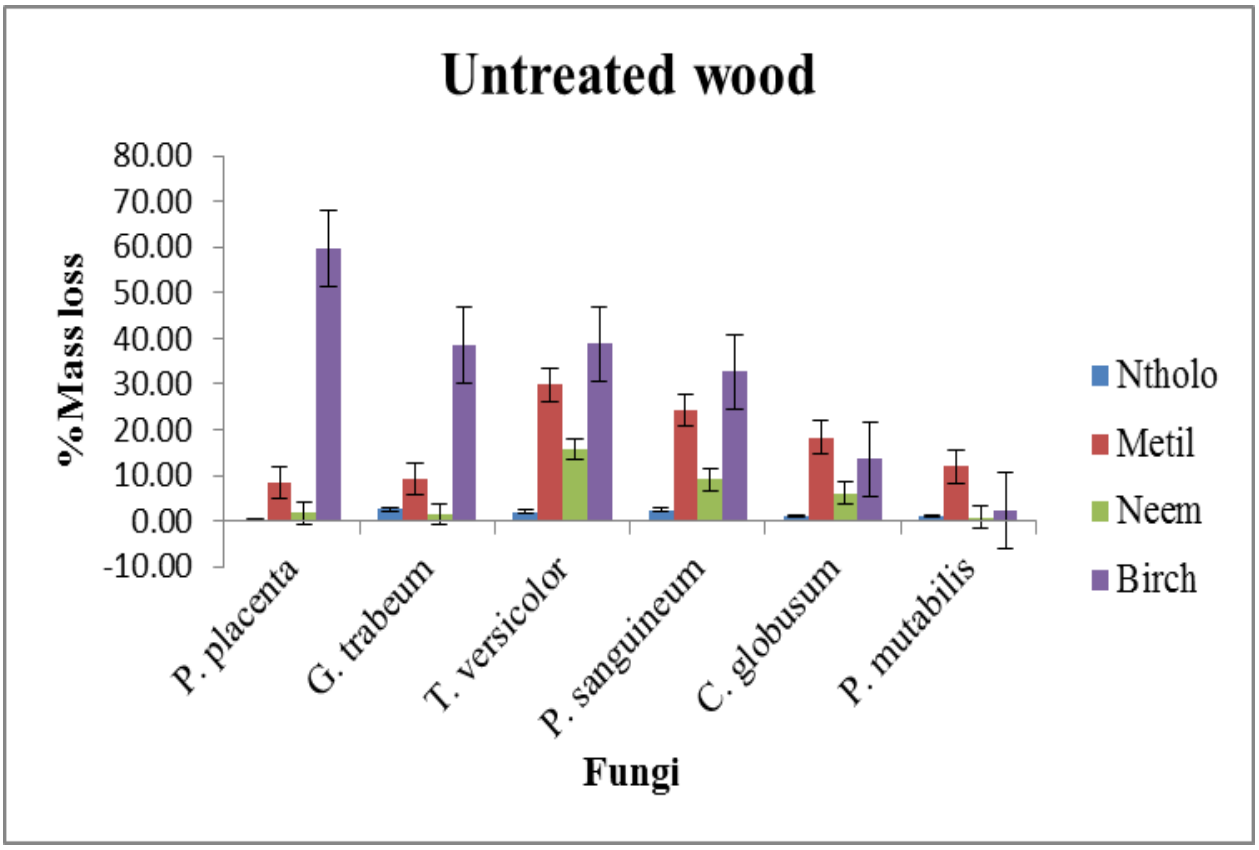


Figure 31. Mass loss of wood species against fungal attack for untreated wood

Ntholo untreated wood was the most resistant against all tested fungal attack. This species has high proportion of fiber tissue and low amount of parenchyma cells, making it the least preferred by wood destroying fungi (Uetimane Júnior *et al*., 2009).

Metil showed high mass loss, with greatest decay by white rot (*T. versicolor*-29.78% and *P. sanguineus*-24.27%) followed by soft rot (*C. globosum*- 18.36% and *P. mutabilis*-11.9%) and brown rot (*P. placenta*-8.44% and *G. trabeum*-9.3%). According Uetimane *et al*., (2009), this species contains a high percentage of parenchyma tissue in structure (about 53%). The high mass loss can be explained by high parenchyma tissue which is responsible for storing nutrients as starch and sugars that are a major food source for wood destroying fungi (Bessa, 2009).

