BIOINCISION OF *Gmelina arborea* Roxb. HEARTWOOD FOR PERMEABILITY IMPROVEMENT TO CHEMICAL TREATMENT

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CERTIFICATION

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DEDICATION

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ABSTRACT

Gmelina arborea is a versatile but moderately refractory timber species whose heartwood is impermeable to chemical treatment due to tyloses deposition which occlude its vessels. This makes its utilisation unsustainable for a broad range of technical applications. Bioincision is an emerging procedure capable of improving the permeability of wood to fluid treatment, but information on its suitability in improving the permeability of *Gmelina arborea* heartwood is limited. Therefore, the effect of bioincision on permeability, anatomical and mechanical properties of *Gmelina arborea* heartwood was investigated.

Five 34-year-old *Gmelina arborea* trees were harvested at the University of Ibadan plantation. A bolt of 300 cm was obtained from the base of each tree. Heartwood of each bolt was extracted and converted into standard dimensions. Wood samples (n=100) were bioincised with two white-rot fungi: *Inonotus dryophilus* (ID, 999) and *Ganoderma adspersum* (GA, CBS109416) for 9 weeks, while unincised samples (n=50) served as control. Ten samples (20 mm x 20 mm x 60 mm) each from bioincised and unincised treatments were pressure-impregnated for 90 minutes with Tanalith (5.5% concentration) for permeability tests. Thereafter, samples from bioincised and unincised (n=30) were pressure-treated with liquid dye and depth of penetration was evaluated. Ten samples (10 mm x 10 mm x 10 mm) each were obtained from bioincised treatments to assess effect of fungi on wood anatomy. Tanalith absorption (Kg/m^3) and retention (Kg/m^3) were calculated. Axial Penetration Depth (APD, mm); Axial Penetration Area (APA, %); Tangential Penetration Depth (TPD, mm); Radial Penetration Depth (RPD, mm); Lateral Penetration Area (LPA, %) were measured. Modulus of Elasticity (MOE, MPa); Modulus of Rupture (MOR, MPa); Maximum Compressive Strength (MCS//, N/mm^2); Janka Hardness (JH, N) of unincised and bioincised samples were determined using standard methods. Data obtained were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Unincised samples had least absorption (112.0±8.5) and retention (6.2±0.5), while GA incised samples had highest (135.0±16.6 and 7.4±0.9), respectively. The APD significantly varied from 7.0±1.7 (unincised samples) to 31.2±4.1 (GA incised samples), implying higher degradation of vessel tyloses by GA. The APA increased from 0.07±0.03 (unincised samples) to 3.1±1.9 (ID incised samples). The TPD ranged from 0.4±0.2 (unincised samples) to 3.0±2.0 (GA incised samples), indicating increased lateral penetration. The RPD were 0.1±0.1 and 4.8±2.2 in unincised and GA incised samples, respectively. The LPA varied significantly from 0.001±0.001 (unincised samples) to 17.0±9.4 (GA incised samples). Both fungi induced delamellation of axial parenchyma cells and degraded tyloses within the wood vessels. Fungal hyphae of GA and ID were predominantly found in vessels and ray parenchyma cells. The MOE ranged from 7305.0±298.1 (GA incised) to 7771.1±256.0 (unincised samples). The MOR varied significantly from 70.8±6.1 to 77.9±3.9 in GA incised samples and unincised samples, respectively. The MCS// were 42.2±1.8 and 43.1±1.4 in GA incised samples and unincised samples, respectively. The JH increased from 2241.0±115.5 (unincised samples) to 2593.1±208.7 (GA incised samples).

Bioincising *Gmelina arborea* heartwood with *Ganoderma adspersum* effectively dissolved occlusions within the vessels. Wood bioincision improved permeability, with negligible effects on the mechanical properties.

Keywords: *Gmelina arborea* heartwood, Bioincision, Wood anatomical and mechanical properties, *Ganoderma adspersum*, Heartwood penetration depth

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CHAPTER ONE

INTRODUCTION

1.1 Background

Wood represents one of the earth's most valuable resources. It is a lignocellulosic material which is made up of three major polymers: hemicellulose, cellulose and lignin; all of which influences its physical, chemical and mechanical properties (Rowell, 1984; Mohebby and Sanaei, 2005). As an engineering material which possesses good technological properties, it exhibits some undesirable characteristics such as dimensional instability, flammability, biodeterioration, etc; all of which limits it for certain applications (Fengel and Wegener, 1989; Mohebby and Sanaei, 2005; Venmalar and Nagaveni, 2005; Adebawo *et al.*, 2016). In terms of wood biodegradation for instance, the hemicelluloses play a major role due to their ease of accessibility, hygroscopicity, and presence of sugar residues which can serve as a recognition point for invasion by biodeteriorating agents (Adebawo *et al.*, 2016). Therefore, meeting the ever increasing demand for this highly sought-after resource requires its efficient utilization and should be accorded precedence in wood utilization.

The shelf life of wood is contingent on the environmental conditions it is being exposed to, together with its inherent wood properties (Lehringer, 2011). Its exposure to moisture, biodeteriorating agents, light and ultra-violet radiation, temperature flux, and mechanical forces generally constitute the stresses which acts on the material, against which the inherent wood properties such as its dimensional stability, natural durability, density, etc must offset (Bergman *et al.*, 2010). It is a resource of global importance, possessing a high strength-to-weight ratio, which is also considered an environmentally sustainable material due to its natural degradability (Rowell, 2005). However, its potential to swell or shrink in reaction to moisture fluctuations (Siau, 1995), depolymerization of its lignin on incessant exposure to UV-radiation from sunlight (Feist and Hon, 1984), and its vulnerability to biodeterioration in the presence of favourable conditions for wood-degrading organisms (Highley, 1999) all hinders it

for a broad range of technical applications, including the drastic reduction of its shelf-life (Lehringer, 2011). These limitations can be addressed through wood modification techniques such as physical (Homan and Jorissen, 2004; Awoyemi, 2006; Hill, 2006) or chemical procedures (Hill, 2006, Islam *et. al*., 2012; Adebawo *et al.*, 2016).

The use of chemical treatments in reducing the aforementioned unfavourable attributes exhibited by wood, in particular, requires an adequate treatment that permits sufficient penetration and an even spread of the chemical in the wood (Hill, 2006; Lehringer, 2011). However, some species of hardwoods and softwoods are refractory in nature, making them difficult to be impregnated with wood modifying substances. In softwoods, this is mostly due to aspiration of pits during heartwood formation, or during seasoning where an irreversible closure of the pit membranes by the torus occur (Flynn, 1995; Fujii *et al*., 1997; Usta and Hale, 2006; Lehringer, 2009a). On the other hand, some hardwood species usually have their conduits occluded by outgrowths from parenchyma cells known as tyloses during heartwood formation, which eventually impedes movement of fluids or gases within the wood (Siau, 1984; Panshin and DeZeeuw, 1980; Kumar and Dobriyal, 1993). In addition to this, pit aspiration and encrustation in the heartwood of hardwoods may also occur, which can contribute to a decline in the permeability of the wood (Panshin and deZeeuw, 1980; Hillis, 1987).

Gmelina arborea, a hardwood species introduced into Nigeria, intended to be a source of short fibre pulp is currently utilized as timber for structural purposes following the operational failure of the pulp and paper industries in the country. It is a non-durable species which therefore requires it to be treated with wood preservatives to ensure its efficient utilization for structural purposes. However, its heartwood is highly impermeable due to extraneous material deposition in the lumen and cell walls, encrustation of the pit membrane surfaces and the numerous tyloses which blocks the vessels, making it difficult to impregnate with wood preservative under various treatment procedures (Owoyemi and Kayode, 2008; Olajuyigbe *et al.*, 2010; Owoyemi, 2010; Adeniyi *et al.*, 2017). In some situations, there might be need for impregnation of the wood with some wood modifying substances in order to improve its wood properties, thereby ensuring its suitability for some certain applications. As such, a sufficient penetration and distribution of these treating substances are needed in the wood (Mai *et al.*, 2004; Hill, 2006). According to Lehringer *et al*. (2009b), specific penetration depths of the treating chemical needs to be achieved for different wood

modification approaches so as to obtain a homogeneous distribution, optimal and lifetime performance of the chemical in the wood. This can be realised through the use of a biotechnological approach such as bioincision.

Bioincision is a process which involves a short-term, controlled microbial decomposition in improving the permeability of wood (Lehringer *et al.*, 2009b). This may include the use of enzymes, bacteria and fungi (Durmaz *et al.*, 2015), which consequently cause some minimal damages to the wood cell wall (Schwarze *et al.*, 2006). It is a biotechnological approach to wood permeability improvement which was patented by Schwarze in the year 2008. Usually, selection of a particular biological species for permeability improvement in a refractory wood species is based on its wood decay pattern and hence, the ability of the bioincising agent to degrade the membranes hindering permeability in the wood. For instance, impermeability in silver fir (*Abies alba* Mill.) and spruce wood (*Picea abies* (L.) Karst.) is caused by excessive aspirated pits, while the bioincision of both wood species with *Trichoderma spp*. and *Physisporinus vitreus*, respectively, caused the selective breakdown of the membranes of the bordered and half-bordered pits in both wood, consequently improving their permeability (Lehringer *et al*., 2009a; Pánek *et al.*, 2013).

Much of the researches on bioincision however have focused on permeability improvement in conifers, with limited studies so far for hardwood species. A study on the mode of decay of *Inonotus dryophilus* (Berk.) Murr., a white-pocket rot fungus in living oaks (*Quercus macrocarpa* Michx. and *Quercus alba* L.), which are both hardwood species showed that the fungus induced some delignification and cell wall degradation in the tree, including the penetration of some tylosic occlusions within the trees' heartwood vessels (Otjen and Blanchette, 1982; Eriksson *et al.*, 1990). Similarly, *Ganoderma adspersum* (Schulz.) Donk. fungus was reported to degrade almost all the tyloses in the vessels and other polyphenolic deposits in other cells within the reaction zones of beech (*Fagus sylvatica*) wood within 14 weeks of exposure (Schwarze and Baum, 2000). For the fact that the degradation of the tyloses by both fungi occurred during incipient stage of decay in the oak woods and beech, it might therefore be logical to expose *Gmelina arborea* heartwood to these fungi species, which are equally anticipated to tunnel through some of the tyloses in the vessels of this wood species in order to improve its permeability. Thus, this study focuses on investigating the

potentials of improving the permeability of *Gmelina arborea* heartwood through bioincision using *Inonotus dryophilus* and *Ganoderma adspersum*.

1.2 STATEMENT OF PROBLEM

It is increasingly being realised that the rate of forest exploitation globally is highly at an alarming rate and currently unsustainable, with the net forest loss estimated to be at 7.3million per annum (Ajake and Enang, 2012). The severity is more pronounced in the tropics where a high percentage of the populace depend on the forest for their existence (Tijani, 2007; Butler, 2012). Reports from UN-REDD (2013) asserts that the Nigerian forests constitute 10% of the country's total land area, which is far below the minimum recommended level of 25% by FAO (Trade Investment Nigeria, 2013). Factors attributed to the unsustainable exploitation of the forests include increasing human population (Unanaonwi, 2015), rising wood and wood products demand consequent on the increasing imperatives for development, inefficient harvesting techniques and utilization (Gerwing *et al.*, 1996), etc. Apparently, the long gestation period of the forest necessitates efficient use of the forest resources in light of the aforementioned factors.

Wood, being the major resource obtained from the forest, concerted efforts must be geared towards its efficient utilization. It is prone to biodeterioration (Adebawo, *et al.*, 2016), dimensionally unstable (Adebawo *et al.*, 2016) and lignin breakdown during usage in structural applications (Feist and Hon, 1984). These issues, if not preemptively addressed in wood before use tend to reduce its service life. However, the successful use of certain wood modifying substances to address these anomalies in wood may be hindered in some "difficult to treat" wood species (Morrell and Morris, 2002), such as *Gmelina arborea*. In addition, this refractory nature of wood inhibits a homogenous and optimum penetration of the treating material in the wood. Thus, procedures such as bioincision may help in addressing the treatability problems of some wood species (Lehringer *et al.* 2009a). However, information on improving substance uptake and deep penetration of chemical substance in *Gmelina arborea* through bioincision with *Inonotus dryophilus* and *Ganoderma adspersum* is lacking.

The pattern of decay in wood by a fungus depends on the anatomical structure of the wood (Eriksson *et al.*, 1990). Usually, fungi spread their mycelia mainly through the

nutrient-rich xylem ray parenchyma cells and find their way into adjoining cells (Blanchette, 1991). In some cases, the wood's chemical content, or the conditions within the wood, which differs between wood species may also have an influence on how a fungus colonizes a wood species and hence, the eventual decay pattern of the fungus (Eriksson *et al.*, 1990; Schwarze and Fink, 1998; Schwarze, 2007). Thus, it is possible that the exposure of *Inonotus dryophilus* and *Ganoderma adspersum* to *Gmelina arborea* wood may alter their wood colonization pattern and type of cellular degradation as compared to the pattern of decay of the fungi reported by Otjen and Blanchette (1982) and Schwarze and Baum (2000) in living trees of *Quercus spp* and *Fagus sylvatica* wood, respectively. Thus, insufficient evidence on the pattern of decay in hardwoods generally by *Inonotus dryophilus* and *Ganoderma adspersum* still persists.

Wood acts as a substrate for fungi from which it derives its nutrition. During wood colonization by fungi, certain cell constituents are degraded, depending on the fungi type invading the wood (Schwarze, 2007). During this process, alteration in the chemical constituents of the wood occurs, which leads to loss in weight and strength loss in wood as a consequence of the breakdown of certain substances within the wood during fungal invasion (Highley, 1999; Lehringer *et al.*, 2010). However, the extent of damage can differ between wood species for a particular fungus species (Kirk and Highley, 1973; Blanchette, 1984a, 1984b). There is therefore a need to investigate the extent of physical and mechanical damage which *Inonotus dryophilus* and *Ganoderma adspersum* will impose on the wood of *Gmelina arborea* during bioincision, as information in this regard is lacking.

The cell wall of wood cells are chiefly made up of cellulose, hemicellulose and lignin, where the lignin acts as a binder of the wood cells together (Schwarze, 2007; Dashtban *et. al.*, 2010). During biological wood decay, alterations in the wood chemical composition occur (Otjen and Blanchette, 1982; Malakani *et al*., 2014). However, for a particular fungus, the quantity of wood chemical component degraded varies depending on the fungus species (Ogunsanwo and Adedeji, 2010) and the wood species (Kirk *et. al.*, 1978). Otjen and Blanchette (1982) reported a 90% reduction in lignin and a 29% in total sugar content of Oaks degraded by *Inonotus dryophilus*. For *Ganoderma adspersum*, there is relatively no information on its effect on the wood chemical composition. Thus, information on the effect of *Inonotus dryophilus* and

Ganoderma adspersum on the chemical composition of *Gmelina arborea* is unavailable.

Worldwide, billions of dollars are lost annually from termite infestation and fungal decay of wood in service (Blanchette, 2005; Oluyege, 2007). Thus, concerted efforts have been directed towards researches into wood protection over the past few decades basically due to the need for the protection of wood in service (Barnes and Murphy, 1995). *Gmelina arborea* is rated as a non-durable to moderately durable timber species (Chudnoff, 1984; Scheffer and Morrell, 1998; Adam and Krampah, 2005). However, its treatment with preservatives requires an adequate absorption and homogeneous preservative penetration within the wood. Information on the efficacy of bioincision in improving preservative absorption and consequently conferring a significant amount of decay resistance on *Gmelina arborea* heartwood wood against wood decay fungi is unavailable.

1.3 STUDY OBJECTIVES

The focal point of this study is to improve the permeability of *Gmelina arborea* heartwood through bioincision using *Inonotus dryophilus* and *Ganoderma adspersum* with a view to making the wood species amenable to chemical impregnation. The specific objectives are to:

1. determine the effect of bioincision on preservative uptake and retention, penetration depth and penetration area of chemical in *Gmelina arborea* wood samples.
2. characterize the activities of *Inonotus dryophilus* and *Ganoderma adspersum* on the anatomical structure of *Gmelina arborea* wood samples.
3. assess the effect of bioincision on weight loss, colour and selected mechanical properties of *Gmelina arborea* wood samples.
4. investigate the effect of bioincision on the alterations in the chemical components of *Gmelina arborea* wood samples through Fourier Transform Infra-red Spectroscopy.
5. evaluate the efficacy of bioincised *Gmelina arborea* wood treated with Tanalith preservative on resistance to white and brown rot fungi attack.

1.4 JUSTIFICATION

In the wake of the increasing rate of deforestation globally, it becomes necessary to develop a sustainable policy for the utilization of the forest. Wood is recognisably the most important forest resource. Its versatility for different uses such as for energy production, pulp and paper, structural materials, etc, makes it in high demand. However, the rate of its exploitation has exceeded its rate of replenishment in the forest due to its long gestation period. Woody biomass constitutes the major source of renewable energy and thus, inducing shortages in wood availability with an attendant increase in prices of raw materials for wood products (Lehringer, 2011). In light of this, approaches to increase the shelf life of wood and wood products become crucial to a sustainable forest management.

Treatment of wood with preservatives or surface modification materials in order to improve wood property for different application has evolved over the years. However, a major challenge confronting the wood industry lies in the fact that not all wood species are readily receptive to these wood property enhancing substances. As such, several procedures have been in use to overcome this challenge. Nonetheless, it is important that not only should wood be permeable to treatments, but the absorption and homogeneity of the impregnated substance goes a long way in determining the optimal performance of the chemical in the wood (Lehringer *et al.*, 2009b). Certain species of hardwoods such as *Gmelina arborea* have low permeability due to the abundant tyloses which plug the vessels in the wood. These vessel occlusions not only reduce permeability in the wood, but also affect the treatment chemical's distribution within the wood. Rupturing or degradation of the tyloses will therefore enhance permeability in the wood. Owoyemi and Kayode (2008) examined the effect of mechanical incision on *Gmelina arborea* wood and detected a significant increase in chemical absorption in the incised wood. However, the impact of mechanical incision on the aesthetic quality and mechanical strength of wood (Morrell and Winandy, 1987; Morrell *et al.*, 1998), coupled with the impossibility of achieving a uniform and deep penetration of the treating chemical within the wood makes it a less attractive procedure in improving wood permeability. Bioincision is an evolving technological friendly procedure utilized in improving permeability of refractory wood species, capable of inducing a homogenous reception and enhancing substantial chemical absorption in impermeable wood species (Lehringer *et al.*, 2009a; Pánek *et al.*, 2013).

Much focus has centred on the bioincision of coniferous species over the years. The target of such studies however have been to degrade the torus membrane which irreversibly aspirates the cross-field pits of tracheids in softwoods. During the bioincision process, other cellular structures are degraded, which vary for different fungus and wood species (Schwarze, 2007; Lehringer *et al.*, 2010). Studies on the characterization of wood degraded by *Inonotus dryophilus* and *Ganoderma adspersum* have only been conducted for oak trees and beech wood, respectively. Therefore, the characterization of the decay pattern of *Inonotus dryophilus* and *Ganoderma adspersum* in *Gmelina arborea* wood will provide adequate information to researchers on the potentials of further utilizing this fungus as a bioincision agent in refractory hardwood species.

It is axiomatic to say that the effect of fungi on wood negatively affects its physical, chemical and mechanical properties as wood materials are removed from the wood during fungi invasion. The extent of degradation of a wood species by fungi is a function of the fungi species, the wood conditions, as well as the inherent wood properties (Schwarze, 2007; Ogunsanwo and Adedeji, 2010). However, the use of fungi in the wood industry in improving wood properties such as aesthetic property (Robinson *et al.,* 2007), acoustic property (Schwarze *et al.*, 2008) and permeability of wood (Lehringer *et al.*, 2009a; Pánek *et al.*, 2013) have all been recent advancement in the wood industry globally. Hence, for a successful utilization of bioincision in enhancing the permeability of refractory species of wood, adequate information is required on the optimal period of wood incubation with the fungus at which a significant improvement in permeability can be achieved, with a minimal or negligible adverse effect on the wood's technical properties.

Wood permeability to fluids and gases generally plays a vital role in quite a number of technical processes like wood drying, pulping process, or in the treatment of wood with preservatives (Hansmann *et al.*, 2002). When considering preservative treatment, the efficacy of a preservative treatment is not only determined by its toxicity, but it also strongly depends on the penetration and homogeneous distribution of the preservative within the wood (FAO, 1986; Olajuyigbe *et al.*, 2010). To enhance the absorption and uniform distribution of preservatives within wood, bioincision is regarded as a promising approach (Lehringer *et al.*, 2009a). Thus, a positive result of bioincision in improving the resistance of treated hardwood like *Gmelina arborea*

against attack by biodeteriorating agents will further consolidate the efficacy of bioincision as a veritable procedure for significantly improving permeability in both refractory hardwood and softwood species.

1.5 SCOPE OF STUDY

This study covered aspects such as the bioincision of the heartwood portion of *Gmelina arborea* with *Inonotus dryophilus* and *Ganoderma adspersum* over four time periods (3, 5, 7 and 9 weeks), the determination of chemical uptake, retention, chemical penetration depth and penetration area of the bioincised wood, determination of weight loss, colour changes, qualitative chemical alterations and mechanical properties of the bioincised wood, examination of the decay pattern of both fungi species (*Inonotus dryophilus* and *Ganoderma adspersum*) on the heartwood of *Gmelina arborea* and the resistance of tanalith-treated bioincised *G. arborea* heartwood to decay by *Trametes versicolor* and *Coniophora puteana* fungi.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wood Structure

Wood is extremely heterogeneous, and the variability which exists in its structure and chemical composition often reflects in its physical properties such as capillary behaviour, permeability, and thermal conductivity (Siau, 1984). The angiosperms or hardwoods have a complex structure and can be distinguished from one another by mere visual assessment. Hardwood species are found in both the temperate and tropical regions, while the conifers predominantly exist in the temperate regions. Due to the greater structural complexity of hardwoods than softwoods, they exhibit high range of variability in their permeability and capillary behaviour. In both the angiosperms and gymnosperms, both the earlywood and latewood can be found. The earlywood usually has a lower density than the latewood. In dried softwoods however, the earlywood usually exhibit lower permeability than the latewood. On the contrary, the earlywood of hardwoods are more permeable than the latewood owing to the larger vessels in the earlywood (Siau, 1984).