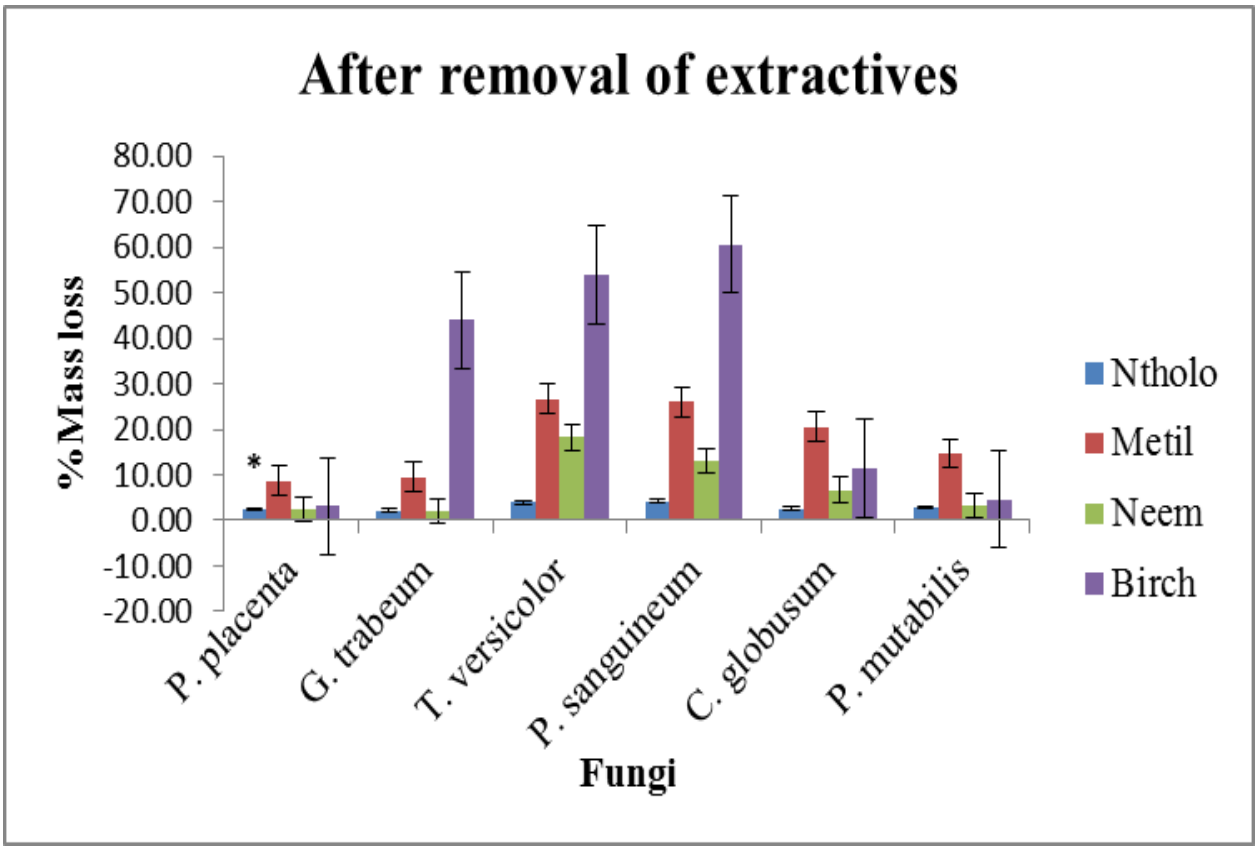
Neem decay was mainly caused by white rot (*T. versicolor*-15.77% and *P.sanguineus*-4.9%) followed by soft rot (*C. globosum*-5.6% and *P. mutabilis*-0.74%) and brown rot (*P. placenta*-1.79% and *G. trabeum*-1.51%). Koyani and Rajput (2015) characterized wood decay of Neem

against white rot (*Irpex lacteus* and *Phanerochaete chrysosporium*) and the mass loss was around 15-19% after 90 days.

According to Hatakka (2001), although lignin is a resistant polymer to biodegradation, fungi that cause white rot are extremely efficient lignin degraders that exist in nature and they also secrete enzymes capable of degrading cellulose and hemicelluloses.

4.2. Resistance against fungal attack after removal of extractives

Extractive removal increased slightly the incidence of attack by wood-destroying fungi. In general, all three species maintained the resistance pattern displayed in untreated wood (Figure 32).



*Fungus poorly grown

Figure 32. Mass loss of wood species against fungal attack after removing extractives

Ntholo experienced more decay as shown by the increase of mass loss: white rot (*T. versicolor*-3.87% and *P. sanguineus*-4.22%) followed by soft rot (*C. globosum*-2.55% and *P. mutabilis*-2.78%) and by brown rot (*P. placenta*- 2.47% and *G. trabeum* -2.17%).
Although metil had the greatest mass loss, the removal of extractives showed little effect, with similar mass loss observed as in untreated wood: white rot (*T. versicolor*- 26.58% and *P. sanguineus* -26.02%) followed by soft rot (*C. globosum*- 20.52% and *P . mutabilis*- 14.69%) and brown rot (*P. placenta*-8.66% and *G. trabeum*-9.49 %). This species has a very low content of extractives (1.11%)

After removing extractives, neem also showed the same trend as ntholo, i.e., the mass loss increased: white rot (*T. versicolor*- 18.19% and *P. sanguineus*-13.19%) followed by soft rot (*C. globosum*- 6.7% and *P. mutabilis*- 3.19 %) and brown rot (*P. placenta* - 2.39% and *G. trabeum* - 1.93%).

Both ntholo and neem retained a considerable amount of extractives in parenchyma tissue (axial and radial) and in some vessel elements even after treatment with solvents (Figure 33). The remaining extractives can be partially attributed to the inefficiency of solvents used in the extraction process.