The cellular composition of hardwoods and softwoods is shown in Plate 2.1. The wood structure of softwoods is relatively homogenous in comparison to that of angiosperms. The xylem is composed of tracheids and parenchyma cells (axial and ray). In addition to the parenchyma cells, resin canals (wood cavities lined with an epithelium parenchyma) can also be found in most conifers (Desch and Dinwoodie, 1996). The tracheids are responsible for water conduction and provision of mechanical support in the wood i.e they perform a dual function in softwoods. Hardwoods, as earlier discussed, are more heterogeneous and the cells exhibit functional differentiation. The fibres are responsible for the provision of mechanical support while the vessels assist in the conduction of water and mineral salts. The parenchyma cells, on the other hand, assist in the storage, conversion and transport of nutrients. The fibers in hardwood and the tracheids in softwood constitute the greatest part of the cell wall and control most of the chemical and physical properties displayed by wood (Hon, 1996).

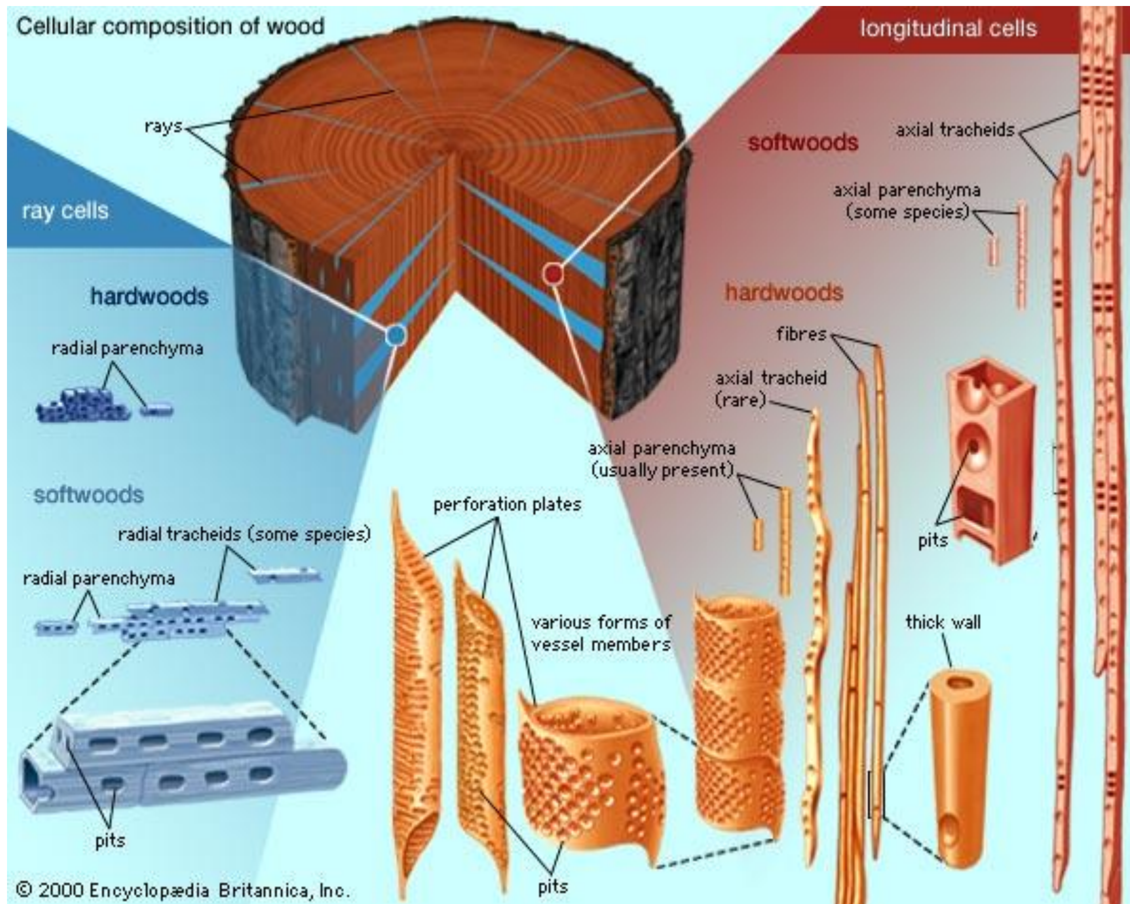


Plate 2.1: Cell types present in hardwoods and softwoods (Encyclopaedia Britannica, 2000).

2.2 Structure of the Plant cell wall

All plants possess a cell wall; the exterior structure of all plant cells which contributes to more than average of the plants' total dry weight (Kubicek, 2013). The cell wall functions by providing the plant its rigidity and strength to endure mechanical stress, while also contributing to the maintenance of the plants' shape (Ibid.). Raven *et al.* (2013) described it as a dynamic and metabolically active structure which is pivotal in the growth, differentiation, communication among and between cells and acts as a pressure vessel that checks the excessive expansion of cells during water intake. The plant cell wall is chiefly made up of three different layers which are the middle lamella, the secondary cell wall and the primary cell wall (Plate 2.2). The latter is a thin and extensible layer produced by growing cells, consists of cellulose, hemicelluloses and pectin (Kubicek, 2013; Chen, 2014). An aggregation of the cellulose gives the microfibrils which are bonded covalently to the hemicellulosic chains to form a hemicellulose-cellulose network that are completely implanted in a pectin matrix (Kubicek, 2013). The secondary cell wall is a thick layer formed to the inside of the primary cell wall immediately after cell growth ceases and it mainly consists of cellulose, hemicelluloses (mainly xylans) and lignin (Kubicek, 2013; Chen, 2014). The lignin component is an encrusting substance which plugs the spaces between the cellulose, pectin components and hemicelluloses of the cell wall and due to its hydrophobic nature, it is responsible for the driving out of water, thereby strengthening the cell wall (Kubicek, 2013). The middle lamella, however, represents the outermost plant cell wall layer, and it is responsible for the binding of the plant cells together (Kubicek, 2013). It is a narrow zone between contiguous cells which is primarily composed of lignin and pectin (Zabel and Morrell, 1992).

In wood, the secondary cell wall consists of about 94% cellulose (Fengel and Wegener, 1989) as shown in Fig. 2.1, and its main function is the provision of high tensile strength to the cell wall. It consists of three prominent layers: S_3 (inner), S_2 (middle) and S_1 (outer) layer which differ from one another on the basis of their thickness and the arrangement of their cellulose fibrils. The S_1 layer, which is a relatively thin layer, has both right and left handed microfibril helices, while the S_2 and the S_3 layers comprise of only a single microfibrillar helix; however, these are opposite to each other (Kubicek, 2013). The S_1 and S_3 layer are relatively thin zones having the orientation of their cellulose microfibrils in a flat helical arrangement; the S_2 layer in

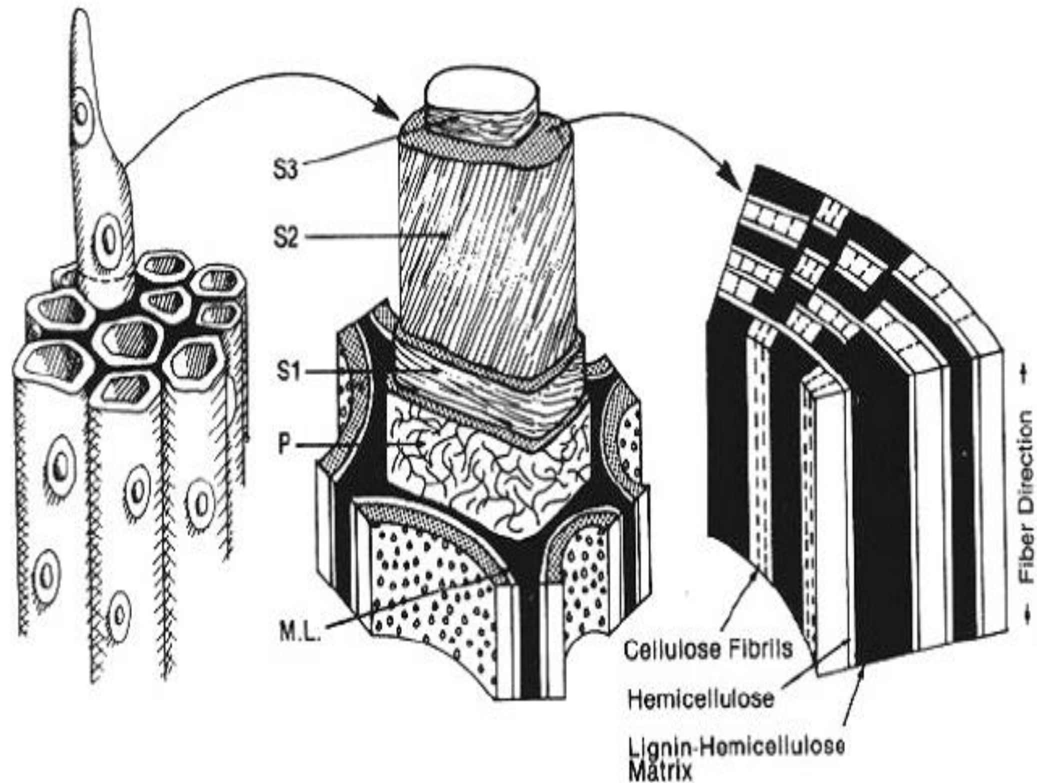


Plate 2.2: A Schematic representation of wood tracheids, secondary cell wall layers and the relationship among the wood polymeric units within the tracheids' secondary wall. ML – middle lamella; P- primary wall; S_1-S_3, secondary wall layers; Adapted from Kirk and Cullen (1998).

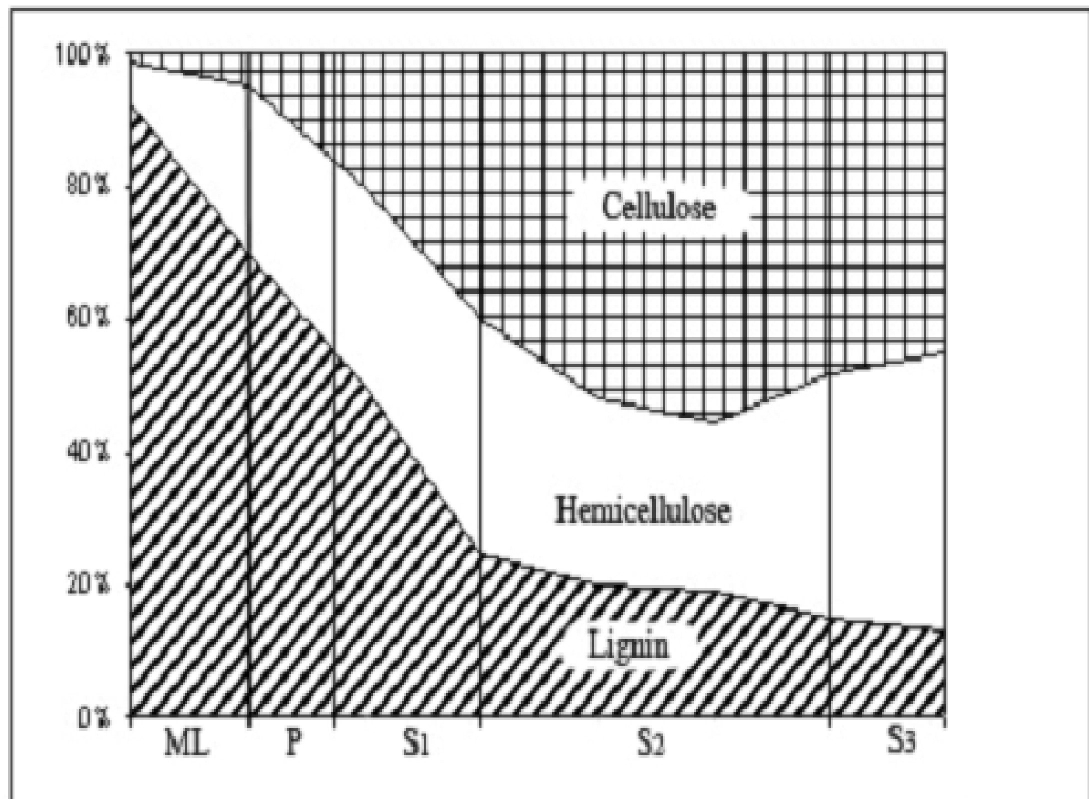


Fig. 2.1: An illustration of the major cell wall constituents' distribution within the several wood cell wall layers (Fengel and Wegener, 1989).

contrast (which is the thickest and constitutes the bulk of the cell wall), has its microfibrils organised in a steep helix, with a near parallel orientation to the axial axis of the cell (Zabel and Morrell, 1992). The secondary layer becomes lignified during lignification, and so are the middle lamella and the primary wall (Eriksson *et al.*, 1990). Within the secondary layer, however, the S_3 layer has the least degree of lignification (Silvester, 1967). This whole organisation allows for the cellulose microfibrils to become implanted within the lignin, which can be likened to steel rods embedded within concrete (Kubicek, 2013). The cell wall chemical composition and the cellulose microfibrils alignment vary significantly within and between species (Witec, 2007).

Hardwood and softwood fibres have closed ends, but they possess a special wall structure which facilitates inter-fibre movement of the tree's sap, movement of the sap between fibres and vessels, and from fibres to the ray cells (Parham and Gray, 1984). These structures line the cell walls of individual cells and are unthickened areas in the secondary walls, technically referred to as the pits (Zabel and Morrell, 1992). They function as a medium for lateral water and various solutes conduction between contiguous cells (Zabel and Morrell, 1992).

The secondary cell wall consists of two types of pits: the simple pit and the bordered pit. Tracheids and vessels of softwoods and hardwoods, respectively possess bordered pits. In this type of pits, the membrane is made up of strands of cellulose known as margo and a centrally thickened region termed a torus (Siau, 1984; Zabel and Morrell, 1992), however, that of the vessels lack the torus (Siau, 1984). The torus is an impermeable structure to fluids, which has been demonstrated by established positive relationship between pit aspiration and low wood permeability (Kollman and Cote, 1968; Siau, 1984). The spaces between the margo membrane are sufficiently large to allow for liquid passage and the movement of particles (up to 1 µm) between adjoining cells (Siau, 1984; Zabel and Morrell, 1992). Extractive deposition during heartwood deposition may often incrust the pits. Each adjoining cell in the pit pair has its secondary cell wall forming a dome-shaped edge or arch around the pit aperture. This feature, however, is absent in simple pits (Zabel and Morrell, 1992; Chen, 2014). After cell death, moisture content change, or alterations in air pressure, the torus membrane may displace against the pit aperture, leading to its blockage; often termed pit aspiration (Zabel and Morrell, 1992; Chen, 2014). Simple pits link contiguous ray

parenchyma cells or are found in fibres; bordered pits acts as a link between adjacent vessels or longitudinal tracheids; while pits which connect parenchyma and vessels or tracheid cells are referred to as half-bordered pits (Kollman and Cote, 1968; Desch and Dinwoodie, 1981; Zabel and Morrell, 1992).

2.3 Chemical components of wood

Defining precisely the wood chemical composition for a particular tree species or for an individual tree is rather difficult. This is because variation exists in the chemical composition of the tree parts (branch, stem, or root), the type of wood (i.e. tension, compression, or normal), climate, geographic location, and the edaphic conditions (Petterson, 1984). There are principally two types of wood chemical components: carbohydrates (65 - 76 %) and lignin (18 - 35 %), both of which are polymeric materials of structural complexity. Cellulose and hemicelluloses both make up the carbohydrates in wood. In addition, little amount of extraneous substances are also present, which can be in the form of proteins (0.03 - 0.1%), organic extractives and inorganic minerals (ash), usually between 4 – 10% (Zabel and Morrell, 1992). Elementally, wood is composed of hydrogen (6%), oxygen (44%), carbon (50%), and several metal ions in trace amounts (Pettersen, 1984; Chen, 2014). These chemical compositions in wood generally vary between softwoods and hardwoods (Sjöström, 1993).

2.3.1 Cellulose

Cellulose constitutes the chief chemical component of the fibre wall, which contributes 40-50% by proportion of the wood dry weight and it is predominantly abundant in the secondary cell walls (Sjöström and Westermark, 1999; Kubicek, 2013). It comprises of linear chains of 1,4-β-ᴅ glucopyranose residues, linked by 1,4-glycosydic bonds, and can attain degrees of polymerization (DP) of 2,000-10,000 and 2,000-6,000 and in both secondary and primary walls, respectively (Kollman and Cote, 1968; Kubicek, 2013). Three exposed hydroxyl groups per anhydroglucose unit at the C2, C3, and C6 positions are present on the surfaces of the cellulose molecules, and are responsible for the structural properties exhibited by the cell wall and the wood's physical and chemical properties (Zabel and Morrell, 1992). Cellulose has a strong propensity for intra- and intermolecular hydrogen bonding to form micelles that aggregate to form microfibrils, which may either be less-ordered (amorphous region) or highly-ordered

(crystalline region) according to Sjöström and Westermark (1999). These microfibrils further aggregate to form fibrils and several fibrils combine to form the cellulose fibres (Sjöström and Westermark, 1999; Kubicek, 2013) as shown in Plate 2.3. Due to the presence of hydroxyl groups on the unbranched celluose chain, cellulose has the tendency form strong hydrogen bonds which gives the linear chain its high stiffness, and is the key reason for the rigidity of the plant cell wall (Klemm *et al*., 2005), and it often promotes its aggregation into a crystalline structure (Klemn *et al*., 2005; Chen, 2014). The relatively unreactive nature and thermal stability of the cellulose fraction of wood is due to its high crystalline nature. However, cellulose may swell when in contact with some solvents, which leads to the formation of hydrogen bonds between the molecules of the solvent present within the cellulose chains. This swelling characteristic of the cellulose molecule is highly temperature dependent. The surface hydroxyl groups on the microfibrils enable water to bind to the cellulose molecule by hydrogen bonds, but penetration of water into the crystalline cellulose is impossible because of the stability created by the hydrogen bonds within the hygroscopic hydroxyl groups of the crystalline region (Zimmermann *et al.*, 2004). It is very challenging to isolate it in its pure form from wood due to its intimate association with hemicelluloses and lignin (Petterson, 1984).

2.3.2 Hemicelluloses

These are complex, non-cellulotic heteropolysaccharides which consists of D-galactose, D-glucose, D-xylose, D-mannose, L-arabinose, glucuronic acid and 4-O-methyl-glucuronic acid (Machado *et al.*, 2016). Their degree of polymerization usually ranges between 200-300. Contrary to cellulose, they are amorphous, branched chain polymers which have frequent substitutions with acetic acid, and they closely interact with the cellulose molecule, thereby providing the aggregated molecule with flexibility and stability (Machado *et al.*, 2016). Some hemicelluloses may occur in an unusually high amount when a tree is under stress; e.g. galactose is abnormally lesser in normal wood than in compression wood (Timell, 1982). Based on the principal sugar unit present, hemicelluloses can be termed as xylans, galactans or mannans. According to Bon and Ferrara (2007), the C_5 and C_6 sugars of the hemicelluloses chain, which are bonded by 1-3, 1-6 and 1-4 glycosidic linkages, frequently get acetylated to form a loose, water-loving structure which functions as a binder between lignin and cellulose. Approximately 20-30% hemicelluloses are present in both hardwoods and softwoods.

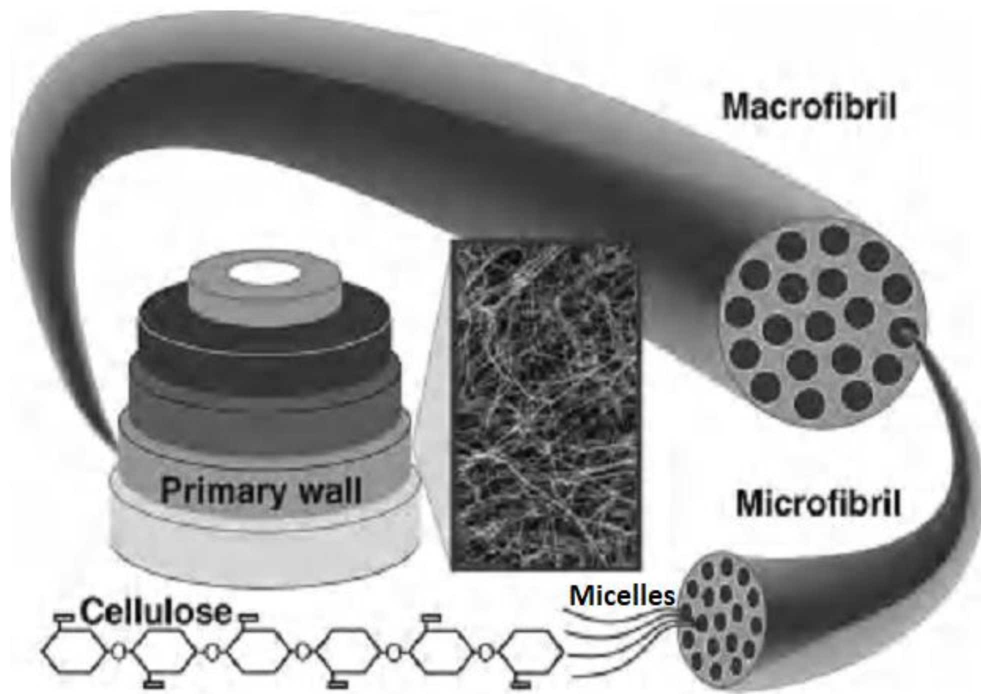


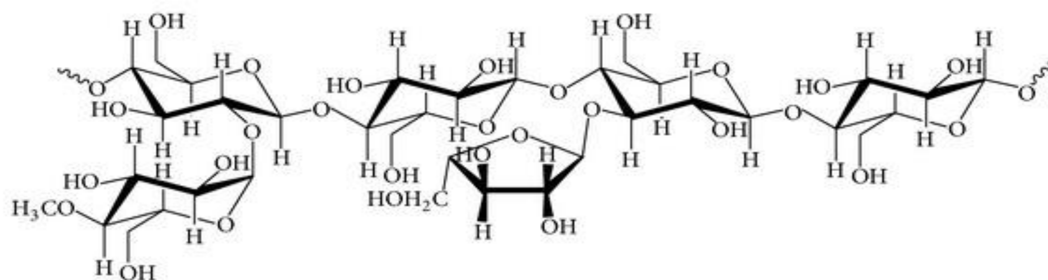
Plate 2.3: Generation of cellulose micro- and macrofibrils (Kubicek, 2013)

In softwoods, the predominant hemicelluloses in their cell walls are galactomannans and galactoglucomannans (12-15%), while in hardwoods, the principal hemicelluloses are the xylans which contain a huge quantities of D-glucuronic acid bonded to the spine and are referred to as glucuronoxylans (Kubicek, 2013). Below in Fig. 2.2 is the chemical structure of the most existing hemicellulose biopolymer. The branched hemicelluloses polymers consist of acidic and/ or neutral groups which render them poorly crystalline, and as such, they appear more like a gel rather than an orderly arranged structure (Watanabe *et al.*, 1989). Thus, the heterogeneous and non-linear nature of the hemicelluloses chain renders it amorphous, allowing for easy accessibility to the hydroxyl groups and other reactive groups within the chain. Unlike cellulose and lignin, they are relatively hydrophilic and easily degraded by heat (Chen, 2014; Adebawo *et al.*, 2016).

2.3.3 Lignin

Lignin is a complex, long-chain, amorphous, aromatic heteropolymer, derived primarily by free radical generation and subsequent oxidative linkage of three major hydroxycinnamyl monolignols or alcohols: sinapyl alcohol, *p*-coumaryl alcohol and coniferyl alcohol (Fig. 2.3), which makes it highly resistant to degradation (Kubicek, 2013; Chen, 2014; Shahzadi *et al.*, 2014; Machado *et al.*, 2016). The coniferyl alcohol and sinapyl alcohol are the major structural backbone of hardwood lignin; p-coumaryl alcohol is a minor originator of both hardwood and softwood lignin, while coniferyl alcohol is the dominant precursor in softwood lignin (Petterson, 1984; Maity, 2015). The equivalent phenylpropanoid units within the lignin polymer are represented as syringyl (S), guaiacyl (G), and *p*-hydrophenyl (H), units, respectively, which are based on the aromatic rings substitution by methoxyl groups (Kubicek, 2013). Thus, the syringyl, guaiacyl and *p*-hydrophenyl units derive their origin from the sinapyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol, respectively. These monomeric units are randomly bonded together by several ether and C-C bonds within the lignin structure (Ralph *et al*., 2004). Lignin is principally deposited in the cell walls which have undergone secondary thickening, providing them with rigidity, strength, and their impervious nature (Kubicek, 2013). They also function as a protective covering for the polysaccharides of the cell wall (Shahzadi *et al.*, 2014). Lignin in gymnosperms is almost exclusively made up of guaiacyl monomers while the angiosperms have lignin consisting of nearly equal amounts of syringyl and guaiacyl (Whetten and Seredoff,

(i) Xylan



(ii) Glucomannan

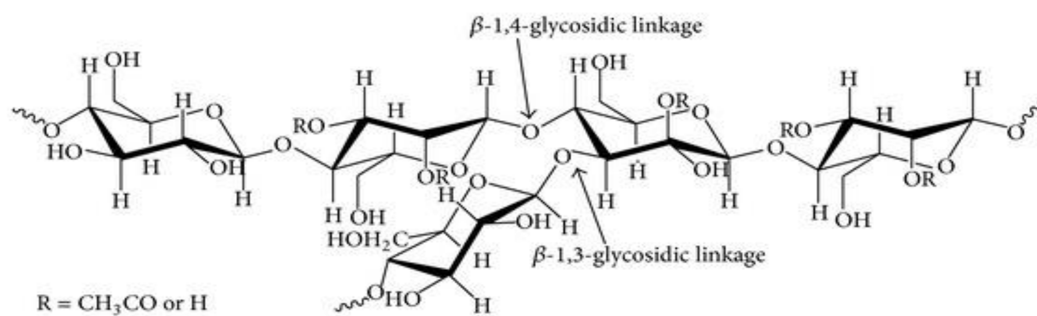


Fig. 2.2: Chemical structure of most existing hemicelluloses biopolymers (Lee *et al.*, 2014)

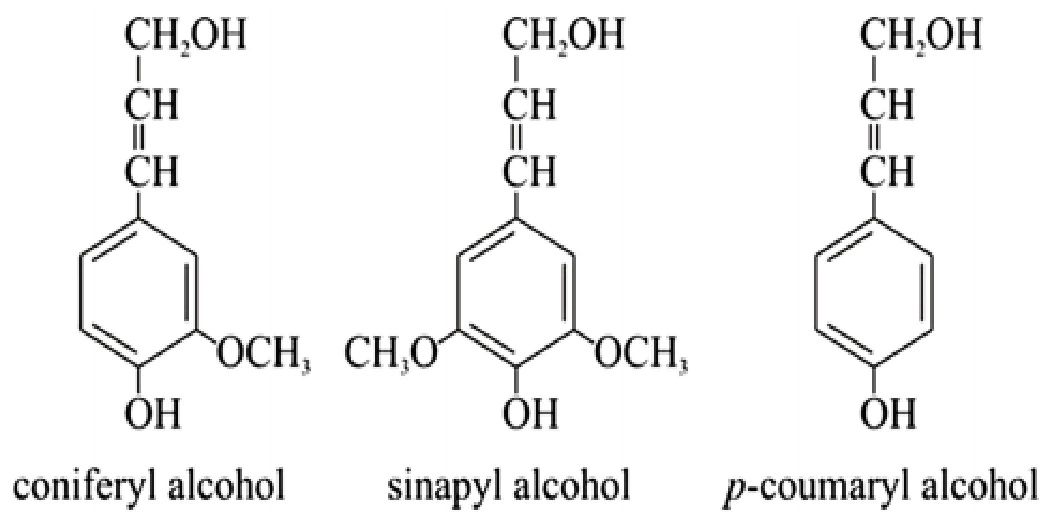


Fig. 2.3: The building blocks/units of lignin (Shahzadi *et al.*, 2014)

1995). Information on the lignin composition of different wood cell types helps in explaining their modes of degradation and also enables the prediction of the susceptibility or resistance of the xylem of a specific host to a certain type of decay (Schwarze, 2007).

Lignin distribution in the wood indicates that the middle lamella contains above 50% lignin (mass ratio), while it is about 20% in the secondary cell wall. Due to the higher bulk of the secondary wall layer in the wood in comparison to the middle lamella, the majority of the lignin concentration resides in the secondary cell wall (Chen, 2014). However, the lignin density of the cell corner of the middle lamella is far greater than that present within the middle lamella, and about four times that of the secondary cell wall (Chen, 2014).

2.3.4 Extractives

These are heterogeneous, low molecular weight group of substances that constitute the non-structural component of wood, of which non-polar and polar solvents can be used to extract them from wood. In wood species within the tropics, they may make up to 20% of the wood's dry weight, while in temperate species, it may range between 4-10%. Telmo and Lousada (2011) described them to include various organic compounds such as proteins, alkaloids, waxes, simple and complex phenolics, mucilages, pectins, simple sugars, glycosides, starches, terpenes, essential oils and saponins. Most of these compounds mainly function as part of the tree's defense mechanism against attack by microbes and energy reserve in trees, while also serving as intermediates in tree metabolism (Rowell, 1984).

2.4 Wood properties

2.4.1 Physical properties

Wood is versatile as demonstrated by its utilization for several products. This is possible due to a wide spectrum of desirable physical properties it exhibits; a combination of some of these properties which often determines its end use (Forest Products Laboratory, 1999). Some of these physical properties are discussed below:

2.4.1.1 Density and Specific gravity

Specific gravity is defined as the ratio of the mass of oven-dried wood to the mass of displaced water by the wood at a specific moisture content (Siau, 1984). Specific gravity has no unit because it is a ratio of masses, it does not have a unit. Numerically, it is equal to the ratio of the mass of oven-dried wood to its moist volume. Therefore, when the bound-water within the wood increases, it will result in an increased wood volume, thus decreasing the wood specific gravity. Wood density, on the other hand, is the mass of the wood per unit volume at a specific moisture content. Increase in wood moisture content usually increases its mass at a rate greater than its volume, resulting in an increased wood density. This increase is much more pronounced above Fibre Saturation Point (FSP) because no swelling occurs beyond this point, while wood mass continues to increase. However, specific gravity and density are equal if measurements are taken at oven-dry states (Siau, 1984). Specific gravity or wood density is a perfect measure of the quantity of wood substance in a wood piece; hence, it is an excellent index for predicting wood strength properties, provided it is a clear wood specimen, straight grained and defect-free (Green *et al.*, 2003). Specific gravity or wood density constitutes one of the most vital wood properties due to its close relationship with most wood properties (Panshin and deZeeuw, 1980; Bowyer *et al.*, 2007).

2.4.1.2 Moisture content of wood

The wood moisture content is defined as the ratio of the weight of the wood moisture to that of the wood oven-dry weight, usually expressed in percentage (Forest Products Laboratory, 1999). Moisture content in wood at times exceeds 100% because it is a measure expressed as the wood oven-dry weight. It is a crucial factor in wood processing because of its influence on wood durability, wood performance, physical and strength properties of wood in service (Kollman and Cote, 1968). In softwood species, the heartwood's moisture content is usually lesser than the sapwood, while in hardwoods, the moisture content difference between these wood zones is contingent on the species (Forest Products Laboratory, 1999).

Moisture in wood can be found as both hygroscopic or bound water, and capillary or free water (Siau, 1984). Bound water exists within the wood cell wall, and it is bounded to the hemicelluloses and cellulose hydroxyl groups of the cell wall, and to the hydroxyl groups of the lignin to a lesser extent, by hydrogen bonds (Siau, 1984).

The quantity of bound water in wood is dictated by the available sorption sites in the wood and the carrying capacity of moisture of each sorption site (Siau, 1984). Free water can only be found in wood when all the sorption sites in the cell wall are completely hydrated; this point is referred to as the Fibre Saturation Point (FSP). The wood moisture content below FSP is a function of the prevailing relative humidity and temperature of the environment. This moisture content in wood at a particular relative humidity and temperature is known as Equilibrium Moisture Content (EMC). At this point, the wood is neither losing nor gaining moisture due to the wood attaining an equilibrium state (Siau, 1984; Forest Products Laboratory, 1999). Several factors such as the temperature, relative humidity, drying history of wood, extractive content, type of applied force, and the wood species affect the EMC of wood (Siau, 1984).

2.4.1.3 Shrinkage and swelling in wood

Wood is a hygroscopic material which responds to humidity fluctuations by either swelling or shrinking. In humid air, wood gains moisture this causes it to swell; when the air is dry, moisture is lost and therefore shrinks (Desch and Dinwoodie, 1981; Forest Products Laboratory, 1999). Shrinkage occurs in wood when the moisture drops below the FSP. Above FSP, wood is dimensionally stable. The anisotropic behaviour of wood causes it to exhibit unequal shrinkage along the various wood planes. This has been attributed to the geometric disposition of the wood cells along the three principal wood directions (Panshin and deZeeuw, 1980). Normal wood usually exhibits the least shrinkage (0.1-0.3%) in the longitudinal plane, although this may be significantly higher in tension, juvenile, compression woods. Tangential shrinkage is usually twice that of the radial shrinkage for most woods (Desch and Dinwoodie, 1981; Siau, 1984). Reasons advanced for the lower radial shrinkage in comparison to the tangential shrinkage, according to Panshin and deZeeuw (1980) include: the presence of high-density latewood and low-density earlywood bands and; the ray restraining influence of the ray cells. Suitability of wood for different structural end uses is highly synonymous with wood having a low Tangential/Radial (T/R) shrinkage ratio (Panshin and deZeeuw, 1980).

2.4.2 Mechanical properties of wood

The source of wood’s strength is from the wood fibre (Winandy and Rowell, 1984). Panshin and deZeeuw (1980) described wood mechanical properties as the behaviour it

expresses under applied stresses. Wood strength refers to its ability to withstand external forces or load that can distort its shape or size (Haygreen and Bowyer, 1996). This change in shape or size of wood is referred to as strain or deformation, while the distributed force within the wood caused by the application of an external force which acts to deform the wood is known as stress (Silvester, 1967). The resistance of wood to different external forces involves various mechanical properties which are measures used in determining the aptness of different timber species for various purposes (Illston *et al.,* 1987). If structural applications of wood are the main subject of interest, the most important attributes to be considered in determining their suitability are their mechanical properties (Haygreen and Bowyer, 1996). A structural application refers to any utilisation for which one of the crucial criteria for material selection is strength.

Like any other construction material, timber has the ability to resist external forces (Farmer, 1972). Farmer (1972) further stated that it is important to consider several other factors when selecting a particular species for a specific purpose, as there are some instances where the choice of a species is less dependent on its mechanical properties. However, for an efficient utilization of timber, a basic understanding of the strength properties is important.

2.4.2.1 Modulus of Elasticity (MOE)

Modulus of elasticity of a material is its capability to retain its previous shape and size after undergoing some applied stress (Panshin and deZeeuw, 1980). The elastic property of a material implies that it completely recovers from deformation induced by minimal stresses after the withdrawal of the loads (Forest Products Laboratory, 2010). However, a plastic deformation or failure occurs at higher loading of stress levels. MOE is the ratio between the stress applied along a particular axis to the strain induced along the same axis. Based on this, three moduli of elasticity are identified which are E_L, E_T, and E_R, denoting MOE on the longitudinal, tangential and radial planes, respectively (Forest Products Laboratory, 2010). The most extensively derived constant from test data is EL (Asafu-Adjaye, 2012), however, others have been mostly developed from material relationships or from relationships between the material behaviour and density using regression equations (Forest Products Laboratory, 2010). MOE is an important property in determining a beam's deflection under a load. The stiffness of a long timber strut or column is very critical in the determination of its

strength (Timings, 1991). As documented by Shrivastava (1997), MOE is a measure of a material's stiffness i.e. the greater the stiffness of the material, the lesser is the deflection observed and hence, the higher the MOE, though the property (MOE) is only applicable within the limit of proportionality.

2.4.2.2 Modulus of Rupture (MOR)

This is a strength property of wood which evaluates its bending strength. It is a measure of the equivalent of stress in the extreme fibres of the wood material at the point of collapse (Desch and Dinwoodie, 1981). According to Panshin and deZeeuw (1996), it is the magnitude of load which produces a failure in bending stresses in the specimen. Shrivastava (1997) describes it as a measure which expresses the maximum tensile or compressive stress in the wood fibres at the point of collapse. It is an index obtained from the static bending property of a material in which the equivalent fibre stress or maximum bending strength at maximum load is measured. MOR is an important strength property in wood members subjected to transverse stresses, which is applicable in the loading of roof trusses (Timings, 1991).

2.4.2.3 Compression Strength

Compression strength, also referred to as maximum crushing strength of wood, is usually considered in directions perpendicular and parallel to the grain (Shrivastava, 1997). When an applied force tends to decrease the length of a material, such material is under compression and the stress is called compressive stress. Compression parallel to grain often results into the buckling of wood during loading such that the wood is subjected to a bending stress rather than to a compressive stress (Desch and Dinwoodie, 1981). The wood resistance when loaded perpendicular to grain is practically important in some building constructions and practical application of this can be found in rollers, railway sleepers, bearing blocks, wedges, and bolted timbers (Kollman and Cote, 1968; Desch and Dinwoodie, 1981). Compressive strength perpendicular to grain of wood is often less applicable in structural applications because it only leads to the densification of the wood under the influence of the compressive force. Hardness and transverse shear are very similar to compression across the grain. This kind of stress in wood comes into play in two ways, namely: (i) with the load acting over the entire area of the specimen, and (ii) with the load concentrated over a portion of the area. The latter condition is commonly met in

practice, and an example is where a post rests on a horizontal sill, or a rail rests on a cross-tie. However, the true resistance of the grain to crushing stress is obtained from the former condition (Samuel, 2004).

Timings (1991) described compressive strength parallel to grain as a measure of the ability of timber to withstand loads applied on the end grain. When wood is subjected to a compressive stress parallel to grain, collapse in the wood is initiated as the cell wall microfibrils begin to fold (Wakefield, 1957). According to Forest Products Laboratory (2010), large distortions result from the internal crushing of wood's complex cellular structure. This strength property is applicable in timbers used as columns, props, and chair legs; hence, high compressive strength parallel to grain is needed in such applications.

The compressive strength of wood is largely provided by its lignin content as the lignin provides the wood with its stiffness by cementing the wood cells together into a coherent mass (Silvester, 1967). The compressive strength of timber perpendicular to the grain is about 15-20 % of its axial compressive strength and it is closely related to the wood hardness (Silvester, 1967).

2.4.2.4 Impact Bending

Impact bending, also known as the maximum hammer drop, is the ability of wood to resist suddenly applied load. According to Desch and Dinwoodie (1981), it is one of the three criteria employed in the laboratory for evaluating toughness. Nonetheless, impact bending strength is the most widely used indicator of the toughness of wood materials. Kollmann and Cote (1968) stated that the amount of shock which a solid body can resist is dependent on its ability to absorb energy and dissipate it before failure. Resistance of wood to impact is very important in applications such as for gymnasium and diving springboards, ladders, scaffold boards etc. (Silvester, 1967).

2.4.2.5 Tensile Strength

This is a measure of the resistance to forces which tends to pull apart the material and tries to stretch or elongate it as a result. Similar to compressive strength, it can also occur either parallel to the grain or perpendicular to the grain. Timber exhibits its greatest tensile strength when loaded parallel to the grain (Silvester, 1967). Tensile strength perpendicular to grain may come into play in building designs involving

connections between wood members. In contrast, tensile strength parallel to grain finds significance in the bottom member of wood trusses and in designs involving connection between structural members (Haygreen and Bowyer, 1996). The tensile strength of wood depends on the intrinsic strength of its fibres and tracheids, their length and their orientation within the wood (Silvester, 1967). Fibre length has been observed to be directly correlated to the angular orientation of the microfibrils with the longitudinal direction of the fibres, with longer fibres having a steeper slope of microfibrils and hence, a higher fibre strength (Silvester, 1967). However, some have reported that the higher wood tensile strength in relation to longer fibres is due to the higher degree of overlap between the fibres in comparison to smaller ones (Desch and Dinwoodie, 1981).

2.4.2.6 Hardness

This is a measure of the resistance of wood to indentation. Kollman and Cote (1968) defined it as the ability of a solid body to resist penetration by another solid body under force. It is comparatively evaluated by a force required to drive in a 11.3mm steel ball to one-half its diameter into the wood (Desch and Dinwoodie, 1981; 1996). Hardness is a strength property of wood which is important for materials used for floorings, paving blocks and bearing blocks (Desch and Dinwoodie, 1996). However, when hardness is to be considered in the selection of wood species for flooring, timbers with high hardness tend to be too slippery to provide an adequate safe walking surface and are usually noisy (Desch and Dinwoodie, 1996). Thus, selected timbers for flooring usually have a moderate hardness (Desch and Dinwoodie, 1996). This property depends on the density, toughness and cohesion between the wood elements (Silvester, 1967).

2.5 Technical challenges associated with wood utilization

2.5.1 Dimensional instability

Wood is a hygroscopic material due to the presence of free hydroxyl groups (OH) on the cell wall polymers, most especially the hemicelluloses. The hydrophilic nature of the hydroxyl groups makes it to attract moisture from the atmosphere and form hydrogen bonds. Depending on the prevailing environmental relative humidity and temperature, a dry wood will absorb moisture until it attains equilibrium moisture content (EMC) with its surroundings. At a lower constant relative humidity,

equilibrium moisture with the atmosphere will be attained by such woods through moisture loss when exposed to the atmosphere (Siau, 1984). Thus, there is a corresponding EMC for any combination of temperature and relative humidity at which there is no exchange of water between the wood and the atmosphere (Desch and Dinwoodie, 1981; Siau, 1984). When a green wood loses moisture, the cell wall volume remains the same until the fibre saturation is reached (Dinwoodie, 2000).

The hygroscopic property of wood renders it dimensionally unstable, and absorption or desorption of atmospheric water vapour occurs in order for a permanent moisture equilibrium to be established between the wood and its environment. Malutan *et al.* (2008) reported that the dimensional stability of wood was significantly influenced by an excessive increase in temperature and humidity. According to Mantanis *et al.* (1994) and Hernandez (2007), wood dimensional stability is a function of its density, species, direction of measurement, temperature, relative humidity, microfibril angle, chemical composition and amount of extractives present. Shrinkage in wood occurs when water is lost from the cell walls, and the shrinkage observed is in proportion with the moisture released below the fibre saturation point. On the other hand, swelling occurs when the wood cell wall absorbs moisture. These two phenomena are reversible processes in wood specimens that are stress-free (Bowyer *et al.*, 2007).

The wood anisotropic property causes it to exhibit differential shrinkage and swelling along the three principal planes of wood. Asides the movement which arises in wood in service, these dimensional changes in wood can lead to distortions during log processing into lumber and seasoning; the distortion or defect observed in the plank is however dependent on its location within the log. Shrinkage in the tangential plane is twice that of the radial plane, while longitudinal shrinkage is insignificant for most normal wood species (Desch and Dinwoodie, 1981; Richardson, 1993; Dinwoodie, 2000; Bowyer *et al.*, 2007; Forest Products Laboratory, 2010).

2.5.2 Wood biodeterioration

Wood degradation or deterioration should not be mistaken for wood biodeterioration (biological degradation). Wood degradation is a holistic term used to refer to the degrading impacts of both biotic factors (biodeterioration caused by fungi and insects) and abiotic factors (weathering - precipitation, ultra violet rays, wind) (Zabel and Morell, 1992). Wood degradation essentially results from the activities of degradation

agents in wood which is manifested in the form of loss of the technical function and or the reduction of the aesthetic value or appearance of wood products infested (Reinprecht, 2016).

Wood decay refers to the wood cell wall deterioration caused by biological agents, which results into the loss of its technical properties. Biological agents such as fungi, bacteria, insects and molds have the capability of causing severe degradation of wood-based materials (Desch and Dinwoodie, 1981). However, fungi and insects constitute the main biological agents responsible for major economic losses incurred from biodeterioration of wood globally (Kollmann and Cote, 1968). Their diversity, coupled with their different diet requirements make wood susceptible to degradation during service or while living. In the forest, recycling of nutrients is a natural process brought about by wood decomposition, but to wood users, decayed wood products implies an added repair or cost of replacement (Clausen, 2010). Of all wood-feeding insects, termites are responsible for the most serious damage (Richardson, 1993; Highley, 1999). They also attack growing trees, agricultural crops, rubber, leather, wool, and some other man-made materials, causing significant damages as a result (Malaka, 1996). However, on a national scale, fungi pose the most serious threat to wood (Highley, 1999).

Fungi attack wood, causing a loss in the wood's physical and mechanical properties due to the weakening or softening of the wood. The growth of fungi is dependent on several factors which are food source (cell wall components), favourable temperature (between 20-32 ^{o}C), sufficient oxygen, moisture (when present above fibre saturation point), an acidic environment (p^{H} of 2-6), and chemical growth factors such as essential elements and nitrogen compounds (Zabel and Morrell, 1992; Bolden and Greaves, 2008; Clausen, 2010; Shmulsky and Jones, 2011). However, degradation by some fungi may continue at moisture content below FSP, though all fungi activity ceases when the wood moisture content drops below 20% (Bolden and Greaves, 2008). When any of these favourable conditions is lacking, decay will be prevented. Thus, the resistance of wood against decay may be improved by either reducing the palatability of the food source or by ensuring wood is dry below 20% moisture content (Bolden and Greaves, 2008).