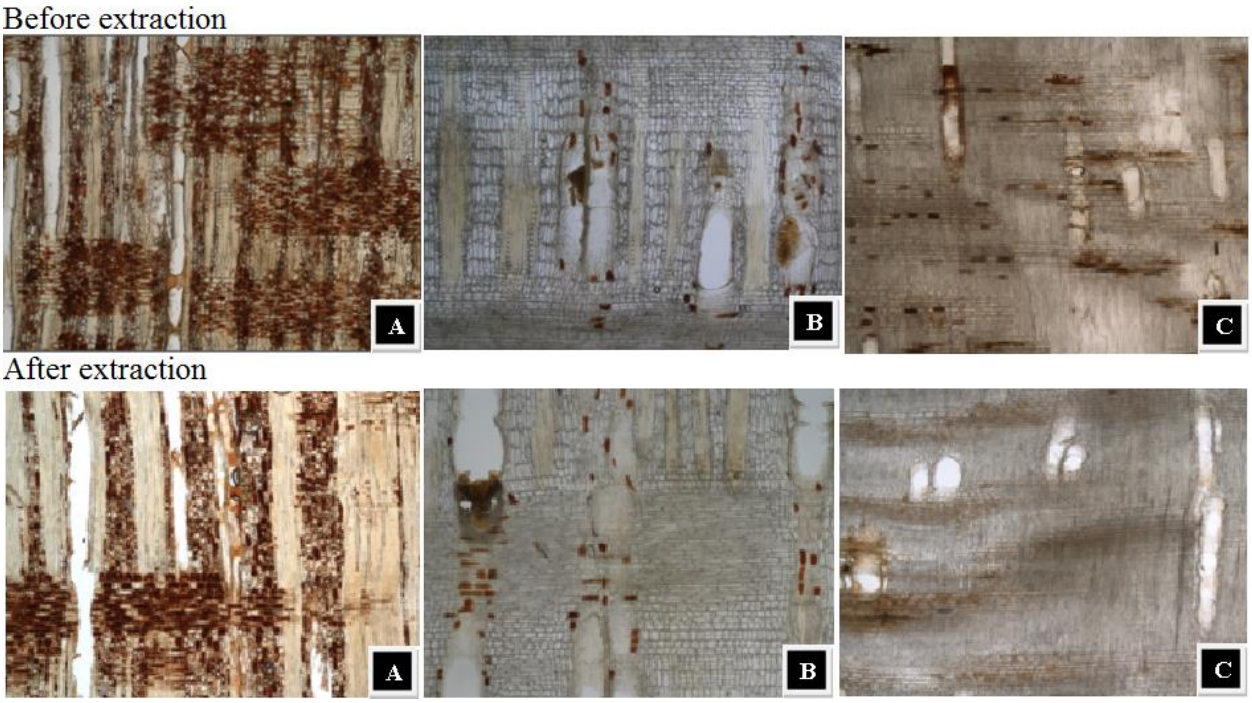


Figure 33. Radial section before and after extractive removal: A- ntholo; B- metil and C- neem

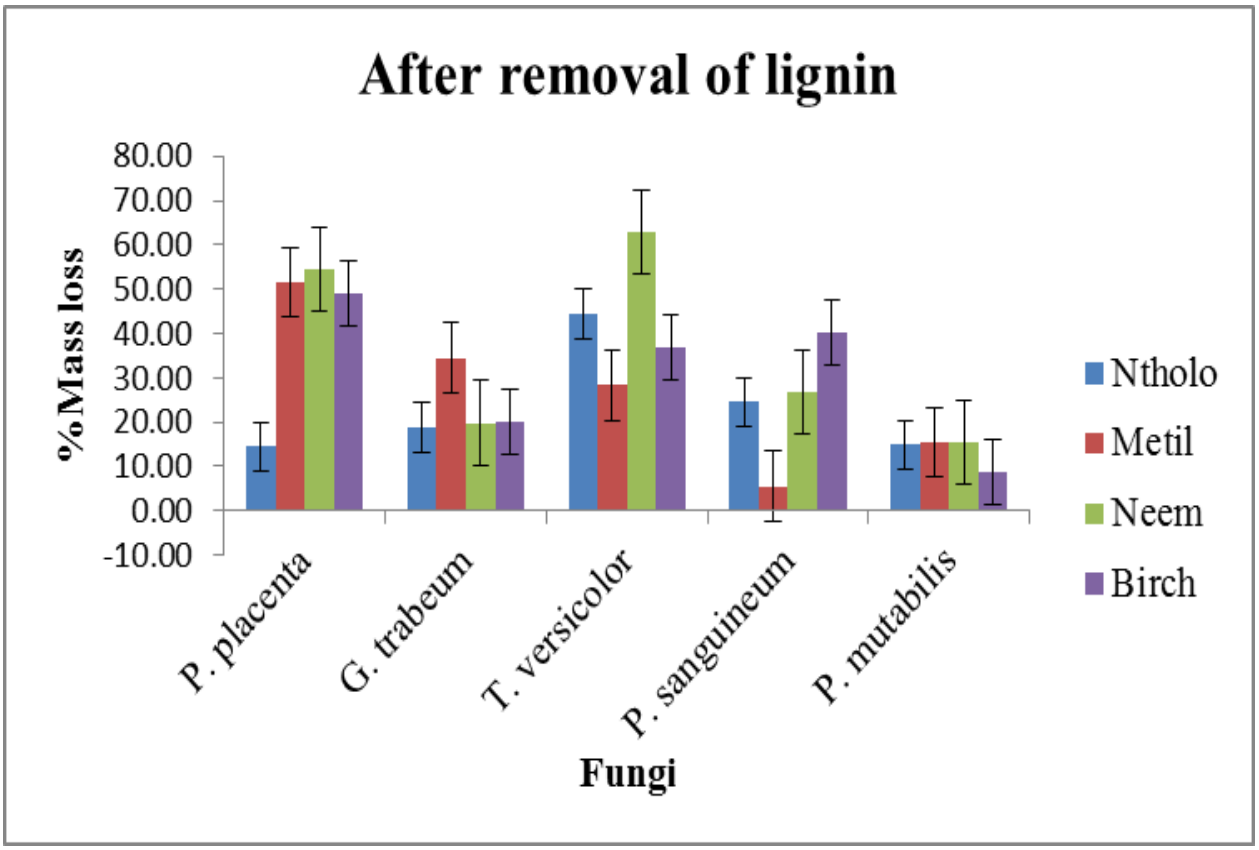
Agatha (2006), reduced the specimens into sawdust and removed extractives using 150 ml of solvent, hexane, acetone and distilled water, at a time and she found that acetone was the most effective solvent to remove extractives from durable woods.

Ntholo and neem which showed low mass loss still had extractives inside the parenchyma cells. Even though metil displayed extractive materials in its structure after extractive removal, it continued to be attacked by wood-destroying fungi. According to Walker (1993) and Taylor (2002), to analyze the natural durability of wood species it is important to analyze the presence, quantity and quality of extractive material.

4.3 Resistance against fungal attack after delignification

The resistance of species after wood delignification are shown in Figure 34. Ntholo was more attacked by white rot fungi (*T. versicolor*-44.43% and *P. sanguineus*-24.49%) with less mass loss for soft rot (*P. mutabilis*- 14.88%) and brown rot (*P. placenta*- 14.47% and *G. trabeum*-

18.85%). Ntholo decay after delignification was clearly higher than untreated wood and after removing extractives.



Note: *C. globusum*- contaminated

Figure 34. Mass loss of wood species against fungal attack after removing lignin

Metil suffered more decay from brown rot fungi (*P. placenta*-51.58% and *G. trabeum*-34.48%). Since most of the lignin was removed leaving an open way for these fungi that consumes preferably cellulose and hemicellulose. The mass loss by white rot (*T. versicolor*-28.26% and *P. sanguineus*-5.57%) was significant for *P. sanguineus*, since this fungus consumes lignin, cellulose and hemicellulose. The mass loss for soft rot was about 15.51% for *P. mutabilis*.

For neem the higher incidence of attack was evident for white rot fungi (*T. versicolor*-62.82% and *P. sanguineus*-26.71%). The attack by brown rot fungi (*P. placenta*-54.47% and *G. trabeum*-

19.87%), also recorded high mass loss comparing with other treatments. The mass loss for soft rot was about 8.6% for *P. mutabilis*.

In this scenario white rot was primarily responsible for the drastic increase of mass loss in ntholo and neem. Probably the lignin was not removed in its entirety or these fungi found in its structure a substrate compatible with their feeding needs other than lignin. Ntholo still had a brownish color after lignin removal (Figure 25). For metil, the removal of lignin increased attack intensity of brown rot which did not need to modify the wood structure to bypass the lignin and reach the substrate. Eaton and Hale (1993) stated that the natural durability of wood depends on both the accessible routes to the fungus and the wood chemical composition. The removal of lignin improved the accessibility to white rot fungi reducing wood durability (Gonzaga, 2006).

4.4. Durability ratings for all treatments per species

The resistance against fungal attack for the tested scenarios is shown in Table 3. In general, all species suffered severe decay when the extractives and lignin was removed.

Table 3. Durability ratings for all treatments per species.

Type of fungal attack\species	**Untreated wood/natural durability**		
	Ntholo	**Neem**	**Metil**
Brown rot	Very durable	Very durable	Durable
White rot	Very durable	Moderately durable	Slightly durable
Soft rot	Moderately durable	Moderately durable	Not durable
Type of fungal attack\species	**After extractives removal**		
	Ntholo	**Neem**	**Metil**
Brown rot	Slightly durable	Slightly durable	Not durable
White rot	Very durable	Moderately durable	Moderately durable
Soft rot	Moderately durable	Slightly durable	Not durable
Type of fungal attack\species	**Delignified wood**		
	Ntholo	**Neem**	**Metil**
Brown rot	Not durable	Not durable	Not durable
White rot	Not durable	Not durable	Slightly durable
Soft rot	Not durable	Not durable	Not durable

Uetimane *et al*., (2009) described anatomical features of ntholo and predicted that it is a very durable due the presence of extractives and thick-walled fibres. Ali (2011) performed a natural durability test using laboratory (test with basidiomycetes) for 8 months using brown rot (*Coniophora puteana*, *Gloeophyllum trabeum* and *Postia placenta*) and white rot (*Trametes versicolor*) and Ntholo behaved as a very durable wood for both brown and white rot.