Decay results from the digestion of wood by fungi (Zabel and Morrell, 1992). Decay fungi are multicellular or single-celled organisms which utilize wood as their substrate source. Their spores are dispersed by wind, soil, water, animals or insects. They grow readily on humid, vulnerable surfaces, with their hyphae spreading within the wood. After inducing severe decay, there may be formation of new spore producing structures or fruiting bodies (Ashaduzzaman *et al*., 2013). These fungi use different mechanisms in degrading wood and are distinguished by how they degrade both the cellulose and hemicelluloses, by their behaviour during invasion and wood degradation, and by the enzymes involved in the degradation process (Kubicek, 2013). Based on these different mechanisms, three classifications of decay types are recognised: white rot, soft rot, and brown rot.

White rot fungi possess the potential to completely degrade wood structure because of their ability to depolymerise lignin through the cleavage of the bond between the Cα and Cβ atoms of the lignin structure (Kubicek, 2013). They are commonly associated with decay in hardwood species, resulting in a uniform whitish appearance or pockets of white rot or patches of selectively decayed areas of the wood (Kubicek, 2013). White-rot fungi are capable of uniform or selective degradation of lignin, cellulose, and hemicelluloses as they can possess both cellulolytic and lignolytic enzymes (Goodell *et al.*, 2008). For brown rot fungi, they are the most predominant in gymnospermous wood attack (Gilbertson, 1980). They remove the hemicelluloses and cellulose component of the wood cell wall but lack the ability to degrade lignin or only degrade a minute fraction of it (Kubicek, 2013). Brown rot is characterized by a brown appearance of the decayed wood, which is usually dry, crumbly and lignin-rich (Kubicek, 2013). Soft-rot fungi preferentially attack wood with low lignin content, and usually need to be in direct contact with the soil due to their high moisture requirement in contrast to the brown and white rot fungi (Kubicek, 2013). A summary of how these fungi utilize wood substrates and the resulting physical appearances of the decayed wood are presented in Table 2.1.

Usually, a decrease in strength occurs when decay fungi metabolize wood. The extent of the wood strength loss varies with the fungi type involved, wood dimension and wood species (Blanchette, 1984a; 1984b; Winandy and Morrell, 1993). However, not all wood attacking fungi induce degradation to the wood cell wall components, as some only cause wood discolouration or stain. These fungi are usually classified as

molds and sap stain fungi, which induces minimal or no wood strength loss (Kollman and Cote, 1968; Blanchette *et al.*, 1992a). This is because they mainly derive their nutrition from the parenchyma cells and not the lignin and polysaccharide components of the wood (Kollman and Cote, 1968; Zabel and Morrell, 1992). However, some literatures have reported some strength loss such as reduced resistance to impact or shock loadings for wood attacked by some stain fungi (Silvester, 1967).

2.5.2.1 White-rot fungi

They are so-called due to the bleached appearance of the wood they attack, and may become more pronounced when the decay of the wood is at an advanced stage. The bleaching may arise as a result of lignin removal, and may be accompanied by the development of black zone lines which are zones of interaction and competition among different fungi species (Eaton and Hale, 1993). They are the most frequently found wood degrading fungi in wood as they attack both hardwoods and softwoods, although they commonly attack hardwoods (Gilbertson, 1980; Kubicek, 2013). White rot fungi are capable of degrading lignin and the wood carbohydrates, which occur at relatively different rates, and this depends on the fungi species and the wood condition (Eriksson *et al.*, 1990). Their adaptation to the heterogeneously structured angiosperm woods, coupled with their ability to induce extensive degradation of all the cell wall constituents, results into multiple decay pattern by these fungi group. Thus, two different mechanistic decay patterns are recognised: selective delignification (sequential decay) and simultaneous (non-selective) delignification (Otjen and Blanchette, 1982; Zabel and Morrell, 1992; Kubicek, 2013).

Table 2.1: White rot, soft rot and brown rot fungi comparison

		White Rot	Soft Rot	Brown Rot
Appearance		Spongy, bleached; strength loss	Crumbly in dry, brownish, soft in moist environments	Brittle. Brown, powdery; strength loss
Wood type and condition	**SR**	Hardwood	Majorly hardwood	Majorly softwood
	SD	Softwood and hardwood	Wet wood, forest ecosystems	Wood in service, forest ecosystems
Cell wall polymer concerned		Lignin, hemicelluloses and cellulose	Hemicelluloses and cellulose, slightly modified lignin	Hemicelluloses and cellulose, slightly modified lignin
Anatomy	**SR**	Proceeds from the cell lumina	Degradation occurs close to hyphae commencing from the cell lumina	Degradation occurs farther away from hyphae
	SD	From the middle lamella and secondary wall		
Fungi	**SR**	*Trametes versicolor* *Phanerochaete chrysosporium* *Heterobasidium annosum*	*Paecilomyces spp.* *Chaetomium globosum* *Thielavia terrestris*	*Piptoporus betulinos* *Laetiporus sulphurous* *Gloeophyllum trabeum*
	SD	*Phlebia tremelosa, Ganoderma austral, P. pini, Pleurotus spp., Ceriporiopsis subvermisporia*	*Trichoderma reesei*	*Postia placenta* *Serpula lacrymans*

Adapted from Kubicek (2013).

SR, Selective rot; **SD**, Simultaneous delignification

The simultaneous rot or non-selective fungi are comprised of a large group of fungi species whose preference are to invade the wood of angiosperms. The reason attributed to their non-selective mode of degradation on hardwood species may be due to the extreme resilience of S_3 layer of softwood tracheids, which inhibits hyphae degradation of the cell wall from within the lumen outwards. Enzymes of brown rot and selective delignifying fungi, in contrast, are composed of low molecular weight substances which easily diffuse through the S_3 layer into the secondary wall. Compared to the wood fibres of angiosperms, the S_3 layer of conifer tracheids is more resistant to degradation. However, brown rot fungi are able to overcome this resistant barrier because of the diffusible secretions produced by their hyphae within the lumen of tracheids; but this layer constitutes a significant barrier to degradation by simultaneous rot fungi. This may be the reason for the predilection of wood of angiosperms over that of gymnosperms by this fungi type, while the brown rot fungi favour the wood of gymnosperms.

In simultaneous rot, wood polysaccharides and lignin are degraded at similar rates by the enzymes secreted by the hyphae growing on the S_3 layer in the cell lumen. This degradation usually occurs very close to the hyphae involved (Kubicek, 2013), culminating into the formation of erosion troughs. The enzymes secreted by this fungi type have the capability to decompose all cell wall components of the lignified cell wall (Eriksson *et al.*, 1990; Kubicek, 2013). Thus, the term "simultaneous rot" is used to express the attribute of these fungi in being able to degrade the cell wall cellulose, hemicelluloses, and lignin at a relatively similar rate, although, they are generally classified as white rot fungi. As described above, the main feature of this fungi decay pattern is usually the formation of erosion troughs where the hyphae are growing on the cell wall, which is in contrast to that induced by brown rot fungi, characterized by a general dissolution of cellulose. A general thinning of the cell wall from the lumina towards the middle lamella as decay progresses is observed due to the coalescence of the erosion troughs induced by the numerous fungi hyphae on the cell wall (Zabel and Morrell, 1992; Schwarze *et al*., 1995).

In selective delignification, lignin removal from the cell wall is more than cellulose or hemicelluloses during incipient stage of decay. In some cases, the hyphal growth occurs in the cell lumen and as such, the lignin of the adjoining cell wall gets dissolved out. Another pattern observed includes the penetration of the cells by the hyphae to

induce an initial lignin removal from the middle lamella which extends towards the secondary wall (Kubicek, 2013), consequently separating the cells due to the delignification of the lignin-rich middle lamella. Thus, the cellulose relatively remains intact during early stages of decay by fungi within this group. The residual wood has a stringy texture due to the significant reduction of its hardness and stiffness, although, the wood still retains a considerable amount of its tensile strength (Schwarze *et al.*, 1995). This is a wide contrast to brown rots where the cellulose is primarily degraded, leaving the lignin component, which makes the residual wood material brittle. Cellulose breakdown in white rot proceeds slowly than in brown rot, which ensures a less drastic reduction in the wood's strength properties. This may be due to the detachment of the glucose or cellobiose molecule from the cellulose chain terminals, thus preserving the long and continuous cellulose fibrils of the wood. An additional reason for the large retention of the wood strength properties may be as a result of the longer persistence of the radial structures within the major strength contributing region in the wood (i.e. the S_2 layer) (Schwarze and Engels, 1998).

2.5.2.2 Brown-rot fungi

The term "brown rot" stems from the colour of the resulting wood material which appears darkened after decay as a result the breakdown of the polysaccharide fraction of the wood and the retention of lignin which has undergone oxidation (Kubicek, 2013). This type of decay in wood is as a result of the activities of fungi belonging to the Basidiomycetes and predominantly occurs in coniferous softwoods (Kubicek, 2013). This group of fungi commonly attack the wood of gymnosperms (Gilbertson, 1980). They are characterized by their rapid and extensive degradation of cellulose, which results into a significant wood strength loss during the incipient stages of decay. They are therefore believed to be the most important of all the types of decay for wood in service. During the incipient stages of decay, cellulose is degraded less rapidly than hemicelluloses (Zabel and Morrell, 1992). In addition to their excessive breakdown of hemicelluloses and cellulose in the wood, the demethylation of lignin is also observed (the extent which is dependent on the fungal species) because they lack the ability to fully depolymerize lignin (Kubicek, 2013), and thus, the lignin usually persists in the wood as an amorphous residue which easily disjoints cubically and disintegrates into powder when dry. The characteristic appearance, colour and consistency of the decayed wood are derived from the modified lignin retained in the wood. It is

presumed that during the pre-cellulotic phase, the formation of hydrogen peroxide occurs which easily permeates the cell wall and in conjunction with the iron ions, resulting in the oxidative depolymerisation of the lignocelluloses matrix (Xu and Goodell, 2001). This assumption may be true because infiltration of the smaller cell wall capillaries by large molecular weight enzymes is impossible. The cell wall penetration is therefore aided by the initial formation of the hydrogen peroxide, while the cellulose is degraded by the large molecular metabolites secreted by the hyphae. Apparently, there is a degeneration of the hemicelluloses which surrounds the cellulose for easy accessibility of the cellulose by the cellulases. Subsequently, the cellulose chain molecules indiscriminately begins to cleave (which occurs at several places on the cellulose chain), forming a series of individual cellulose chain fragments. Brown-rot fungi commences its invasion of wood by first attacking the cell wall's S_2 layer and then extends to the S_3 and S_1 cell wall layers as decay advances (Nilsson, 2009). According to Green and Highley (1997), the S_3 layer only becomes attacked during the latter stages of decay of the wood, with no alteration to the porosity of this layer. The degradation of the long cellulose chain molecules into smaller fragments is accompanied by a drastic reduction in bending strength and toughness with little weight loss (less than 5%) after a short period of decay (Wilcox, 1978; Curling *et al*., 2001).

Available detailed evidence on the molecular mechanisms involved in brown rot suggests that it is brought about by both oxidative and hydrolytic attack. The oxidative or non-enzymatic involvement in the process occurs during the early stage of decay primarily because the fungi attack cellulose initially, with the hemicelluloses and lignin unaffected, and factual evidence further provides that the extracellular enzymes secreted by these fungi are too big, preventing the infiltration of the cell wall in order to access the cellulose, hence, the need for the oxidative mechanism (Kubicek, 2013). Thus, their hyphae produce both low molecular weight chemicals and enzymes which are channelled into the cell wall to break down the cell wall components (Nilsson, 2009). For the non-enzymatic decay, which occurs during early wood decay, brown rot fungi produce hydroxyl ion radical, which is explainable by the proposed degradation pathway illustrated by the Fenton reaction. According to Xu and Goodell (2001), the Fenton reaction explains that extracellular hydrogen peroxide produced by the fungi

reacts with ferrous iron (Fe^{2+}), which creates the hydroxyl radicals capable of permeating through the cell wall to degrade hemicelluloses.

2.5.2.3 Soft-rot fungi

This type of rot in wood is a typical attribute of the Ascomycotina and Deuteromycotina (Eriksson *et al.*, 1990). Degradation by these fungi has been observed to be of two types: a decay pattern where the fungus creates cavities vertically within the secondary wall, often referred to as a type 1 soft rot and, a type 2 soft rot where there is a complete erosion of the secondary wall (Schwarze *et al.*, 2000a; Kubicek, 2013). The main characteristic feature of soft rots, when examined under the microscope, is the formation of cavities; however, many of the fungi also cause the erosion of the cell wall which is similar to the pattern of simultaneous white rot fungi, most especially in hardwoods. The term “soft rot” stems from the wood appearance after degradation by these fungi group, often having a soft, decayed surface, while the wood below the decayed surface remains sound. Studies have revealed that this form of rot requires a high moisture for it to occur (Eriksson *et al.*, 1990), however, it has been recently discovered to also occur in dry environments, where the wood macroscopically appears like that of a brown rot (Kubicek, 2013). Although decayed wood by soft rot fungi usually have a soft texture, studies which investigated the attack of soft rot fungi on CCA treated timber showed that the degraded wood remained hard, however, the term soft rot is widely accepted (Rayner and Boddy, 1988). Soft rots often appear as white rot but they differ in that the soft rot fungus does not degrade the middle lamella, with no involvement of lignases during the degradation of the wood (Kubicek, 2013). The only enzymes involved are cellulases and hemicellulases which are responsible for the wood polysaccharides degradation and consequently resulting in a considerable strength reduction of the decayed wood (Eaton and Hale, 1993; Kubicek, 2013). The decay rate of wood by soft rot fungi is inexorably linked to the lignin content and its spatial distribution, as well as the guaiacyl fraction of softwood lignin, which apparently is a major barrier to the fungi (Eriksson *et al.*, 1990).

The wood decay mechanism of soft rot fungi involves the use of their specialized microhyphae (usually 0.3-0.4 µm thick) which negotiate the cell S_2 layer from the lumen and subsequently realign along the cellulose microfibrils by a characteristic L-

bending (unidirectional growth) or T-branching (growth in two opposite directions). The cavities produced on the cell wall are usually aligned in a parallel orientation to the cellulose microfibrils. After the fungus microhyphae growth, the occurrence of the T-branching or L-branching of the hyphae on the S_2 layer spans over a defined distance, where release of enzymes around the hyphae causes the formation of cavities. After the cavity formation, new microhyphae originate from the initial hyphae and the sequence of the characteristic branching and cavity formation is repeated. The persistent enlargement of these cavities and the development of new ones results in the total degradation of the cell wall S_2 layer.

In soft rot, both the S_3 layer and the middle lamella are easily penetrated, but the latter is not degraded while the attack is usually mild in the S_3 layer of softwoods. This limited attack on both layers is due to to the thickness and the lignin content of the layers. The type 2 soft rot is characterised by the thinning of the wood fibre walls from the cell lumina in a similar pattern to that observed in white rot decay and some higher ascomycetes fungi such as *Xylaria*, *Hypoxylon*, *Daldinia* (Zabel and Morrell, 1992). As pointed out earlier, soft rot fungi causes a less considerable decay in softwoods than hardwoods which is believed to be due to the generally lower syringyl-guaiacyl type of hardwood lignin in comparison with the higher lignin content as well as the high content of the lignin type (guaiacyl) in softwoods (Schwarze *et al*., 2004).

2.6 Wood natural durability

Natural durability of wood refers to its inherent resistance to attack by biodegradation agents such as insects and fungi under favourable conditions for attack (Tsoumis, 1991). This wood quality is usually exhibited by the heartwood of most wood species except for those species where there is no clear delineation between the sapwood and heartwood (Wong *et al.*, 2005). According to Eaton and Hale (1993), sapwood is generally considered to be non-durable (having a service life of below 5-7 years) due to their vulnerability to biological attack, while heartwood is considerably resistant due to extractive deposition which are toxic to biological agents. Variation in the natural durability of wood also occurs radially from pith to bark, with the inner heartwood closer to the pith being less durable than outer heartwood (Wong *et al.*, 2005).

The management objective of most plantations under short rotation focuses less on quality but rather on yield maximization. Hence, fast grown wood species typically

have higher sapwood proportions and poorly developed heartwood, making plantation timbers less inferior to those obtained from natural forests in terms of durability (Hill, 2006). Studies have demonstrated over the years that heartwood durability is particularly due to the toxicity of their extractives to biological agents; coupled with the higher permeability and presence of stored carbohydrates in the sapwood (Kollmann and Cote, 1968). However, recent discoveries have also shown that the antioxidant activity of the extractives, in addition to their biocidal characteristics, is also a factor (Schultz and Nicholas, 2000; Hill, 2006). Heartwood formation in the wood results in decrease in wood moisture content and in some hardwood species, tyloses develop in the vessels, while encrustation of the pit membranes and pit aspiration are common occurrences in sapwood species (Siau, 1984; Usta and Hale, 2006; Panek *et al*., 2013). Thus, the durability of heartwood is complex and several factors may be involved in the exhibition of this wood property i.e. antioxidant, biocidal, moisture exclusion, cell wall moisture exclusion and metal chelating mechanisms. Several studies have investigated the effect of isolating wood extractives like flavonoides, terpernes, quinines etc. on the durability of the wood (Dungani *et al*., 2012). According to Schultz and Nicholas (2000) flavonoids prevent the degradation of the heartwood using a dual mechanism of free radical scavenging (antioxidation) and fungicidal means. According to Gupta and Prakash (2009), flavonoids are able to scavenge free radicals and possess natural antioxidant attributes. Thus, this antioxidant properties and free radical scavenging of heartwood extractives may be so crucial in controlling wood decay by brown rot fungi whose degradation mechanism utilizes free radical systems and oxidative mechanisms. The ability for the synergistic effect of the fungicidal and non-biocidal properties (free radical scavenging/ antioxidant property) of heartwood extractives in inhibiting fungal colonization and degradation of wood led Schultz and Nicholas (2002) to propose the development of alternative approach into wood protection through further studies on the natural durability mechanisms of the heartwood of some species of wood. Decay resistance has equally been reported to be associated with density (Wong and Ling 2009), though this is very much associated with the wood extractive content of tropical species (Haygreen and Bowyer, 1996).

The evaluation of the natural durability of timber species under terrestrial conditions are conducted by several standard and non-standard procedures of field and laboratory examinations, which is used to rate timbers into various durability classes (Findlay,

1985; Eaton and Hale, 1993) as shown in Table 2.2. From field tests, the durability of wooden stakes belonging to the same durability class varies between tropical and temperate climates due to the different rates of biodeterioration between both regions (Eaton and Hale, 1993). This may even occur with wood species that are non-indigenous to a particular area. For example, Harris (1971) reported that failure may occur to a timber which is resistant to termite in one area when it is exposed to different species of termites from other localities. This, therefore, necessitates the need to acquire information on the performance of a wood species within a locality before adopting it for certain applications in other regions or localities (Eaton and Hale, 1993). While heartwoods are generally considered to have high resistance against biodeteriorating agents, not all wood species have naturally durable heartwood (Thomasson *et al.*, 2006), and may require extra protection through the application of protective chemicals to protect them from attack against biodeterioration when exposed to adverse environment. For example, *Gmelina arborea* wood falls into this category and as such, treatment with wood preservatives is problematic due to deposition of extraneous materials and tyloses within the heartwood (Owoyemi and Kayode, 2008; Olajuyigbe *et al.*, 2010). The treatment of refractory, non-durable species of wood is one of the major problems confronting the wood preservation industries globally.

2.7 Wood preservation

Wood preservation broadly implies the protection of wood by any physical or chemical treatment against any factor that may degrade and eventually destroy it (Kollman and Cote, 1968; FAO, 1986). During wood processing, prevalent conditions permit the development of wood biodeteriorating agents and as such, it is imperative to protect wood during processing, merchandising and utilization (Highley, 1999). The main objective of preservative treatment of wood is to extend its shelf life during service which helps in reducing the product's ultimate cost and prevents the need to replace them frequently (FAO, 1986). The sapwood portion of all species is vulnerable to degradation by biodeteriorating agents but the heartwood is somewhat more resistant to decay. While the heartwoods are sufficiently durable in most cases for use in adverse situations without the application of preservatives, some are just as vulnerable to deterioration like the sapwood. Even the so-called naturally durable wood species are subject to eventual breakdown by wood degrading organisms, though they usually have a longer service life (Kollmann and Cote, 1968; Thomasson *et al.*, 2006). When a

Table 2.2: Natural durability classes for tropical and temperate exposure and laboratory exposure

Durability class	Shelf life under		
	Tropical conditions (years)	Temperate conditions (years)	Laboratory conditions (% weight loss)[a]
Perishable	< 2	< 5	> 30
Non-durable	2 – 5	5 – 10	10 – 30
Moderately durable	Not given	10 – 15	5 – 10
Durable	5 – 10	15 – 25	1 – 5
Very durable	10+	25+	< 1

[a] 16 weeks incubation at 22°C with the following test fungi: *Serpula lacrymans*, *Antrodia* (*Fibroporia*) *vaillantii*, *Coniophora puteana*, *Pycnoporus* (*Polystictus*) *sanguineus*, *Gloeophyllum trabeum*, *Trametes* (*Coriolus*) *versicolor* (Findlay, 1985)

tree trunk is processed into sawn wood, the timber usually consists of both the sapwood and heartwood. However, the presence of a durable heartwood portion within the sawn timber doesn't make the entire sawn wood durable as there is the presence of a considerable proportion of sapwood. For instance, Morrell (2011) reported that the presence of heartwood in the Western Cedar wood induced no durability effect on the adjacent sapwood. For such wood to meet its end-use requirements, it will need to be properly preserved in a situation where it is being utilized in a high risk environment.

2.8 Wood preservatives

Wood preservatives refer to chemical substances which are toxic to wood decaying agents after their incorporation into wood and are capable of inhibiting their growth and activity in wood-based materials (FAO, 1986; Eaton and Hale, 1993). They also include wood protection coating substances which seals the wood pores and its surface to help minimise or prevent weathering, fire effects and maintain the dimensional stability of the wood. Wood preservatives helps in extending the useful service life of valuable biomaterials and therefore saves consumers money and time, mitigates deforestation and unnecessary perishable goods consumption (Freeman, 2003).

Timber in service is exposed to a broad spectrum of hazards both in severity and type, and these range of hazards include attack from insects, marine borers, fungi, etc. Thus, there is no single wood preservative which can be considered universal in the protection of wood from all these range of hazards. (FAO, 1986; Desch and Dinwoodie, 1996). Eaton and Hale (1993) broadly classified wood preservatives into three major groups which are discussed below.