The same experiment performed by Ali (2011), found that metil was also durable against brown rot (*Coniophora puteana*, and *Gloeophyllum trabeum*) but very durable against *Postia placenta* (a brown rot fungi), very susceptible to fungal attack, especially to soft rot and white rot (*Trametes versicolor*), being a part of not durable timber. According to Uetimane *et al*., (2009), metil had thin-walled fibres and no extractives which may lead to its low durability.

Neem has been recommended in pest's management thanks to its toxicity present on leaves, roots and wood against xylophages agents (Soares *et al*., 2009 and Paes *et al*., 2012). Paes *et al*. (2007) also determined natural durability of neem to brown rot (*Postia placenta*) and white rot (*Polyporus fumosus*) and classified has a durable wood.

4.5. Extractives content

Figure 35 shows the amount extractives for ntholo, metil and neem. Ntholo (3.95%) has the highest extractives amount, metil and neem had 1.11% and 1% respectively.

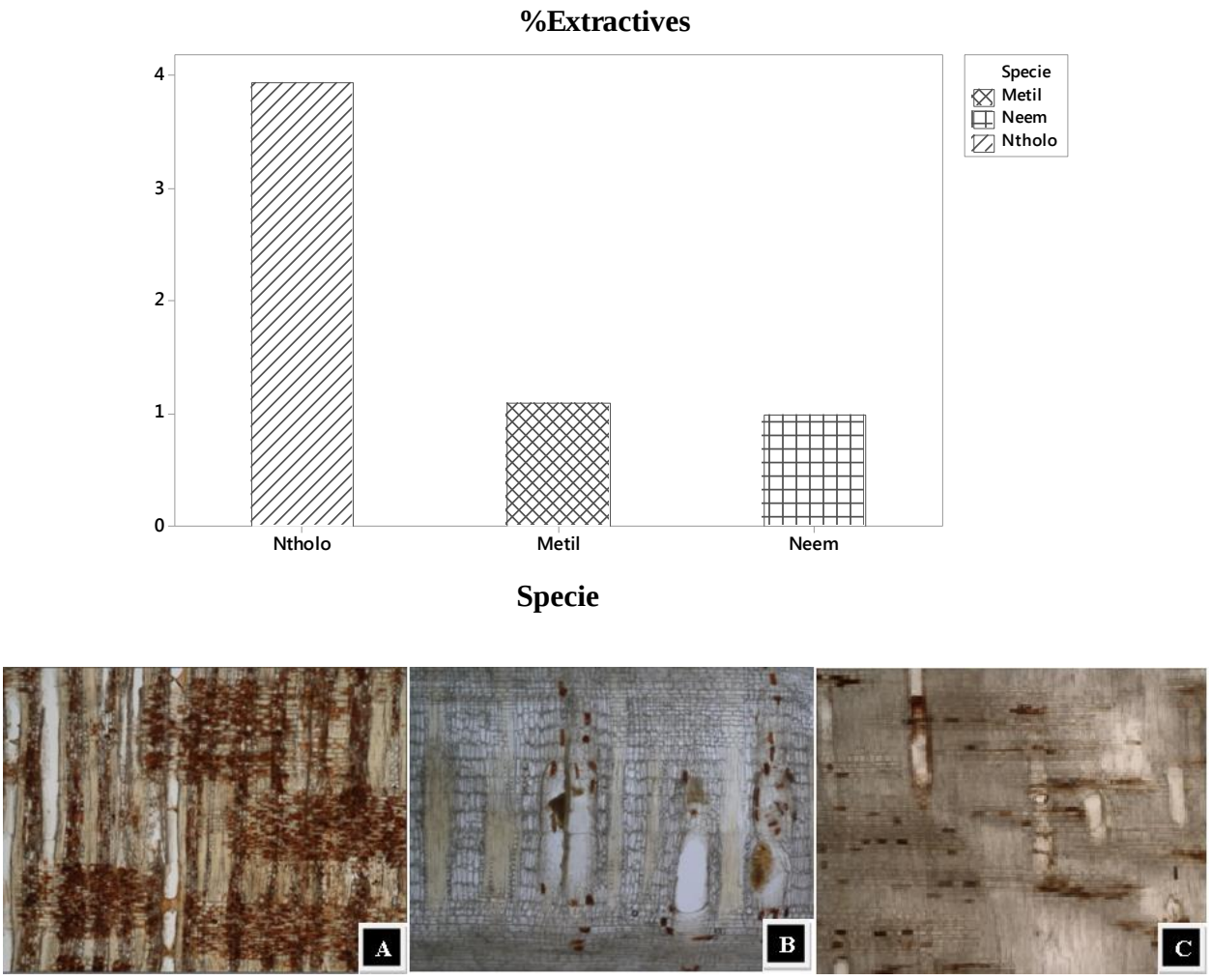


Figure 35. Amount of extractives; A- ntholo; B- metil and C- neem

Lhate (2011), removed extractives from ntholo and metil with acetone and estimated the percentage of extractives and found similar results as obtained in this study, i.e., 3.67% and 1.98% respectively, despite using only acetone as a solvent. Bergstedt and Lyck (2007), Lhate (2011), and Antwi-Boasiako *et al.* (2010) confirm that the natural durability increases with the amount of extractives and is also influenced by the type of lignin, density and wood anatomy (permeability and pH).

The amount of extractives obtained for neem (1%) was very low compared to Araújo *et al*. (2000), who determined extractives amount of neem according to standard ABNT NBR 8112 (1986) and found about 8.46% of extractives content. This difference can be related to the solvent used in extraction.

Although neem and metil have almost the same amount of extractives, neem resisted more to fungi attack. Probably neem extractives have greater toxicity to fungi than metil. Kabir *et al.* (2008), isolated the extracts of neem leaves to treat wood. Neem extractives have raised the interest of researchers in seeking for preservatives from the biological source which are eco-friendly.

4.6. The role of extractives and lignin against fungal attack

The role of extractives and lignin in the overall durability of the three species has been depicted through analysis of variance (Table 4) at 5% confidence interval. The results showed significant differences ($P<0.05$), amongst overall mass loss means of untreated wood, removal of extractives and delignified wood against all tested fungal attack in all three species.

Table 4. ANOVA

Source	DF	Adj SS	Adj MS	F-value	P-value
Treatment	2	7364.8	3682.4	21.12	0
Wood species	3	18315.1	6105	35.02	0
Fungi	5	10810.6	2162.1	12.4	0
Replication	3	334	111.3	0.64	0.591
Error	224	39049.9	174.3		
Total	237	78772.3			

A Fisher pair wise comparisons using the Least Significant Difference (LSD) statistical test (Figure 36) was performed to assess which treatment was different for each species. There are no statistical differences between mass loss of untreated wood and extracted wood for ntholo and neem. Mass loss of metil showed no statistical difference for all treatments.