2.8.1 Tar oil preservatives

These types of wood preservatives belong to the group of preservatives commonly used during the times of old, and are commonly utilized in a wide range of applications. They are preservative oils originally gotten from wood tar, but are presently being derived from coal tar (Eaton and Hale, 1993). The commonly used oil-borne preservatives types are pentachlorophenol, copper naphthenate and creosote. Both pentachlorophenol and creosote solutions are usually utilized in applications which involve rare human contact. However, copper naphthenate is an exception to this and it is a recently developed preservative which is not as widely utilized as the

other two (Shupe *et al*., 2008). Creosote and other heavy petroleum oils that are less volatile are effective in protecting wood from weathering, but they render treated wood dirty and negatively influence its colour, paintability, gluability and fire resistance (FAO, 1986). The earlier used tar oil wood preservative was coal tar, which is obtained from coal distillation (FAO, 1986; Eaton and Hale, 1993; Richardson, 1993). However, it was replaced by creosote because creosote is less viscous and better penetration was achieved under pressure impregnation (FAO, 1986). Creosote, being a mixture of different organic chemicals in varying proportions, offers good protection to wood against wood-degrading organisms provided a deep penetration and uniform distribution is obtained in the wood (FAO, 1986; Shmulsky and Jones, 2011). It is a brown or black oily liquid with a pungent smell and relatively viscous at low temperatures (Eaton and Hale, 1993). Creosote has the following advantages: (1) highly poisonous to wood-degrading organisms; (2) easy to apply in wood; (3) insoluble in water and evaporates slowly, which ensures a great degree of permanence in the treated wood under varying conditions; (4) depth of penetration in wood is visible; (5) relatively cheap; (6) renders the treated wood dimensionally stable and resistance to splitting due to its insolubility in water. However, creosote treated wood gives an unpleasant odour that are harmful to humans and plants. It is recommended that foodstuffs which are sensitive to odour should be placed far away from wood treated with creosote. Freshly-treated timber with creosote can easily be ignited, but after several months of seasoning the treated wood, it becomes difficult to ignite such wood due to the evaporation of the more combustible and volatile fractions of the creosote (FAO, 1987; Eaton and Hale, 1993; Shupe *et al*., 2008).

2.8.2 Water-borne preservatives

The use of water-borne preservatives emanated from the knowledge of the bio-toxicity of metallic salts and fluorides (Hartford, 1973). For this type of preservatives, water is the preservative carrier and they are the most common solvent carrier utilized by the wood preservative industry due to their low cost and availability. However, they exhibit some major drawbacks in that they reduce the wood's mechanical property (Winandy, 1995), are corrosive to wood fasteners, require redrying of the wood, cause swelling in timber, and consequently distort parts of the wood which has been machined to a particular specification (Findlay, 1985; FAO, 1986; Eaton and Hale, 1993). Water-borne preservatives are soluble in water during formulations. They are of

two types: the fixed water-soluble preservatives which react chemically to form insoluble compounds that become fixed to the wood after treatment; and the unfixed water-soluble preservatives which do not bond to the wood after treatment. The former do not result into a loss of preservatives during subsequent rewetting of the wood while the latter are easily leached out of the wood and should not be utilized in applications where treated timber will be consistently wet (Eaton and Hale, 1993). The most commonly utilized water-borne preservative is that containing formulations of copper and organic biocides. They belong to the fixed water-soluble group and are efficacious in protecting timber against a broad range of bio-degrading organisms such as fungi and insects. Borates are another type of water-borne preservative, although they are non-fixed water borne preservatives, and provide adequate wood protection against boring insects. Being a non-fixed water-borne preservative, they can leach out of wood when used under severe wet conditions and so, they are mostly employed on wood to be utilised for interior applications like framing and flooring (Freeman, 2003). The commonly utilized non-fixed water-borne wood preservatives include fluor-chrome-arsenate-phenol (FCAP), chromated zinc chloride (CZC) and Borates. Metallic salts dissolved in water have been in use for a very long time in preserving wood, most especially for wood to be used for exterior applications. Most of these salts contain a significant quantity of alkaline chromate which acts as the fixative agent, fixing other chemicals to the wood and ensuring their permanence in the wood during service (Eaton and Hale, 1993).

The commonly used water-borne preservatives are based on mixtures of salts or oxides of copper/ chromium, with the copper/chromium/arsenic (CCA) the most common. CCA is often marketed under the 'Celcure' or 'Tanalith' trademarks (Desch and Dinwoodie, 1996). Historically, CCA was developed from the Forest Research Institute, Dehra Dun, India in the 1930s. It is the most widely utilized water-borne preservatives globally over the years. Standard CCA solutions contain Chromium (Cr), Arsenic (As) and Copper (Cu) in the form of CrO_3, As_2O_5 and CuO, respectively. The CCA formulation contains a primary fungicide (copper), a secondary insecticide and fungicide (arsenic), and an oxidizing or fixative agent, which is chromium (Desch and Dinwoodie, 1996), and offers protection against ultraviolet light. However, the inorganic arsenic and chromium are major sources of health and environmental problems (Solo-Gabriele *et al.*, 2004). Due to the health concerns and environmental

impacts posed by the utilisation of CCA, several countries have restricted or outrightly banned their use (Hingston *et al*., 2001; Evans, 2003; Kim *et al*., 2008). The advantages of water-borne preservatives are: (1) paintability of the treated wood after the water dries off (2) they can readily be mixed with fire retardant chemicals (3) they are odourless (4) the treated wood is usually left clean and are not unpleasant to handle (5) formulations of water-borne preservatives are easy to prepare (6) ease of transportation either in solid or concentrated form and can be prepared for use with the cheapest of all solvents – water (Findlay, 1985).

2.8.3 Organic solvent preservatives

These types of preservatives consist of active chemicals which are poisonous to bio-deteriorating agents, and are usually dissolved in an organic solvent like petroleum distillate (Desch and Dinwoodie, 1996). The organic solvent (diluent) is usually non-polar and may either be volatile or non-volatile (Eaton and Hale, 1993). According to Eaton and Hale (1993), preservative formulations using organic solvents can be prepared with the following solvent carriers: light type (white spirits), medium-heavy type (kerosene or petrol), and oily type (heavy fuel oil). These organic solvents are usually of low viscosity and have the ability to penetrate rapidly into dry wood, which makes them appropriate for usage in preservative formulations intended for superficial application by brushing, immersion and spraying. In this type of preservative, the organic solvents are non-toxic and perform no preservative function while the preservative action is performed solely by the toxic deposits of the chemical combined with the organic solvent. They function by acting as a carrier (solvent) and depositing the toxicant in the wood, while the solvent volatilizes after treatment (Richardson, 1993). The organic solvents are usually expensive, and therefore, their use is justifiable only when the other preservative types are unsuitable for the intended use of the timber. One of the main factors which affect the volatility of the organic compounds is the molecular weight (Richardson, 1993). Higher molecular weight of the solvent implies a higher activity, but a lower volatility. Hence, consideration is often given to compounds which possess an optimum combination of activity and permanence (Richardson, 1993). Some organic compounds have been discovered to possess a combination of toxicity, volatility and water solubility. For example, when phenol is chlorinated, compounds which consist of one to six chlorine atoms are formed. However, since the extent of chlorination increases toxicity and reduces volatility and

water solubility, chlorinated phenol with five chlorine atoms (pentachlorophenol) was discovered to be the preferred organic compound possessing optimum activity and permanence (Richardson, 1993). Other types of organic-solvent based preservatives include lindane, dieldrin, tributyl tin oxide (TBTO), copper 8-quinolinolate and copper napthenate. Just like CCA, the use of dieldrin and some other organochlorines like DDT have been banned in several countries (Desch and Dinwoodie, 1996). Organic solvent preservatives are usually applied to wood using non-pressure systems such as spraying, brushing, dipping or low pressure processes like double vacuum. Usually, the treated wood is left in a clean condition, they do not leach out, penetrate deeply into easily treated timber, they do not cause wood distortions or swelling, treated timber can easily accept finishes and glue after the solvent has evaporated, and are very useful in remedial treatment of attacked woods which are already part of a building because they are easily absorbed by wood when applied by spray or brush. However, their demerits are that they increase wood flammability immediately after the application of preservative treatment, are relatively costly, and can taint some certain foodstuffs, with the exception of copper 8-quinolinolate (FAO, 1986).

2.9 Wood treatment methods

The methods used in introducing chemicals into wood are dependent on the species of wood, the imminent deterioration hazards the wood will be subjected to, the type of chemical, and the expected service life of the wood (FAO, 1986; Hihara *et al.*, 2013). Wood to be treated must be dried before the application of the preservative except the treatment procedure is specifically designed for the treatment of freshly felled timber (Desch and Dinwoodie, 1996). Two types of wood treatment methods are generally recognised which are: (1) pressure treatment, which involves the impregnation of wood in closed vessels at levels above atmospheric pressure, and (2) non-pressure treatment, which are considerably variable in both the procedures and equipment used (Ibach, 1999).

2.9.1 Pressure treatment

The pressure treatment involves the placement of wood in a pressure vessel or cylinder capable of withstanding high pressures and the application of pressure or vacuum (Richardson, 1993). It has, so far, been the most successful procedure in the treatment of wood (FAO, 1986). It allows for a more uniform and deeper preservative

penetration within the wood, while the wood preservative retention is also controllable (Eaton and Hale, 1993). Here, the wood is treated by placing it in a high-pressure tank containing preservative, followed by the application of pressure to force the preservative into the wood. There are several variants of the pressure processes which follow a similar principle but only differ in details. The wood is placed on metal buggies or trams, and is run into a long steel cylinder, which is shut before introducing the preservative into it. The preservative is subsequently driven into the wood by introducing pressure until the desired absorption by the wood is achieved. There are generally three major types of pressure treatment methods that are commonly employed which include the empty cell, full cell and modified full cell (Richardson, 1993). They are usually utilised in wood treatment where the risk of biological attack to timber is high (Desch and Dinwoodie, 1996).

2.9.1.1 Full Cell

The full cell method, also known as Bethell procedure, marked the first significant utilization of pressure for the treatment of wood (Freeman *et al.* 2003). This procedure was patented by John Bethell in 1838 (Eaton and Hale 1993; Richardson 1993). The aim of this process is to ensure that maximum preservative retention in the wood is achieved, which includes both the cell walls and cell lumens being totally filled with the preservative (FAO, 1986; Richardson, 1993). It is a standard technique commonly used for timbers that require creosote treatment or water-borne preservatives. If creosote is to be used, it is first heated to a temperature between 60-80°C in order to improve penetration by reducing its viscosity (FAO, 1986; Richardson, 1993). For the water-borne preservatives, it is often warmed to prevent sludging, crystallization or freezing in cold conditions (Richardson, 1993). With waterborne preservatives, the net retention of the preservative is contingent on the concentration of the treating solution and this can be regulated to obtain the final desired preservative retention in the wood (Richardson, 1993).

2.9.1.2 Modified Full Cell

This process is very similar to the full-cell procedure but differ in the quantity of initial vacuum applied and the intermittent utilisation of a prolonged final vacuum. This process entails the application of an initial vacuum at a lower level, where the exact level is determined by the desired final retention, the wood species and wood

dimension (FAO, 1986). This method of pressure treatment is commonly employed when lumber are to be treated with waterborne preservatives (Richardson, 1993).

2.9.1.3 Empty-Cell

The aim of this process is to achieve a low net preservative retention whilst ensuring a deep penetration of the preservative chemical in the wood (FAO, 1986; Richardson, 1993). In this case, the preservative initially within the lumen are recovered under pressure while the cell walls are relatively filled with the preservative (Richardson, 1993). The empty cell process produces treated wood which have the least tendency of bleeding during service (Richardson, 1993). The empty-cell processes are of two types which are the Rueping and the Lowry process (FAO, 1986; Richardson, 1993). Both procedures utilize the expansive force of compressed air to eject some of the preservative absorbed during the pressure application phase. They are generally utilized for creosote treatments but can be used for water-borne preservatives which fix slowly, especially for those which fix when a component of the chemical preservative is lost, as in the case of ammonia-based preservatives which fix during an alteration in the pH when the ammonia component evaporates (Richardson, 1993).

2.9.2 Non-pressure methods

The effectiveness of non-pressure treatments depend on the wood moisture content, type of wood being treated, method and treatment duration as well as the preservative type used (Chirra, 1995). They are mainly intended for superficial protection of the wood and chemical penetration depth are generally shallow (Hihara *et al.*, 2013). Non-pressure treatments, especially the ones which involve surface applications on wood, usually give poorer results than those of pressure treatments (Richardson, 1993). There are several types of non-pressure treatments which generally consists of (1) steeping in solutions of water-borne preservatives or soaking in preservative oils, (2) surface application of preservatives by brief dipping or brushing, (3) vacuum treatment, and (4) diffusion processes with water-borne preservatives

2.9.2.1 Surface applications

The simplest procedure in treating wood is by brushing or dipping. In this case, preservatives which do not congeal or become viscous when cold should be used, unless they can be heated without affecting the biocidal efficacy of the preservative.

For the best result when applying the preservative by brushing, the preservative should be flooded all over the wood surface to ensure that checks and depressions are filled with preservatives. Dipping, however, involves submerging the wood in the preservative for a few seconds to several minutes. Compared to brushing, dipping gives a slightly higher penetration of the preservative into the wood. Both procedures may not be ideal for refractory wood species or where treated timber is to be used in ground contact applications or where it would be exposed to very moist conditions (Ibach, 1999). In general, they are extensively used in timber treatment where the end use involves a low risk of biological attack (Eaton and Hale, 1993).

2.9.2.2 Cold soaking and steeping

Cold soaking involves submerging well-dried wood in preservative oils of low viscosity for several hours or days, while **steeping** involves submerging dried or green wood in water-borne preservatives for several days. Cold soaking timber yields significantly higher retention and penetration levels of preservative than those treated by dipping for similar species. When steeping seasoned wood with water-borne preservatives, the wood absorbs both the dissolved preservative salt and the water. For wood in the green state, only the preservative salt is taken up by diffusion into the saturated wood. Penetration and retention results of steeped wood vary considerably and it is recommended only when other reliable treatments are impracticable (Ibach, 1999).

2.9.2.3 Diffusion processes

These processes, just like the steeping procedure, are applicable for green wood. They are applied for water-borne preservatives whose dissolved salts diffuse into the water in the green wood from the treating solution. Double diffusion, a type of the diffusion process, involves a two-stage dispersion of a preservative liquid into the treated timber. It involves steeping partially dried or green wood firstly in a preservative solution, and then into another chemical solution. During this process, there is a reaction between two chemicals which diffused into the wood to produce a preservative precipitate in the wood which becomes fixed to the wood and highly resistant to leaching. An example is the steeping of the wood firstly in a solution of sodium fluoride and then in copper sulphate solution, which eventually precipitates leach resistant copper fluoride preservative in the wood (Ibach, 1999).

2.9.2.4 Hot and cold open tank process

This method utilizes the use of less sophisticated equipment in achieving an effective treatment of timber, which is the most attractive procedure being adopted in developing countries (Eaton and Hale, 1993). Timber so treated with this method can be used where there is the likelihood of high biological attack as penetration and retention of the preservative is relatively high. Since it involves the application of heat, preservatives which easily decompose when heated are not appropriate for this method. In order to obviate this problem, the timber is usually heated in hot water, steam or hot air and immediately placed in the tank containing the cold preservative to cool. Also, organic solvent preservatives or highly volatile preservatives are not suited to this procedure as there is a risk of fire outbreak (Desch and Dinwoodie, 1996). This procedure entails immersing seasoned timber in a tank containing a cold preservative and then heating it afterwards to a temperature of 85-95°C for 3 hours. As the preservative is heated, heat is transferred into the wood and this causes the expansion of the air in the wood cell voids, and consequently getting expelled from the wood. When the heat source is removed and the temperature of the preservative and wood drops, the residual air in the wood contracts and absorbs the preservative into the wood to fill up the empty spaces in the wood. Re-heating the preservative for like 1-3 hours can induce a 'kickback' as the residual air within the wood expands and pushes out excess preservative in the wood. In this case, the penetration is the same but the retention is lower than the first treatment phase. The wood is removed from the hot preservative and exposed in open air to cool (Desch and Dinwoodie, 1996).

2.9.2.5 Vacuum process

Vacuum process, also referred to as VAC-VAC, is used in the treatment of wood with water-borne preservatives or water-repellent preservatives like pentachlorophenol solution. The aim of this process is to treat wood using a restricted amount of a water-repellent preservative and obtain similar penetration and retention levels to those acquired for treated wood dipped in preservatives for 3 minutes. It involves using a brief, low initial vacuum, followed by the introduction of the preservative into the cylinder until it is filled up under vacuum, releasing the vacuum and soaking the wood in the preservative under or slightly above atmospheric pressure and a final vacuum is subsequently applied. A better retention and penetration result is yielded using this

treatment when compared to the 3-mins dip procedure, also facilitating quick drying of the wood surface which allows for the wood to take appropriate finishes. They are utilized for wood where desired retention levels are lower than those obtained for pressure treatments. However, preservative retention levels are difficult to control for vacuum treatments as opposed to empty-cell treatments. A good control over the amount of preservative retained in the treated wood for vacuum process with water-borne chemicals is possible by adjusting the concentration of the preservative (Ibach, 1999).

2.10 Preservative effectiveness

The necessity for the wood preservation industry arose due to the dwindling nature and unavailability of naturally durable timber, coupled with the impossible task of controlling the environmental conditions under which a wood would be subjected (Freeman *et al.*, 2003). Ever since the discovery that no single preservative is perfect for all situations due to the broad spectrum of hazards to which wood is subjected under different applications, much emphasis has therefore focused on utilizing preservatives which are more suited to particular applications (Goodell *et al.*, 2003). The effectiveness of a preservative in protecting wood from degradation however, is dependent on the treatment results and the preservative system used. Treatment results, in this case, are a measure of the penetration, retention, and distribution of preservative in the wood (Arsenault, 1973). Furthermore, Archer and Lebow (2006) stated that the efficacy of a preservative is measured by the preservative toxicity against the target organisms, its resistance to leaching and other forms of degradation posed by the environment, amongst others. An effective preservative does not imply complete protection of the wood when sub-standard retention and penetration levels of the preservative in the wood is obtained. According to Ibach (1999), variables which influence treatment results include the wood species, moisture content, heartwood penetrability, proportion of sapwood and heartwood, etc.

2.11 Treatability of wood

The term treatability is can be defined as the ease of impregnation of timber with treatment solution such as a wood preservative (BS EN 350-2, 1994). On the other hand, permeability, as defined by Nicholas and Siau (1973) and Siau (1984), is a measure of the ease of movement of fluids and gases through a porous solid when

subjected to a pressure gradient. The permeability of wood is of major importance in the wood industry as it finds application in several technical processes such as wood drying, pulping, or preservative treatment of wood (Lehringer *et al.*, 2009b). Generally, it is easy to treat the sapwood of most timber species (though there are exceptions like the spruces), while the heartwood are very difficult to impregnate (Wang and DeGroot, 1996; Forest Product Laboratory, 1999). Reasons attributed to the refractory nature of heartwood from an anatomical standpoint include the irreversible nature of the pit after aspiration (Thomas and Kringstad, 1971), small pore sizes in the heartwood (Petty and Preston, 1969), the quantity and type of extractive deposition on pit membranes during heartwood synthesis (Siau, 1984), and in addition, formation of tyloses in the heartwood of hardwoods (Panshin and Dezeeuw, 1980; Siau, 1984). Thus, reduced treatability of wood species with non-durable heartwood can result into a lower durability of the heartwood in comparison to the sapwood (Wang and DeGroot, 1996). Poor treatability, however, may be a less critical problem in wood species with naturally durable heartwoods. For an effective wood treatment, it is necessary that the treating chemical penetrates to some given depth into the wood (Wang and DeGroot, 1996). Due to its good permeability, sapwood samples have majorly been utilized in assessing the efficacies of conventional and newly developed preservatives, though varying results in penetration have been obtained due to the numerous wood specific factors affecting the permeability and treatability of different wood species (Comstock, 1970; Lande *et al*. 2009). Larnøy *et al*. (2008) and Lande *et al*. (2009) identified some anatomical factors believed to influence the permeability of softwood species, and these include the aspiration of bordered pit pairs of tracheids, composition of wood components, amount and constitution of heartwood, ratio of earlywood/latewood, size and frequency of radial and axial resin canals, parenchyma rays, half boarded pits between parenchyma rays and tracheids, and presence and characteristics of resins in the sapwood. In order to achieve deeper penetration of chemicals in wood during treatment, pressure treatment procedure is most often adopted; the penetration of impregnating chemical is mainly through bulk flow which occurs via the interconnecting spaces of the wood structure (Siau, 1984). The anatomical structure of wood is one of the primary structural factors which influence wood permeability. Treatability of wood is lower in the transverse direction than the axial direction due to the length and narrow configuration of the longitudinally oriented cells such as fibres, vessels, and tracheids (Nicholas and Siau, 1973). Radial

flow is generally due to the ray cells while tangential flow is basically due to the location of pit pairs, as the concentration of most pits are situated on the radial surfaces of adjacent tracheids (Nicholas and Siau, 1973). Studies have often reported lower permeability in the tangential direction than the radial direction, but significant variation exists among wood species and contrary results have been reported in some species (Comstock, 1970).

BS EN-350-2 (1994) classified wood types into four treatability classes based on vacuum pressure impregnation as shown in Table 2.3. Treatability of wood has generally been empirically assessed by the gross uptake and the penetration depth of the treating fluid in the wood (Kumar and Dobriyal, 1993; Lehringer *et al.*, 2009a; Schwarze and Schubert, 2009; Schubert *et al.*, 2011; Thaler *et al.*, 2012; Panek *et al.*, 2013; Emaminasab *et al.*, 2015). However, considerable differences have been observed in the gross retention and penetration of wood species belonging to the similar treatability group (Kumar and Sharma, 1982), while some species of hardwood having similar gross preservative uptake have equally been discovered to exhibit variable performance (Purushotham *et al.*, 1967; Chaubey *et al.*, 1986). For instance, early failure was discovered in some species after preservative treatment (Greaves, 1977; FAO, 1986); the cause of this was due to heterogeneous preservative penetration within the various wood cells, particularly in the fibre cells, which are the main structural component of hardwoods (Dickinson and Sorkhoh, 1976). Greaves (1977) noted that the size variation and different pattern of distributions of structural wood tissues are mainly responsible for the variation in flow and distribution pattern of treating substances in wood; which may also be observable within a species. Thus, it has been suggested that quantitative indices such as gross uptake and depth of chemical penetration may not be sufficient in describing the treatability of a wood species as they tend to neglect the micro-distribution of the chemical and consequently exaggerate the anticipated performance of treated wood (Kumar *et al.*, 1990; Kumar and Dobriyal, 1993; Sint *et al.*, 2011). A more robust treatability index known as penetration index has therefore been introduced, which is a measure of the average penetration of the different wood components and described to be a better quantitative approach in determining wood treatability (Kumar *et al.*, 1990; Kumar and Dobriyal, 1993; Sint *et al.*, 2011).

Table 2.3: BS EN 350-2 treatability classes based on observation of vacuum pressure treatment processes

Treatability class	Definition	Description
Class 1	Permeable	Easy to treat
Class 2	Moderately resistant	Moderately easy to treat
Class 3	Resistant	Difficult to treat
Class 4	Extremely resistant	Very difficult to treat

Source: BS EN 350-2 (1994)

Since both softwoods and hardwoods have different anatomical structures, an indepth analysis into the treatability of each of this wood type, as affected by their wood structure would be separately conducted.

2.11.1 Softwood permeability

Wood cells in softwoods consist of about 5 to 10% rays, 0.1 to 1% longitudinal resin canals, and the predominant cell type being the longitudinal tracheids, which is about 90 to 94% (Wang and DeGroot, 1996). Panshin and Dezeeuw (1980) stated that there is a negligible amount of axial parenchyma in softwoods. The tracheids are close-ended, and movement of materials among tracheids and rays occurs mainly through the bordered or non-bordered pit pairs, most of which are situated on the tracheid radial walls or radial walls of rays (Panshin and Dezeeuw, 1980). As the mean diameter of the pit pores is considerably minute than that of the lumen of tracheids, wood treatability largely depends on the size and condition of the pit structure in this type of wood (Wang and DeGroot, 1996). Thus, parameters like number of pit openings, effective pore size, encrustation of the pit membranes, probability of aspiration and deaspiration, and tracheid length are major indices which determine the treatability of softwood species (Wang and DeGroot, 1996). Also important is how the tiny pores in the cell walls function, as they significantly influence treatability when preservative types are polar solvents (Nicholas and Siau, 1973). However, due to their smaller pores when compared to that of the pit pores, their role in influencing treatability may be less pronounced when the pit openings are more effective in fluid transport (Wang and DeGroot, 1996).

Sapwoods generally have high permeability when its pits are unaspirated. During drying, prior to the application of preservatives, the pits of some softwoods species greatly aspirate during water removal from the cells, resulting from the sealing of the pit aperture by the torus membrane (Wang and DeGroot, 1996, Usta and Hale, 2006; Lehringer *et al*., 2009a; 2009b Pánek *et al.*, 2013). Pit aspiration occurs as a result of hydrogen bonding formation between the pit opening and the margo/torus as shown in Plate 2.4 (Lehringer *et al*., 2009b). However, these adhesion forces are not too often overcome during processing when high pressure is applied (Thomas and Kringstad, 1971). The latewood of softwoods have been observed to have a higher permeability than the earlywood (Siau, 1995). This is due to the characteristics of the pits which

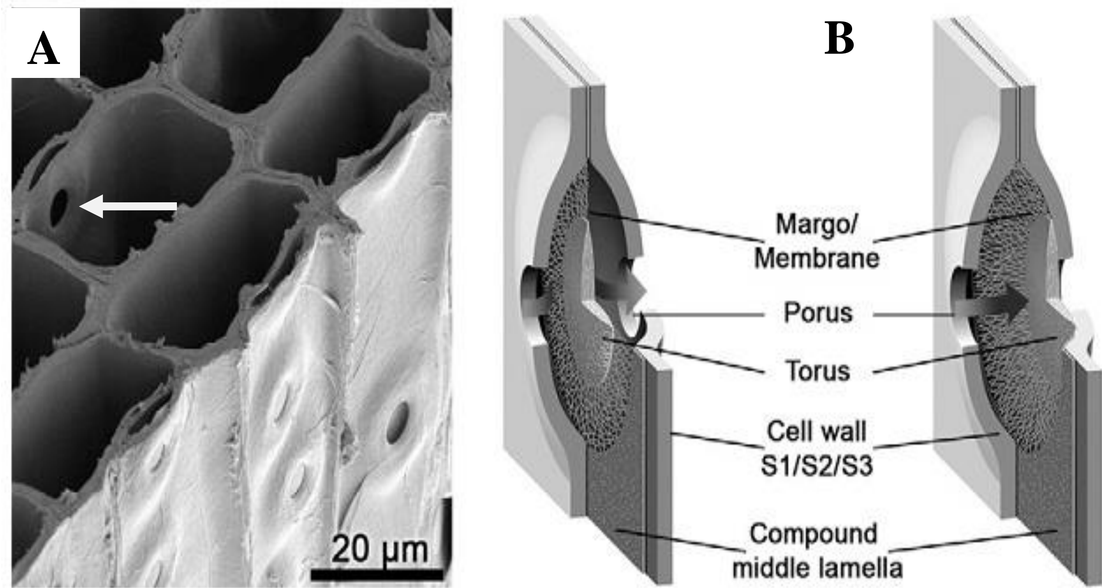


Plate 2.4: (A) Bordered pits of adjacent tracheids acting as interconnecting voids between the cells; (B) Bordered pit aspiration due to displacement of torus against the pit aperture (Source: Lehringer *et al.*, 2009b).

include the thicker strands of the pits, higher lignification, a tighter margo strand, smaller pit diameters, and denser organization of the pit chamber, consequently increasing its stiffness than that of the earlywood (Lehringer *et al*., 2009b). In addition, the thicker walls of the latewood cells necessitate that a greater distance must be covered by the torus for aspiration to occur. Thus, fewer pits become aspirated during drying due to the need for higher forces to cause a displacement of the torus against the pit aperture (Lehringer *et al*., 2009b). This is only observed after seasoning the wood, as the earlywood is more permeable and permits higher bulk flow than the latewood in the green condition due to the larger pits and the unaspirated state of the pits (Flynn, 1995; Lehringer *et al*., 2009b).

The extent of pit aspiration, as well as the encrustation of the pit membrane varies with wood location in the tree. For instance, Harris (1954) investigated 200 trees of 27-year old *Pinus radiata* in Australia and discovered a considerable radial variation, where an average percentage of 30%, 90%, and 96% of pits were aspirated in the sapwood, transition zone and heartwood, respectively, with the increase in pit aspiration along these zones being relatively gradual. Similar variations in the percentages of encrusted pits have also been reported by Yamamoto (1982) in trees of *P. densiflora, Pinus strobus* and *P. Banksiana*, where he observed that the extent of encrustation in the sapwood bordered pits was approximately zero, with a gradual increase through the transition zone to approximately a complete encrustation of the heartwood bordered pits. While some of the aspirated sapwood bordered pits can relatively be deaspirated through some treatments like resoaking in water, aspiration of the heartwood bordered pits is more or less irreversible due to the adhesion of the extractives between the pit opening and the aspirated pit membrane (Thomas and Nicholas, 1966). Thus, reduced treatability in the heartwood than sapwood is as a result of the combined effect of both pit aspiration and pit encrustation in the heartwood (Krahmer and Cote, 1963; Liese and Bauch 1967).

Furthermore, rays can constitute major pathways for the radial flow of liquids due to the higher concentration of pits on their radial surface than the tangential surface (Cote, 1963; Wang and DeGroot, 1996). As documented by Panshin and deZeeuw (1980), ray tracheids are common features present in the woods of the genera *Larix, Pinus, Pseudotsuga, Picea, and Tsuga*. Thus, radial permeability is higher than tangential permeability in some species such as *Pinus sylvestris* due to the conductive

role performed by the ray tracheids (Banks, 1970). However, the effectiveness of the ray tracheids in enhancing wood treatability may depend on their abundance and their condition in the wood. Baines and Saur (1985), in their study of the treatability of spruce wood suggested that the low percentage of ray tracheids may be a major contributory factor towards its refractory nature. Similarly, the refractory nature of *Picea abies* was also reported to be due to the low volume percentage of ray tracheids in the wood (Liese and Bauch, 1967). In general, the ray tracheids are more effective than ray parenchyma cells in enhancing radial flow in wood (Cote, 1963; Erickson and Balatinecz, 1964). This conclusion may further be supported where ray tracheids closely associated with ray parenchyma cells in preservative treated woods often get impregnated, while little or no trace of the preservative is found in the ray parenchyma cells (Liese and Bauch, 1967).

2.11.1 Hardwood permeability

In most hardwoods, four major cell types are recognized which are present in varying percentages in different species. The cells include vessels (18 - 54.9%), fibres (26.6 - 68%), axial parenchyma (0.1 - 13.5%), and rays (11.9 - 21.4%) (Panshin and deZeeuw, 1980). In hardwoods, virtually all the cells contribute to fluid flow, and hence, treatability of the wood (Siau, 1984; Wang and DeGroot, 1996). However, studies have shown that the vessels are the major path flow for fluids in hardwood species (Wang and DeGroot, 1996). Thus, treatability of hardwoods is being determined by factors such as the distribution, size and conditions of the vessels (Wang and DeGroot, 1996). On the contrary, recent studies have shown that vessels may not be the dominant path for chemical flow in hardwoods. For instance, Tripathi (2012) reported that considerably higher penetration depth of boron was observed in the end-grain sealed wood of yellow and red Meranti than the unsealed wood species, indicating the higher importance of transverse flow in both species. Also, there was no sign of chemical penetration in the vessels of *Ailanthus excelsa* Roxb. despite the absence of occlusions in the vessels of the wood species (Kumar and Dobriyal, 1993).

In ring-porous species, the average diameter of vessels vary between 20 – 100 µm, while considerable variation can be observed between the springwood and latewood of diffuse-porous species; earlywood (50 – 400 µm) and latewood (20 – 50 µm) (Wang and DeGroot, 1996). In the transverse section of hardwoods, the concentration of

vessels is about 15,000 cm^{-2} (Siau, 1984). Vessels are generally made up of individual vessel elements, having an end to end connection through perforation plates (Wang and DeGroot, 1996). In some species, they can be long and continuous, and Thomas (1981) reported vessels attaining about three metres in length. However, the lumina of vessels within the heartwood and transition zones often contain various resinous, gummy, and chalky exudates, as well as tyloses (Owoyemi and Kayode, 2008). These occlusions in the vessel lumen greatly limit the permeability of the heartwood and transition zones of hardwoods (Kumar and Dobriyal, 1993). Teesdale and Maclean (1918) were able to confirm the influence of these vessel occlusions on hardwood treatability, where comparisons were made between treatability of wood species with vessels containing tyloses and those without tyloses. Further studies have equally confirmed the reduced treatability of hardwoods whose vessels contain tyloses (Thomas, 1976). The refractory nature of hardwoods with heavy tylosic occlusions have found application in the wood industry where species such as white oaks are used in making barrels for wine and whisky (Cote, 1990). As mentioned earlier, the presence of gummy and chalky extractives may also be observed, which also contributes significantly to reduced treatability of hardwoods (Siau, 1984). Thus, a combination of tyloses and extractives in the heartwood of hardwood species are major factors responsible for impermeability of the heartwood (Panshin and deZeeuw 1980; Wang and DeGroot, 1996).

Tyloses are suberized structures which arise from ray parenchyma cells intrusively growing through pit pairs into adjoining vessels and tracheids, resulting into a ball-like structure which occludes the lumen of the vessels and tracheids (Harper *et al.*, 2012). Tyloses are commonly observed in the heartwood vessels of many hardwoods species, but they can also be found in the sapwood of some of hardwood species and in some softwood species (Siau, 1984; Harper *et al.*, 2012). They occur during the compartmentalization of decay in living trees (Shigo, 1984) and usually function as barriers to retard or avoid the spread of pathogenic agents within trees (Yamada, 2001; Harper *et al.*, 2012). They may also form around wounded regions of trees in the heartwood in order to avoid loss of water, even when the wood is not being decayed (Yamada, 2001; Harper *et al.*, 2012). According to Talboys (1964), tyloses can be formed in uninfected wood areas immediately the tree is being invaded by a fungal pathogen. The frequency of tyloses formation in trees has been reported to vary

positively with increase in fungal invasion as discovered by Collins and Parke (2008) in *Lithocarpus densiflorus* wood, basically for the building up of mechanical barriers against the advancing fungal hyphae. Bell (1980) also suggested that as part of their defensive function in blocking the pathway against the movement of the invading pathogens within the wood vessels, they also allow for the accumulation of toxic extractives, preventing them from dilution by transpiration stream. While tyloses are typically mechanical barriers produced in response to invading fungi or pathogens within wood in order to curb their spread, their effectiveness in functioning in this regard may be limited as certain species of fungi have the ability to penetrate through this barrier (Harper *et al.*, 2012). This has been observed to be as a result of these strains of fungi being able to produce enzymes capable of breaking down suberin in plants (Ofong and Pearce, 1994).

In situations where the vessels contain these various occlusions, available evidence from literature indicated that fibres and rays could act as alternative channels for fluid conduction in hardwoods. Greaves and Levy (1978) in their study observed high concentrations of CCA and creosote in the ray tissues of some treated hardwoods. Behr *et al*. (1969) also affirmed the importance of ray parenchyma cells in radial flow in some hardwood species. On the contrary, Teesdale and Maclean (1918) noted that ray cells in hardwoods play an insignificant role in lateral distribution of creosote preservative. Fibres, on the other hand, have also been reported to be the essential pathway for treatment uptake in the heartwood of hickory (Teesdale andMacLean, 1918; Thomas, 1976) where factors such as pit and vessel blockage, vessel isolation and low vessel percentage are present in the wood. Fibres are elongated, thick-walled cells with small lumen diameters, having length ranging between 600-2,300 μm (Wang and DeGroot, 1996). Inter-fibre movement of treating fluids via pits has also been reported by Behr *et al*. (1969).

Transportation of fluids between cells occurs through the bordered and half bordered pit pairs in hardwoods (Siau, 1984). There is the absence of the torus membrane in the bordered and half-bordered pits of hardwoods as opposed to those of the softwoods (Kollmann and Cote, 1968; Siau, 1984; Butterfield, 2006). In addition, the pits of hardwoods are generally smaller than those found in softwoods (Siau, 1984; Wang and DeGroot, 1996). Despite these structural differences between hardwood and softwood pits, reports from research studies reveal that the hardwood pits are nonetheless

permeable (Thomas, 1976; Cote, 1963; Siau, 1984). However, pit aspiration is also a prominent occurrence in hardwoods, but it is mostly restricted to the heartwood, together with the encrustation of the pit membranes (Panshin and deZeeuw, 1980). The interplay between these factors and the presence of cell inclusions in vessels aforementioned generally render the heartwood of hardwoods difficult to treat (Cote, 1963; Krahmer and Cote, 1963; Kumar and Dobriyal, 1993; Tripathi, 2012).

2.12 Procedures for improving wood permeability

The factors which affect wood permeability can be grouped into four: structural (wood properties such as earlywood-latewood ratio, sapwood-heartwood ratio, void volume, pit aspiration, pore size, presence of cell occlusions), chemical factors (chemical composition of the pit membrane, extractive content), physical factors (method of treatment application), and properties of the treating substance (viscosity, polarity); the former being as a result of the structural features in wood which inhibits easy movement of chemicals within wood, while the latter involves the characteristics of the treating substance that affects normal fluid flow within the wood (Nicholas and Siau, 1973; Nicholas, 1977). While the physical factors and characteristics of the treating substance can easily be modified in order to achieve a relatively satisfactory chemical penetration into the wood, both the wood structural and chemical factors still pose a serious challenge to the wood preservation industry. As the permeability of wood plays a critical part in the efficacy of a preservative treatment, development of methods which can improve the treatability of refractory wood will therefore significantly impact the wood preservation industry (Nicholas, 1977). However, Mai *et al*. (2004) noted that anatomical factors are mainly responsible for the reduced permeability of dried wood. Since pit aspiration is a main cause of impermeability in softwoods, means of reversing the aspiration; preventing its occurrence, or degrading membranes of the aspirated pits have been underlying focus of methods already developed (Nicholas and Siau, 1973). Hardwoods, on the other hand, are usually rendered difficult to treat when occlusions are present in considerable amount in the vessels and rays. However, research on improving the treatability of the impermeable hardwood species has received little attention. A number of studies have investigated various physical and mechanical procedures which can be employed to surmount the challenge of poor permeability in some commercial wood species (Mia *et al.,* 2004). So far, majority of these procedures are unable to considerably enhance the

permeability of the studied wood species to allow for satisfactory penetration depth of the impregnating substance (Mia *et al*., 2004). Nicholas and Siau (1973) reviewed the basic methods utilized in enhancing the treatability of impermeable wood species and grouped them into three, which include altering the wood characteristics, altering the treating process, and altering the treating solution. However, a broader review will be discussed here due to the introduction of some newly developed procedures.

2.12.1 Compression

This method is based on the ability to improve wood treatability by inducing damages to the pit membranes through mechanical compression (Nicholas and Siau, 1973; Eaton and Hale, 1993). Research investigations by Cech and Huffman (1970) and Cech (1971) revealed that wood seasoning and treatability can be considerably improved by dynamic transverse compression, which involves passing a board through rollers which reduces the thickness to 5 - 15 percent of its original thickness. It was concluded by Cech (1971) that the improvement in treatability was as a result of the mechanical damage to the pit membranes, evident from the splits in the pit membranes of the vessels of compressed yellow birch (*Betula alleghaniensis* Britton) as revealed by electron micrographs. This was similar to observations reported by Nicholas (1971) for the heartwood of Douglas fir and Gunzerodt *et al.*, (1986) for the heartwood of *Nothofagus fusca*. However, the work of Nicholas (1971) revealed that penetration of FCAP solution into Douglas fir heartwood could be influenced by different levels of compression as well as the plane of the applied compression. Results of compression on wood treatability are also affected by the wood moisture content, where lower moisture contents give a better treatability result after compression (Cech, 1971). Improved permeability in *Nothofagus fusca* heartwood was due to the combination of the rupturing of the intervessel pit membranes and tyloses in the vessels (Gunzerodt *et al.*, 1986).

Considering the effect of the direction of the applied compression on the wood, higher retentions of chemicals were obtained for the radially compressed wood than the tangentially compressed ones. This supports the theory by Cech (1971) that the improved treatability is due to the induced damages to the pit membranes. For the rupturing of the pit membrane to occur, the compression must be applied at right angle to the pits. Since the pits are majorly concentrated on the radial surfaces of tracheids,

therefore, application of the compression in the radial axis will result to more damages to the pit membranes (Nicholas and Siau, 1973). This procedure is advantageous over incising in wood treatability improvement as it avoids the damages to the wood aesthetic quality. However, it causes a significant strength loss similar in magnitude to that caused by incision (Nicholas and Siau, 1973).

2.12.2 Steaming

Steaming is one of the procedures with great potentials in opening up the wood structure of refractory wood species due to its easy adaptability in commercial processes (Nicholas and Siau, 1973). It is particularly applied to green southern pine before treatment basically because it helps in the drying process and also contributes to its improved permeability (Nicholas and Siau, 1973). For an effective result, it has been established that the treatment must be applied to green wood (Kang *et al.*, 1997). The fact that the best result is obtained when applied to wood in green condition suggests that it interferes with the process which reduces permeability during drying (Nicholas and Siau, 1973). This view was echoed by Comstock (1968) that steaming does not necessarily improve the permeability of the wood but rather operates in retaining the green permeability of the treated wood after drying. Further evidence was provided by the work of Erickson and Crawford (1959) where they observed no improved permeability of green sapwood after steaming. However, improved permeability was noticed in the steam-treated wood after drying when compared to the unsteamed wood, though the permeability was lower than the initial green wood. This only provides supporting evidence to the suggestion by Comstock (1968) on its interference with the process of pit aspiration, which is a major cause of reduced permeability in softwoods during drying. Further to this, Nicholas and Thomas (1968a), in their study, observed that the steam treatment causes an acid hydrolysis of the pit membrane components, which considerably reduces the effectiveness of pit aspiration due to the formation of weaker bonds during the process. This enables the easy reversibility of the aspirated pits during treatment, consequently improving the wood treatability (Nicholas and Siau, 1973).

However, little success of steaming in improving the sapwood and heartwood of other refractory wood species have been reported (Erickson and Crawford, 1959; Comstock, 1968). This may be as a result of variations in the chemical constituent of the pit

membranes in different species (Nicholas and Siau, 1973). For example, the pit membrane of the heartwood contains lignin-like components which are resistant to enzymes (Cote and Kramher, 1962; Nicholas and Thomas, 1968b), which implies that the pit membranes are protected by these lignin-like components against the mild hydrolysis induced by steaming. However, Kang *et al.* (1997) noted an improved treatability of pre-steamed Japanese larch heartwood and attributed the improved permeability to hemicelluloses degradation in the membrane of the aspirated pit and cell wall without the elimination of extractives from the pit membrane.

An additional drawback associated with the use of this procedure is a considerable loss in mechanical strength (Hatt, 1906; Kang *et al.*, 1997). In the work of Hatt (1906), where the effect of steaming on the Modulus of rupture, compressive strength parallel to grain, and impact strength of loblolly pine was investigated, he observed that the optimal steaming conditions for the species was 274°F (134^{0}C) for 4 hours or 259°F (126^{0}C) for 6 hours. However, AWPA Standards (1971) gave a maximum steaming temperature recommendation ranging between 104^{0}C and 116^{0}C, prohibiting the steaming of certain wood species and the use of steaming for some applications. According to Stamm (1964), most of the strength loss during drying of wood in kilns occurs above fibre-saturated point during which the wood is surrounded by steam, citing a loss of 2.5 percent in modulus of rupture for softwoods dried at 160°F (71^{0}C) for 2 days. Kang *et al.* (1997) reported a considerable improvement in the treatability of Japanese larch heartwood when steaming was performed under pressure conditions compared to when steaming under atmospheric conditions.

2.12.3 Extraction

Several studies have demonstrated that wood permeability can be significantly enhanced by extraction with various solvents such as strong acids, alkalis or chelating agents (Kramher and Cote, 1963; Mia *et al.*, 2004). This procedure improves permeability by the removal of extractives from the pit membrane, and consequently increasing the effective pit-pore size (Nicholas and Siau, 1973). This indicates that this procedure may only be effective in heartwood of refractory species. Although, a degree of success has been achieved with this procedure, the result of the treatment is usually accompanied by some unwanted side-effects such as strength losses, cell wall degradation, and some swelling of the wood (Tschernitz, 1973; Kumar and Morell,

1993). Also, there have been arguments against the use of this procedure on a commercial scale before subsequent treatment of the wood with preservatives which is associated with the cost of the solvents (Nicholas and Siau, 1973). The minimal improvement in wood treatability obtained through the use of this method cannot be justified owing to the high cost of the solvents, prolonged time for treatment, and the inclusion of an additional step in the wood treatment process (Nicholas and Siau, 1973).