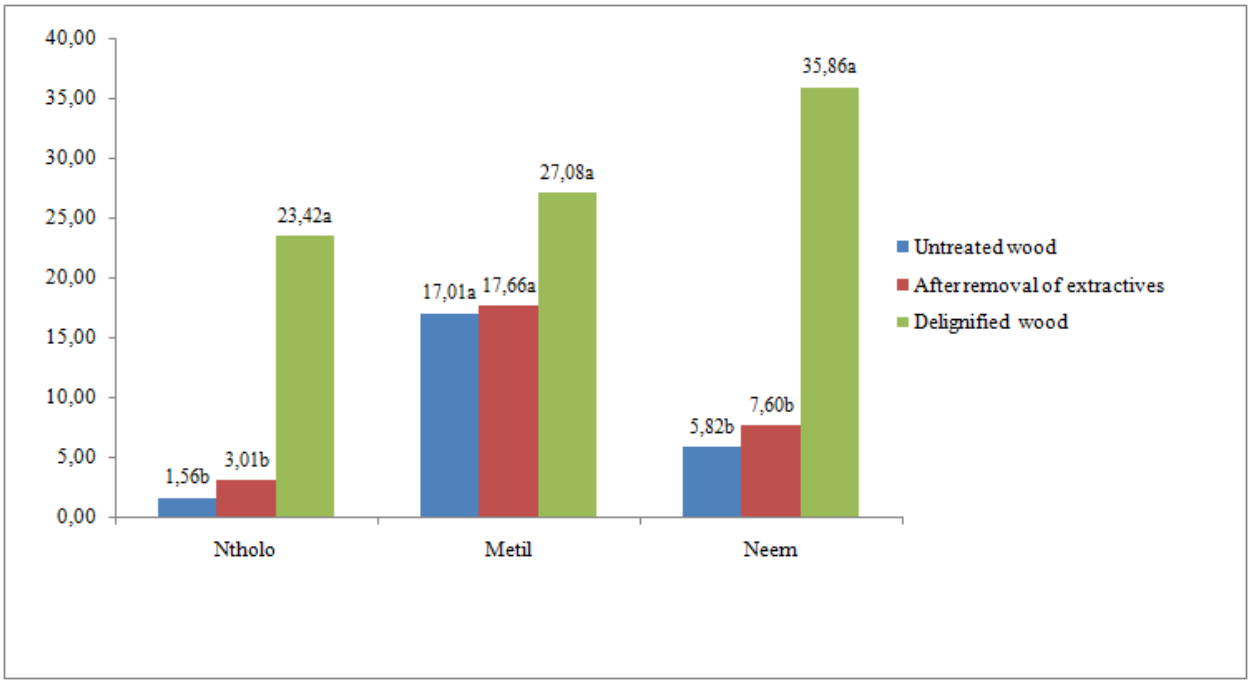


Figure 36. LSD between overall mass loss means for all fungal attacks per treatment

Mass loss was low for untreated wood and after extractive removal did not show a significant difference in decay probably due to an ineffective extractive removal process. Delignified samples however, resulted in significant mass loss. The present findings are consistent with that of Pereira *et al*. (2009) who states that extractives plus the phenolic nature of lignin offers protection to wood inhibiting enzymatic activity in fungi including insects.

Least Significant Difference (LSD) between types of fungal attack (Table 5) shows that ntholo and neem have similar behavior against fungal attack. The attack of brown rot, white rot and soft are not significantly different for untreated wood and after lignin removal but the attack of brown rot and soft rot are statistically different from white rot attack after extractives removal. Untreated wood and after extractives removal of Metil showed that attack of brown rot and white rot are statistically different and soft rot attack is not statistically different from brown rot and white rot attack.

Table 5. LSD between means for all type of fungal attack per species and treatment

	Untreated wood/natural durability		
Type of fungal attack\species	Ntholo	Metil	Neem
Brown rot	1,45a	8,867b	1,648a
White rot	2,191a	27,02a	12,41a
Soft rot	1,0552a	15,13ab	3,39a
	After extractives removal		
Type of fungal attack\species	Ntholo	Metil	Neem
Brown rot	2,316b	9,074b	2,162b
White rot	4,047a	26,301a	15,69a
Soft rot	2,665b	17,6ab	4,94b
	After lignin removal		
Type of fungal attack\species	Ntholo	Metil	Neem
Brown rot	16,66a	43,03a	37,2a
White rot	34,46a	16,9a	44,8a
Soft rot	14,88a	15,51a	15,43a

CONCLUSIONS

This study was aimed at assessing the role of lignin and extractives in the overall performance of lesser known timber (ntholo, metil and neem) against fungal attack, namely, brown, white and soft rot fungi based on accelerated laboratory tests.

White rot fungi caused more decay for all wood species in all treatments of untreated, after removing extractives and delignified wood. Metil is the most perishable species followed by neem, while ntholo showed superior durability. Delignified samples of the three species suffered more decay compared to untreated and after removal of extractives. Both extractives and lignin seem to play an important role in the resistance against wood destroying fungi but the lignin was the main source of wood natural durability. In terms of extractives, ntholo showed the highest content (3.95%) followed by metil (1.11%) and neem (1%).

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