2.12.4 Chemical modification

It is believed that this procedure works by either increasing the effective pore size of the pre-treated wood or through the modification of the chemical components of the pit membrane after pre-treatment with a chemical reagent (Nicholas and Siau, 1973). The use of chemical reagents such as sodium chlorite, pulping liquors, acids, and bases have proven to be effective in this regard (Kramher and Cote, 1963). However, a major demerit of the process is that it results into significant strength loss in the wood (Nicholas and Siau, 1973). Another alternative of the chemical modification process which has been studied is the gas-phase treatment. For this procedure, it is more advantageous than the use of liquid reagents because of its compatibility with a commercial treating operation due to the rapid penetration of the gas through the wood, leaving no residual liquid in the wood that must be expunged before the application of preservative treatment (Nicholas and Siau, 1973). A study by Lantican *et al.* (1965) revealed an improvement in permeability of western redcedar heartwood when treated with ozone. However, ozone is detrimental to wood in that it also attacks the cell walls, causing a significant loss in strength properties as a result (Lantican *et al.* 1965; Nicholas and Siau, 1973). The inability of these chemical reagents to induce a selective degradation of the pit membranes during wood pre-treatment therefore makes the procedure partially acceptable. While the treatment still induces more attack on the pit membranes than the other wood cells, the approach is still of great potential and further research may identify a better way of utilizing the process in wood treatability improvement (Nicholas and Siau, 1973).

2.12.5 Incising

This process involves the mechanical rupture of wood cells by drilling or making series of slit-like holes on the wood surfaces at periodic intervals either along or across

the grain direction of the wood (Nicholas and Siau, 1973; Eaton and Hale, 1993; Richardson, 1993). It commonly employs the use of a mechanical incising machine which consists of teeth made up of either needles or knives that are driven into the wood during the incising process (Rahman *et al*., 2011). It is one of the most potent procedures of improving wood treatability, while it is also cost-effective (Nicholas and Siau, 1973). The depth of incision in the wood varies with the wood species as well as the timber thickness. This ranges from 4 mm for timber of small dimensions to 20 mm for large size timbers over 75mm in thickness (Eaton and Hale, 1993). For poles, where the priority is the protection of the portion of the wood in contact with the ground, the incision may be restricted to the portion of the wood around the ground-line zone (Eaton and Hale, 1993). Generally, this procedure is particularly important in improving the permeability of species with good longitudinal permeability but poor with lateral permeability (Eaton and Hale, 1993). The penetration depth is dependent on the depth of incisions, with the treatment usually extending slightly below the depth of incision (Eaton and Hale, 1993; Morrell *et al.*, 1998). However, of paramount interest is that the preservative should be uniformly distributed within the incised area (Eaton and Hale, 1993). It is also advantageous in that it helps to relieve internal stresses built up within the wood during drying and thus, reduces the risk of checks in wood during service (Eaton and Hale, 1993). However, it has some disadvantages such as the production of rough surfaces on the wood, loss in strength in timber with small dimension, and creation of water traps by the incision holes which constitute potential avenues for fungal invasion of the wood (Nicholas and Siau, 1973; Lehringer, 2011, Thaler *et al.*, 2012). Depending on the wood's end use, this procedure may still be adopted as the above disadvantages may not be of significance in some applications. The development of a more sophisticated incising technique using laser incision systems has recently gathered momentum (Goodell *et al.*, 1991).

2.12.6 Microorganisms and enzymes

Increase in the permeability of ponded logs during storage has been reported due to the build-up of bacteria within the wood during ponding (Eaton and Hale, 1993). A reduction in the bacteria degraded wood pectin content was reported, and anatomical investigation further provided evidence of this as there was degradation of the pectin-rich bordered pits of softwoods species from scanning electron microscopy (Greaves, 1970). Several studies have further investigated the use of microorganisms and

enzymes in opening up the wood structure of refractory wood species (Schmidt and Liese, 1994; Lehringer *et al*., 2009a; Panek and Reinprecht, 2011; Pánek *et al.*, 2013). Research in this area was initiated by Lindgren and Harvey (1952) after discovering that the permeability of the sapwood of southern pine was enhanced by *Trichoderma* mold. Subsequent works in this line of wood permeability improvement have also demonstrated that bacteria and fungi are effective in improving wood permeability (Yildiz *et al*., 2012; Panek *et al*., 2013). Observations from these studies have pointed to the fact that permeability improvement in the studied wood species was due to the degradation of the ray cells and pit membranes. Further investigation revealed that the degradation induced on the ray cells and pit membranes by these organisms was by enzymatic activities (Nicholas and Siau, 1973). It was also reported that permeability improvement was, in addition, due to the opening of some liquid flow pathways such as resin channels and intercellular spaces within the wood (Militz, 1993b). Since these microorganisms operate through enzymatic activities in wood, this led to the use of enzyme treatments in improving wood permeability. The compositions of the pit, which are mainly pectins, various hemicelluloses and cellulose (Schwarze *et al.*, 2006), have further encouraged research in this area as they can easily be degraded by a host of enzymes. A study conducted by Nicholas and Thomas (1968b) revealed a considerable rise in the sapwood permeability of loblolly pine owing to the attack induced on the pit membranes by several enzymes. In order to reduce the long period needed for the permeability improvement process, Militz and Homan (1993) proposed the mixing of the various hydrolytic enzymes with the liquid wood preservative such that the activity of the enzymes are not nullified, as this will make it more practically feasible. The use of microbes and enzymes in improving wood permeability holds great potentials in the wood industry simply because of their selective ability during attack, which consequently causes negligible strength losses (Nicholas and Siau, 1973). However, the major limitation observed from most of these studies on the use of microorganisms and enzymes in wood permeability improvement is that there is little activity in the heartwood of impermeable wood species as compared to that of the sapwood. This makes them not too suited in improving heartwood permeability, although, a few studies have reported otherwise (Schwarze *et al.*, 2006). The influence of ponding on permeability improvement of some spruces has been extensively studied (Unligil 1971; Banks, 1970; Fowlie and Sheard, 1983). A full sapwood penetration, increase in creosote and CCA retentions by 179% and 50%, respectively, and a 5.4%

reduction in modulus of rupture after 4 months of ponding was reported by Unligil (1971). According to Fowlie and Sheard (1983), the ponding process was considered too slow to be adopted commercially. Bacteria and enzyme treatment of refractory species could not be commercially adopted as it takes a long time to achieve a significant permeability improvement, coupled with the difficulty in achieving a uniform preservative distribution within the wood as a result of the neutralizing effects of the adhering extractives to the aspirated pits on the rapid and homogenous ingress of the introduced enzymes and microbes (Mai *et al.*, 2004; Lehringer *et al.*, 2009b). However, a report by Panek and Reinprecht (2011) slightly contradicts the assertion that bacteria takes long time to improve the permeability of ponded wood as they observed a significant wood permeability improvement in the sapwood of spruce wood after one week of attack by the bacterium *Bacillus subtilis*, while the heartwood remained unchanged. Further exploration of the use of these microorganisms for wood permeability improvement continues to show great promise in the wood preservation industry.

2.13 Fungal bioincision of refractive woods

An advantage of the use of fungi over isolated enzymes and bacteria in wood permeability improvement lies in their higher mobility (Lehringer, 2011). White-rot fungi are capable of conveying their enzymes deep into the wood using their hyphae during wood colonization (Lehringer *et al*., 2009b). During the substrate colonization, the fungal hyphae primarily move through the parenchyma (which are rich in nutrients) and ray cells to link with the adjacent tracheids through the connecting simple and bordered pits. An alternative pathway during colonization also includes boring through the cell walls (Lehringer *et al*., 2009b). In hardwoods, however, fungi often invade the wood by moving through the vessels and wood rays (Zabel and Morrell, 1992). During incipient attack, they selectively degrade the pit membranes by modifying their chemical structure, thereby causing little or no substantial loss in strength (Lehringer *et al*., 2009b). Trichoderma species increased some wood species' permeability (Johnson and Giovik, 1970). A screening programme conducted on the Trichoderma species revealed that they produce cellulase, pectinase and amylase enzymes, and among all the Trichoderma species examined, only *T. viride* and *T. aureoviride* showed promise for further wood permeability improvement studies (Mai *et al.*, 2004). Uptake of preservative was substantially improved in the fungal

inoculated sapwood of spruce (*P. abies*) logs after 4 weeks of storage at 25°C, but no improvement was observed in the heartwood, even after extended exposure periods (Rosner *et al.*, 1998). Further experiments investigated the effect of *Dichomitus squalens* and *Phanerochaete chrysosporum* (both are selective delignifying white rot fungi) on permeability of spruce wood. Both the sapwood and heartwood had a significantly higher preservative uptake after 2-3 weeks of incubation (Rosner *et al.*, 1998). Rapid colonization of the wood substrate by selected fungi species such as Phanerochaete, Dichomitus, Trichoderma, Gliocladium species can be enhanced by the thermal sterilization of the wood surface (Mai *et al.*, 2004). Similarly, Norway spruce and white fir exposed to *Physisporinus vitreus* (a white rot fungus) for 6 weeks had both their sapwood and heartwood permeability significantly improved, with an insignificant drop in the impact bending strength (Schwarze *et al.*, 2006). This process was afterwards termed "bioincising" and patented by Schwarze (2008).

Bioincision, as defined by Lehringer *et al.* (2009b), is a short-term, controlled, microbial decomposition of wood, geared towards improving its permeability. Enhanced preservative uptake of about 200-400% was observed in Norway spruce and Fir heartwood after bioincising with *Physisporinus vitreus* (Schwarze *et al.*, 2006). This was reported to be due to the selective degradation of the membrane of the simple and bordered pits of the ray parenchyma cells and tracheids, respectively, brought about by the enzymatic secretions of the fungus. The authors further suggested that the enzyme secretions by the fungus contained polygalacturonase and oxalic acid, which promoted the hydrolysis of the pectin-rich membranes of the bordered pits during incipient wood decay. This fungus species has been observed to possess a high tolerance for extremely moist conditions (Lehringer *et al.*, 2009b). During the first period of wood colonization, selective degradation of the pit membranes occur, which is thought to be a strategy to elevate the substrate moisture content (Schwarze and Landmesser, 2000), in order to facilitate the rapid and efficient hyphae distribution within the wood (Lehringer *et al.*, 2009b). The removal of the pit membranes and the increase in the moisture within the substrate also enhances the distribution of lignolytic enzymes (Lehringer *et al.*, 2009b). During the second period of substrate colonization, however, cell wall degradation is initiated, which incurs substantial weight and strength losses in the wood (Lehringer *et al.*, 2009b). Thus, the bioincision process is typically limited to the early period of wood colonization (incipient decay) where

severe damages to wood is prevented, but strictly restricted to changes within the wood that significantly improves the wood permeability (Lehringer *et al.*, 2009b).

The dimension of the wood specimen has been reported to influence a homogenous permeability improvement in wood bioincised with fungi (Schwarze *et al.*, 2006). They used two dimensions of Norway spruce in their investigation: 100 mm x 10 mm x 15 mm (15 cm^3) and 100 mm x 25 mm x 15 mm (37.5 cm^3), both aligned in the longitudinal, tangential and radial axis, respectively. When these samples were bioincised with the fungus *Physisporinus vitreus*, a more heterogenous fungal activity was noticed in the larger wood specimens. Also, the directional plane of the wood has equally been observed to influence the bioincision process (Kleist and Seehann, 1997; Schwarze *et al.*, 2006). A higher preference for the tangential walls degradation of Norway spruce than the radial walls by the white rot fungus *Stereum sanguinolentum* was observed in the study conducted by Kleist and Seehann (1997). It is believed that the structural attributes of the tangential walls are responsible for the preferential degradation of this wood axis (Kleist and Seehann, 1997). This observation was corroborated by reports of schwarze *et al.* (2006) where they discovered that *Picea abies* and *Abies alba* wood specimens were homogeneously colonized when incubated with their tangential surfaces, in comparison with those incubated with their radial surfaces. Thus, it is believed that the radial surfaces of wood are somewhat resistant to fungal attack (Kleist and Seehann, 1997; Schwarze *et al.*, 2006). Since bioincision is a short-term incubation with fungi, faster wood colonization and shorter periods of bioincision is therefore recommended, which could be achieved by incubating wood with fungi with their tangential surfaces (Lehringer, 2011).

Whilst a considerable number of research have been conducted on the improvement of permeability of refractory softwoods through bioincision, difficult to treat hardwoods have received little or no attention with this procedure of wood permeability improvement. Fungi species identified to be suitable in bioincising softwoods for improved permeability may not be appropriate for permeability improvement of refractory hardwoods, as the main cause of their impermeability differ (Kumar and Dobriyal, 1993; Flynn, 1995; Wang and DeGroot, 1996). For instance, the fungus *Physisporinus vitreus* have been reported suitable for bioincising some common refractory softwood species, but it negatively affected permeability in the normal and tension wood of Poplar, which is a refractory hardwood species (Emaminasab *et al.*,

2015). Owing to this, identifying fungi species potentially suitable for improving the permeability of refractory hardwoods and softwoods usually considers the decay pattern of the fungus and its ability to degrade the structures responsible for poor permeability in the wood (Emaminasab *et al.*, 2015), although some authors base their selection on the fungus ability to rapidly colonize the wood species to be bioincised (Thaler *et al.*, 2012).

In a research work conducted by Emaminasab *et al.* (2015) to enhance the radial permeability of normal and tension wood of poplar which is considered to be difficult to treat due to the vessel characteristics in the tension wood zone (Tarmian and Perre, 2009), they bioincised both wood type with *Xylaria longipes* (soft-rot) and *Physisporinus vitreus* (white-rot) fungi for 15, 30 and 45 days. Their choice of *Physisporinus vitreus* fungus was due to the fungus ability to selectively degrade pectin, which has been reported by different studies which investigated its suitability in bioincising refractory softwoods (Thaler *et al.*, 2012; Panek *et al.*, 2013; Emaminasab *et al.*, 2016). On the other hand, *Xylaria longipes* fungus was equally investigated because it has been described as a selective fungus with a type II soft rot, often neglecting the middle lamella of the wood during degradation. It was observed that both fungi further caused a permeability reduction in the wood due to the blocking of the vessel lumina by their hyphae. *Physisporinus vitreus* fractured the gelatinous cell wall layer and slightly degraded the middle lamella and cell wall, while *Xylaria longipes* degraded the wood cell wall and middle lamella, with both fungi inducing a significant loss in mass and compression strength in both bioincised wood species. Thus, both fungi were concluded unsuitable for the bioincision of poplar wood.

However, an interesting aspect of selecting fungi species for bioincision is the possibility of fungi species altering their decay pattern when colonizing different wood substrate. This makes it difficult to conclude on the suitability of a fungus species for bioincising a broad range of refractory hardwood or softwood species, despite it showing an encouraging wood permeability improvement for a particular wood substrate. A major reason attributed to this has been the differences in the anatomy and wood conditions of different wood substrates (Schwarze and Fink, 1998). For example, Schwarze and Fink (1998) demonstrated the different pattern of decay induced by isolates of *Meripilus giganteus* (white-rot fungus) in beech wood and large-leaved lime wood. In their study, they reported that the fungus degraded preferentially the pectin-

rich regions within the substrates, but there was homogenous breakdown of the middle lamella in the large-leaved lime xylem rays due to the concentration and homogenous distribution of pectin within the middle lamella, while that of beech was more localized. Differences in decay pattern were equally reported for the same fungus in the normal wood and tension wood of both species, as well as in the artificially inoculated and discoloured wood (living tree) of both species. Also, studies conducted on the bioincising potentials of *Xylaria longipes* and *Physisporinus vitreus* in permeability improvement of tension and compression wood of poplar and Douglas fir, respectively, by Emaminasab *et al.* (2015). Emaminasab *et al.* (2016) revealed some slight changes in the degradation mode of both fungi species. In poplar, there was degradation of middle lamella by both fungi with the absence of cell wall cracking observed, while in Douglas fir, the middle lamella remained relatively unaffected with micro-cracks to large cracks observed in the cell wall after incubation for 45 days for both fungi. These slight changes in the activities of a fungus within different wood species may induce unwanted degradation in the wood, which may make fungus unsuitable for the intended purpose. Therefore, additional researches need to be embarked on so as to determine the suitability of a particular fungus for several wood species.

Furthermore, Eriksson *et al.* (1990) reported that anatomical differences in wood govern the mode of degradation of white rot species. The fungus *Inonotus dryophilus*, a white rot fungus species notable for heart rot decay in living trees of Oaks (*Quercus spp*.) causes a white-pocket rot within the tree. The decay in the wood commences with the attack of the large earlywood vessels and penetration of the tylosic occlusions within the vessel lumina (Otjen and Blanchette, 1982; Eriksson *et al.*, 1990). An identical decay mode has been reported for *Ganoderma tsugae* (a white rot fungus) in the wood of *Tsuga Canadensis* (Blanchette, 1984a). Contrary to this pattern of degradation, *Phellinus pini*, a white rot fungus which commonly causes heart rot in living trees of gymnosperms, commences the wood degradation by preferentially attacking latewood cells in Pinus and Larix species, leaving the early wood cells intact even during advanced stage of decay (Blanchette, 1980a). This implies that the different variations in the anatomical structures of different wood species can influence the decay pattern of different fungi. Varying mode of decay for different fungus-host combinations have also been documented by Schwarze *et al.* (2004). Similar to reports

on the degradation pattern of *Physisporinus vitreus*, consistent observations on the pattern of degradation of a particular fungus for a broad range of wood species is essential to help ascertain its suitability for the bioincision of refractory wood species.

Information on the permeability improvement of quite a number of economic refractory hardwood species still remains inadequate. An example is *Gmelina arborea* heartwood, which has been reported to be poorly permeable to chemical impregnation (Owoyemi and Kayode, 2008; Olajuyigbe *et al.*, 2010; Owoyemi, 2010; Moya and Berrocal, 2015). The reasons attributed are due to the presence of vessel tyloses, and extractive depositions which incrust and encrust the wood cells (Sulaiman and Lim, 1989, Ahmed and Chun, 2007; Adeniyi *et al.*, 2017). Ahmed and Chun (2007) reported that there was good longitudinal permeability of safranin within the impregnated wood but the sapwood was more permeable than the heartwood. Olajuyigbe *et al.* (2010) observed a marked difference in the chemical absorption between the wood of Obeche and Gmelina after treatment with Cuprinol clear and heartwood extracts of *Tectona grandis*. This difference in absorption was attributed to the differences in their permeability, as Obeche wood is described as a highly permeable wood (Adetogun and Adegeye, 2002). Similarly, the weight percent gain of *Gmelina arborea* sapwood impregnated with different methanolic concentrations of cashew nut shell liquid (CNSL) resin was condiderably lower than that of Scots pine and Obeche (Ashaduzzaman *et al.*, 2013). The inability to properly impregnate this species has great implications on its utilization for outdoor usage, most especially for structural ground-contact applications. In an attempt to improve its impregnation with preservative treatment, Owoyemi and Kayode (2008) investigated the effect of mechanical incision on the preservative absorption of the heartwood when impregnated with CCA, creosote and CNSL. They reported an increase in preservative absorption in the incised wood for all the preservative types. However, the observed increment in the incised wood for each of the preservative class was minimal, implying that this procedure may not be that efficient in enhancing adequate chemical uptake for a better field performance of the wood (Hill, 2006; Lehringer *et al*., 2009b; Lehringer, 2011). Hence, bioincising the wood with a fungus species capable of degrading these occlusions responsible for the poor permeability of the wood can be a significant development in the preservative wood industry.

2.14 Effect of fungal bioincision of wood on wood properties

Fungal attack on wood leads to the degradation of the wood constituents, principally the cellulose, hemicelluloses and lignin, consequently affecting the wood properties (Zabel and Morrell, 1992). Bioincision is a short-term degradation of wood by microbes such as fungi, and as such, the wood pre-treatment with fungi is limited to the incipient stage of decay. At this stage of decay, a visible evidence of wood degradation is absent (Zabel and Morrell, 1992), although, significant changes in some wood properties may occur (Otjen and Blanchette, 1982; Malakani *et al*., 2014). It should however be noted that the extent of these wood property changes depends on the period of exposure to decay (Curling *et al.*, 2002; Lehringer *et al.*, 2010). The effect of fungal incision on wood permeability has been extensively discussed in the preceding sections of this paper but other alterations in wood properties during and after wood bioincision with fungi are discussed below.

2.14.1 Physical properties

Most of the physical property alterations in the wood are brought about by chemical and anatomical changes in the wood during the process of decay (Zabel and Morrell, 1992). Weight or mass loss is one of the alterations that occur in the bioincised wood physical properties (Curling *et al*., 2002; Lehringer *et al.*, 2010). Also, there is notable reduction in the density of the wood. As reported by Zabel and Morrell (1992), there is a significant weight loss and little reduction in wood volume of white rot fungi degraded wood while a considerable wood volume change is notable in wood invaded by brown rot fungi, making comparability of density reduction between these two decay types difficult.

Furthermore, the hygroscopicity of the wood is altered when fungi degrade the lignin-polysaccharide wood components. When this occurs, the water-holding capacity of the cell wall of the wood is affected, which in turn, influences the EMC of the wood (Zabel and Morrell, 1992). However, the normal wood EMC is usually higher compared to that of a brown-rotted wood, while it is lower than that of wood degraded by white-rot fungi when wood weight losses are beyond 60% (Zabel and Morrell, 1992). This reduction in EMC of brown-rotted wood is thought to be due to the preferential degradation of amorphous cellulose, which is considered to have a higher moisture-holding capacity than the crystalline cellulose. For the white-rotted wood,

EMC is relatively stable during incipient decay, possibly as a result of the uniform decomposition of all the wood cell wall constituents. However, an increase in EMC during the latter decay stage suggests a preferential degradation of the crystalline cellulose (Zabel and Morrell, 1992).

2.14.2 Calorific value, Acoustic and Electrical properties

Microbial action on wood substrate results in the production of microbial biomass (ingested as food), water, carbondioxide and metabolic wastes products (Zabel and Morrell, 1992). This tends to have an effect on the calorific value of the wood. Scheffer (1936) stated that the decrease in density of the wood should be directly proportional to the reduction in calorific value of the decayed wood. Also, the ability of wood to transmit or emit sound is negatively affected after fungal colonization (Noguchi *et al.,* 1986). For instance, Schwarze *et al.* (1995) noted a 52% decline in the sound velocity of beech wood after incubating it for 12 weeks with *Fomes fomentarius* (a white-rot fungus). Thus, the selection of bioincising fungi capable of improving wood acoustic properties and preserving the wood stiffness are often restricted to soft-rots and some white-rot fungus strains capable of inducing a soft-rot type degradation (Liese and Pechmann, 1959). Similarly, the electrical property of wood is altered after fungal attack (Zabel and Morrell, 1992). Being a good insulator in comparison to other construction materials like steel, degradation in wood induced by fungal attack increases its electrical conductivity (Richards, 1954).

2.14.3 Anatomical properties

The anatomical structures of wood are affected after fungal degradation of the wood. However, the degree of degradation of different cell types and cell wall layers varies for different fungi species (Gilbertson, 1980, Adenaiya *et al.*, 2016). Investigations on the wood-fungus interaction have indicated that fungi induce some damages to the cell wall structure and pit membrane degradation, which may be in the form of delignification, degradation of the pectin-rich membranes of the pits and the cell wall carbohydrates (Schwarze and Landmesser, 2000; Lehringer *et al.*, 2010). In some species with occlusions, reports of the degradation of tyloses in some hardwoods have also been documented (Otjen and Blanchette, 1982; Schwarze and Baum, 2000). These varying fungal activities in wood substrates have implications on the resultant

properties of the wood such as weight loss, effect on permeability and acoustic properties, etc (Thaler *et al*., 2012, Emaminasab *et al.*, 2015; 2016).

2.14.4 Mechanical properties

The negative impact of fungal degradation on wood is inevitable; however, this can be minimized using suitable fungus species for bioincision. According to Wilcox (1978), incipient wood decay may be characterized by significant decrease in some of the wood mechanical properties, accompanied by slight losses in the chemical components and minute alteration in its appearance. Since different wood chemical components are responsible for the different strength properties of the wood, major breakdown of these components will strongly alter the different wood strength characteristics. Cellulose and hemicelluloses are principally responsible for the wood tensile and elastic properties (Fengel and Wegener, 1989; Klemm *et al*., 2005), while the lignin is mainly responsible for the wood compression strength (Silvester, 1967). Thus, a strong decline in the cellulose and hemicelluloses of the wood during fungal colonization will significantly affect the wood MOE, MOR, tensile strength and impact bending strength, while lignin removal will negatively affect compressive strength of the wood. For instance, negligible strength reduction in impact bending strength and MOR of heartwood and sapwood of Norway spruce was reported by Panek *et al*. (2013) after bioincising with *Trichoderma viride* fungus as a result of the preferential degradation of the bordered pit membranes and without any changes to the cell wall tracheids. Contrary to this, Thaler *et al.* (2012) observed a considerable loss in in compression strength (20% and 30%) of Norway spruce wood bioincised with *Antrodia vaillantii* (brown rot) and *Hypoxylon fragiforme* (white rot) fungi, respectively, after 30 days. Similar reports were documented by Emaminasab *et al.* (2015), where a loss in compressive strength of about 51% was observed in the tension wood of poplar exposed to the soft rot fungus *Xylaria longipes* after 45 days due to excessive cell wall degradation and delamination of the wood cells.

2.14.5 Chemical composition

A wood species' chemical composition is one of the factors which affect the rate of decay of the wood by a decay fungus (Scheffer and Cowling, 1966). Brown-rot fungi principally degrade wood hemicelluloses and cellulose with minimal lignin loss (Curling *et al*., 2002). Their activity is mostly pronounced in the wood cell wall S_2

layer during incipient wood decay due to the lower lignin concentration in this region, consequently making the polysacharride components easily accessible to the fungi (Curling *et al.*, 2002). White-rot fungi, on the other hand, predominantly degrade the lignin component of the wood or may degrade the lignin and carbohydrate fraction at relatively similar rates (Eriksson *et al.*, 1990; Kubicek, 2013). This indicates that during wood decay, there is a commensurable loss of the wood chemical components, irrespective of the fungus type attacking the wood. Lehringer (2011) reported reduction in the lignin, glucan, xylan, mannan, arabican, galactan composition of the heartwood and sapwood of Norway spruce bioincised with *Physisporinus vitreus* between 3 weeks and 9 weeks. This was similar to the observations reported by Winandy and Morrell (1993) and Bari *et al.* (2015).

2.15 Factors influencing wood bioincision

The growth rate of wood decay fungi changes over a period of time, and this growth rate is best illustrated by a sigmoid growth curve (Zabel and Morrell, 1992; Lehringer, 2011) as shown in Figure 2.4 below. Generally, a rapid growth rate and a brief lag phase of the bioincising fungus are required in order to achieve an efficicient bioincision (Lehringer, 2011). The lag phase connotes the time interval between the decay fungus introduction on the wood and the time an exponential breakdown of the wood becomes obvious. The incubation period for the bioincising process usually occurs within this phase and needs to be terminated at the end of this phase, or in some cases, may extend to the early log phase. Extending the incubation period to later stages may incur major wood strength losses (Lehringer, 2011).

The lag phase is characterized by low wood degradation, as the fungus at this stage adapts to the environmental conditions within the wood substrate. A moderate secretion of enzymes and the metabolism of freely available carbohydrates also occur, which causes insubstantial damages to the wood cell wall. During the log phase, however, there is a dramatic surge in fungal activity within the wood substrate. At this stage, the mycelium of the fungus is well established and hyphal growth proceeds rapidly through the axial parenchyma and vessels in hardwoods, or tracheids in the

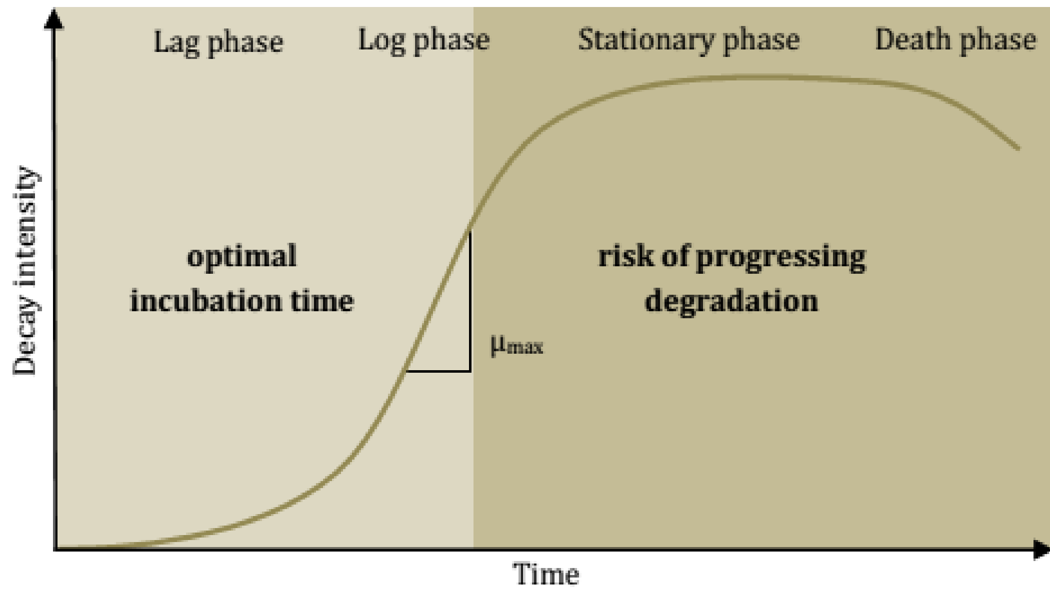


Fig. 2.4: Growth curve of a decay fungus (Lehringer, 2011)

softwoods, until the intensity of decay attains the stationary phase. At the stationary phase, the activity of the fungus is at a stable level, where subsequent nutrient limitation within the wood substrate causes a decline in hyphal growth and intensity of decay (Lehringer, 2011).

An understanding of the critical factors which facilitates wood degradation by fungi is particularly important, as this can help to proffer control or preventive measures in reducing wood degradation. Also, knowledge of the growth requirements of fungi is essential for the optimization of industrial processes such as in fermentation and other biotechnological processes involving the use of fungi (Zabel and Morrell, 1992). Fungi, like all living organisms, require some essential growth conditions to enable them to thrive well. These factors are discussed below:

2.15.1 Water

Water plays a key role as a reactant in the process of hydrolysis during the extra cellular digestion of the cell wall carbohydrates, enabling the break down of the glycosidic bonds of the wood cell wall carbohydrates. It also constitutes the medium for the secreted hydrolytic enzymes from the fungal hyphae as well as for the dissolved substrate molecules produced after hydrolysis of the cell wall carbohydrates which are subsequently absorbed by the fungus. Furthermore, it represents the solvent for the several enzymes present in the fungal hyphae needed to drive the process of cell metabolism such as synthesis, growth and respiration of the fungus. Water is also important as a swelling agent in the wood capillaries, which enables the deep penetration of the hydrolytic enzymes and free water into the cell wall of the wood (Ericksson *et al.* 1990; Zabel and Morrell, 1992). However, it must be noted that bound water in wood is usually unavailable to most fungi species and thereby affects their optimal growth (Zabel and Morrell, 1992). Thus, for fungi to develop effectively in the wood substrate, there must be presence of some free water in the wood cells. On the other hand, water saturation of the wood deprives the wood of the required oxygen for growth. This implies that optimal fungal growth (depending on the species) occurs within a moisture range in wood, which is estimated at 60-80% (Scheffer, 1973).

2.15.2 Oxygen

As most fungi are obligate aerobes, free oxygen is required to carry out various metabolic reactions (respiration) such as energy synthesis and release (Zabel and Morrell, 1992). During this metabolic process, oxygen serve as a proton acceptor, thereby driving the oxidation of the cell wall carbohydrates and leading to energy liberation (Zabel and Morrell, 1992). The quantity of oxygen consumption is directly proportional to the quantity of carbondioxide generated. This relation between the oxygen consumption and carbondioxide production have been applied in decay studies, often used in the comparison of decay rates of fungi on different substrates (Toole, 1972). The oxygen content of wood or that in a culture flask is usually expressed in atmospheric pressure in millimetres of mercury. Several studies have investigated the effect of atmospheric gases (mainly CO_2 and O_2) on decay rates or growth of decay fungi (Toole, 1972; Scheffer, 1986). Findings from these studies revealed that oxygen is essential for fungi growth, though the oxygen concentration reported for their growth is very low (1%). However, several fungi species grow optimally at oxygen levels greater than 20% of ambient air (Zabel and Morrell, 1992).

2.15.3 Temperature

Temperature plays a major role in many fungi metabolic activities, which include respiration, assimilation, digestion, transpiration, and synthesis, which are enzyme-mediated. Within optimal temperature ranges, the rate of metabolic reaction rises as temperature increases, until some reactions occurring within the sequence begin to limit the reaction rate, or due to the denaturation of the enzymes by heat (Zabel and Morrell, 1992). Each fungus species has three cardinal growth temperatures: an optimal level (best growth), a maximal level (growth termination) and a minimal level (growth initiation). Usually, the optimal temperature skews towards the maximal temperature, most especially for fungi species with optimal growth levels. Generally, the temperature range for most fungi species falls between 0° and 45^oC. Within this temperature range, fungi species have developed an adaptation to utilize several substrates under various temperature regimes (Zabel and Morrell, 1992). Based on their temperature requirement, fungi can be grouped into three main classes: psychrophiles, mesophiles, and thermophiles. The psychrophilic (cold-loving) fungi are those considered to have a minima below 0° and a maxima of 20^oC, but have an

optimal range between mesophiles 0° and 17°C (Zabel and Morrell, 1992). This group of fungi are mainly associated with spoilage of some refrigerated foods at temperatures below 0°C, and a few fungi in the genera Cladosporium, Sporotrichum, and Thamnidium (Cochran, 1958). Thermophilic fungi are those considered to have a minima of or above 20°C and a maxima of above 50°C (Coonie and Emerson, 1964). Some fungi which belong to this group include *Chaetomium thermophila* and *Penicillium duponti*, which are often found in decomposing composts, where there is a heat build-up during the deterioration of biomass (Zabel and Morrell, 1992). Most fungi, however, belong to the mesophilic group, and this also includes the wood-inhabiting fungi species. They thrive best within a temperature range of 15 to 40°C, but they are considered to have a minima of above 0° and a maxima below 50°C Generally, wood-decay fungi are highly intolerant to high temperatures even after short exposures but are resistant to long exposures to low temperatures (Zabel and Morrell, 1992).

2.15.4 Substrate

Fungi species require a suitable substrate which provides them with the required energy (derived from the oxidation of carbon compounds); essential vitamins, nitrogen, CO_2, and minor elements; and various metabolites required for synthesizing different compounds (glucans, enzymes, proteins, chitin, etc.) essential for their growth and development (Zabel and Morrell, 1992). Several fungi possess the ability to breakdown and utilize carbohydrates, which includes cellulose. However, only the wood-decay fungi (quite a significant proportion of them) possess the ability to decompose and use up stored sugars in the cellulose-hemicellulose-lignin complex within the cell wall of wood (Zabel and Morrell, 1992). Many fungi have the ability to use carbohydrates such as cellulose, starches, and hemicelluloses but the presence of lignin which acts as a protection around these carbohydrates greatly limits the accessibility of enzymes and attack by microbes to a narrow range of wood degrading microorganisms (Zabel and Morrell, 1992).

2.15.5 pH

There is a particular pH at which optimal growth of fungi occurs, while there is a minimum and maximum pH where fungal growth ceases. The pH affects several aspects of fungal growth and development which often results into the production of

bimodal pH growth curves. For instance, the sporulation and spore germination process require a more restrictive pH. Also, high pH levels may cause the inhibition of minor metal soubilities, while low levels may lead to the alteration of the activities of exoenzymes (Zabel and Morrell, 1992). Generally, a pH range of 3 to 6 is believed to be the optimal pH for fungal growth. For brown-rot fungi, a minimum pH of 3 is required while wood-stain fungi are highly sensitive to pH, with growth cessation occurring at pH above 5 (Zabel and Morrell, 1992). During the decay process, a reduction in pH occurs due to the activities of wood-decaying fungi, and this relationship between fungal activity and pH forms the foundation of various chemical indicator tests recommended for identifying early-stage decays in utility poles and pulpwood (Zabel and Morrell, 1992).

2.15.6 Chemical growth factors

Chemical growth factors such as vitamins, nitrogen, and minor metals, and light play a key role in fungal growth. Nitrogen is essential for protein synthesis and other components such as chitin and enzymes. Fungi mostly utilize nitrates, nitrites, urea, and ammonia as their major sources of nitrogen. Minor metals and vitamins constitute the essential enzyme systems components. While some fungi possess the ability to manufacture their essential vitamins, some are deficient in some vitamins and require an external source for the synthesis of the deficient vitamins (Zabel and Morrell, 1992). Trace quantities Minor metals play a crucial role in several enzymatic reactions. For instance, manganese and iron (ferric ion) are believed to be important in the decay by brown and white-rot fungi (Zabel and Morrell, 1992). Light, on the other hand, is considered to be unfavourable to the vegetative growth of wood-decay fungi, possibly as a result of the damaging effect of the UV spectrum of light at elevated intensities (Zabel and Morrell, 1992). A study by Duncan (1967) suggested that the decay rates of fungi however, may be increased by a periodic exposure to light, where wood specimens in proximity to a periodic light source recorded about double the weight loss of specimens placed farther away from the light source. Scientific description for this occurrence is unknown as of yet, but it is thought to be as a result of temperature rise due to greenhouse effect caused by the light source (Zabel and Morrell, 1992).

2.16 *Inonotus dryophilus*

Inonotus dryophilus (Berk.) Murr. is a white-rot fungus, notable for causing a white pocket rot in the heartwood of living Oaks (Otjen and Blanchette, 1982). Morphological characteristics of the degraded cell walls of *Quercus macrocarpa* and *Quercus alba* heartwood when observed under the scanning electron microscope indicated a selective delignification of the wood. Also, middle lamella was eroded between the cells of the degraded wood, coupled with the removal of lignin from the cell walls (Otjen and Blanchette, 1982). Decayed wood, when viewed in the longitudinal plane, showed white pockets within confined areas of the degraded wood and bordered by sound wood, composed of latewood fibres and medullary rays (Otjen and Blanchette, 1982). Chemical analyses of the decayed wood revealed a massive decline in the wood's lignin content from 24.99% in the sound heartwood to 2.59% in the degraded wood. Anatomical examination of the decayed wood depicted rapid colonization of the large earlywood vessels and axial parenchyma, while the thick-walled fibres and medullary rays were unaffected, both appearing to constitute an obstacle to decay (Otjen and Blanchette, 1982). The heartwood of Oak woods are known to contain numerous tyloses in their vessels, and the anatomical investigation showed that the fungus moved through the vessels of the large earlywood and penetrated the vessel tyloses during the early stage of decay. During advanced decay of the wood, the fungus colonized and subsequently degraded the occluded cells, leading to the coalescing of the white pockets (Otjen and Blanchette, 1982). It was however discovered that the fungus was capable of exhibiting two different types of cell wall deterioration within the wood. Degradation of the axial parenchyma in close proximity to the rays followed a typical simultaneous white rot decay pattern while a selective delignification of those further away from the rays was observed (Otjen and Blanchette, 1982). The ability of this fungus to overcome mechanical and chemical barriers such as tyloses in the vessels and chemical depositions in the medullary rays of the Oak wood, therefore, makes it a potentially suitable fungus for the removal of these structural barriers in impermeable hardwoods.

2.17 *Ganoderma adspersum*

Ganoderma adspersum is commonly known to cause white heart rot in trees of oaks, limes, beech, sycamore, birch, etc (Mattheck and Weber, 2003). When incubated with beech wood (*Fagus sylvatica*), *G. adspersum* degraded polyphenolic deposits and

tyloses in the axial parenchyma and fibre-tracheids' lumina of the wood (Schwarze and Baum, 2000). In addition, it was also observed to penetrate through occluded pit chambers of the wood. Interestingly, the degradation of these occlusions within the wood cells occurred prior to it attacking the secondary cell walls, which occured at a later stage. The degradation of the polyphenolic deposits and tyloses was discovered to be almost complete after 14wks of incubation with the wood, after which the fungus switched to its typical white rot mode of decay, leading to a considerable rupturing of the axial parenchyma, bore-hole formation and the widening of the simple pits of the wood (Schwarze and Baum, 2000). This implies that this fungus has the potentials of being utilized in the bioincision of refractory hardwood species as it tends to selectively degrade occlusions within the wood cells during incipient stage of decay.

2.18 *Gmelina arborea*

Gmelina arborea Roxb. is a fast growing tree species belonging to the family Verbenacea and was introduced to Nigeria from Burma. The species is commonly found within the rainforest and deciduous forest and is tolerant to a wide range of conditions from sea level to 1200m elevation and annual precipitation from 750 to 5000mm. *G. arborea* grows best in climates with average temperature of 21-28^{0}C (Jensen, 1995), and grows best on deep, well drained and alkaline soils with a range of pH between 5 to 8. Plantation establishment of this tree species is usually geared towards the production of raw materials for saw logs, pulp production and energy (Rasineni *et al.*, 2010). It is suitable in tropical wood utilization for particle boards, plywood core stock, as pit prop timber in Enugu coal mines, matches and splints and sawn timber for light constructions. It is also useful in furniture production, manufacture of boxes and as a general utility timber for joinery (Adeniyi *et al.*, 2017).

The sapwood is whitish in colour and sometimes has a yellowish or greenish tinge while the heartwood is pale brown to yellowish brown in colour and undistinguishable from the sapwood (Adam and Krampah, 2005). It has a straight to interlocked grain with a coarse texture. The wood is generally light, having a density of ranging between 400 to 510 kg/m^3 at 12% moisture content (Adam and Krampah, 2005). *Gmelina arborea* wood has a low shrinkage when dried to 12% moisture content from green state. Okon (2014) reported a mean tangential and radial shrinkage of 6.46% and 4.47% for a 25-year-old *Gmelina arborea* wood harvested from Oluwa forest reserve

in South west Nigeria. The wood seasons well, athough it is vulnerable to slight warping or collapse and is generally stable in service when dry (Adam and Krampah, 2005).

At a moisture content of 12%, *G. arborea* wood has MOE of 5500–10,800 N/mm², MOR of 55–102 N/mm², cleavage of 12–15 N/mm, Janka hardness of 2335–3380 N, shear strength of of 5–11 N/mm², and compressive strength parallel to grain of 20–39 N/mm². The wood is easily sawn and only has a slight blunting effect on tools (Adam and Krampah, 2005). The wood is considered non-durable (Scheffer and Morrell, 1998; Adam and Krampah, 2005) or moderately durable (Chudnoff, 1984) and is not recommended for applications involving ground contact. However, the heartwood is reported to be moderately durable. It has a poor resistance to attacks by termite, marine borer and fungi (Adam and Krampah, 2005). The heartwood is highly resistant to preservative treatment due to the occlusions present in the wood (Adam and Krampah, 2005; Adeniyi *et al.*, 2017).

The growth ring boundaries of the wood may be distinct or indistinct. The wood is usually diffuse porous and the vessels perforations are simple. The intervessel pits are alternately arranged and are polygonal in shape. The mean tangential diameter of the vessel lumina is between 100 – 200 µm. The vessels contain tyloses and the vessel per square millimetre ranges from 5 – 20. Fibres have simple pits and are usually thin to thick-walled. The axial parenchyma is diffuse and vascicentric. The rays are are usually procumbent and few may be upright. There is also the presence of acicular crystals in the wood (Adam and Krampah, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling site and sample collection

Five (5) 34-year-old *Gmelina arborea* trees were harvested at the University of Ibadan Gmelina plantation located in Ajibode, Ibadan, Oyo State. Ajibode is a village located within Ibadan, which falls between longitudes 3° 52' and 3° 54' East of the Greenwich meridian and latitudes 7° 27' and 7° 28' North of the Equator (Fig. 3.1). The area is well drained and the pattern is dendritic (Adewuyi and Oladapo, 2011). The rainy season starts and terminates in April and November, respectively with usually a brief dry spell in August. The dry season falls between November and February. The average annual precipitation varies between 788 mm to 1884 mm (Farinde and Oni, 2015).

The trees were selected based on the absence of excessive knots and absence of reaction tendencies. Bolts of 300cm long were extracted from the trunk of each of the felled trees at the basal portion (0.3m from ground level). The bolts were subsequently transported to the Wood workshop Unit of the Department of Forest Production and Products (DFPP), University of Ibadan (U.I) for further conversion.

3.2 Preparation and stabilization of test blocks

The heartwood portion of the bolts were sawn into dimensions 6cm x 2cm x 2cm (length x width x thickness), 30cm x 2cm x 2cm (length x width x thickness) and 15cm x 2cm x 0.6cm (length x width x thickness) such that their radial and tangential faces were revealed, and the grains of the wood samples were longitudinally oriented (BSI, 1961). The latter sample dimensions were used only for the tensile strength test, the 30cm x 2cm x 2cm samples were used to evaluate the impact bending, MOE and MOR, while the 6cm x 2cm x 2cm were used for the evaluation of the Maximum compressive strength (//) and Janka hardness. The clear wood samples were oven-dried at 105^0C for 24 hrs to determine their initial dry weight (W_1), and were then stored in a

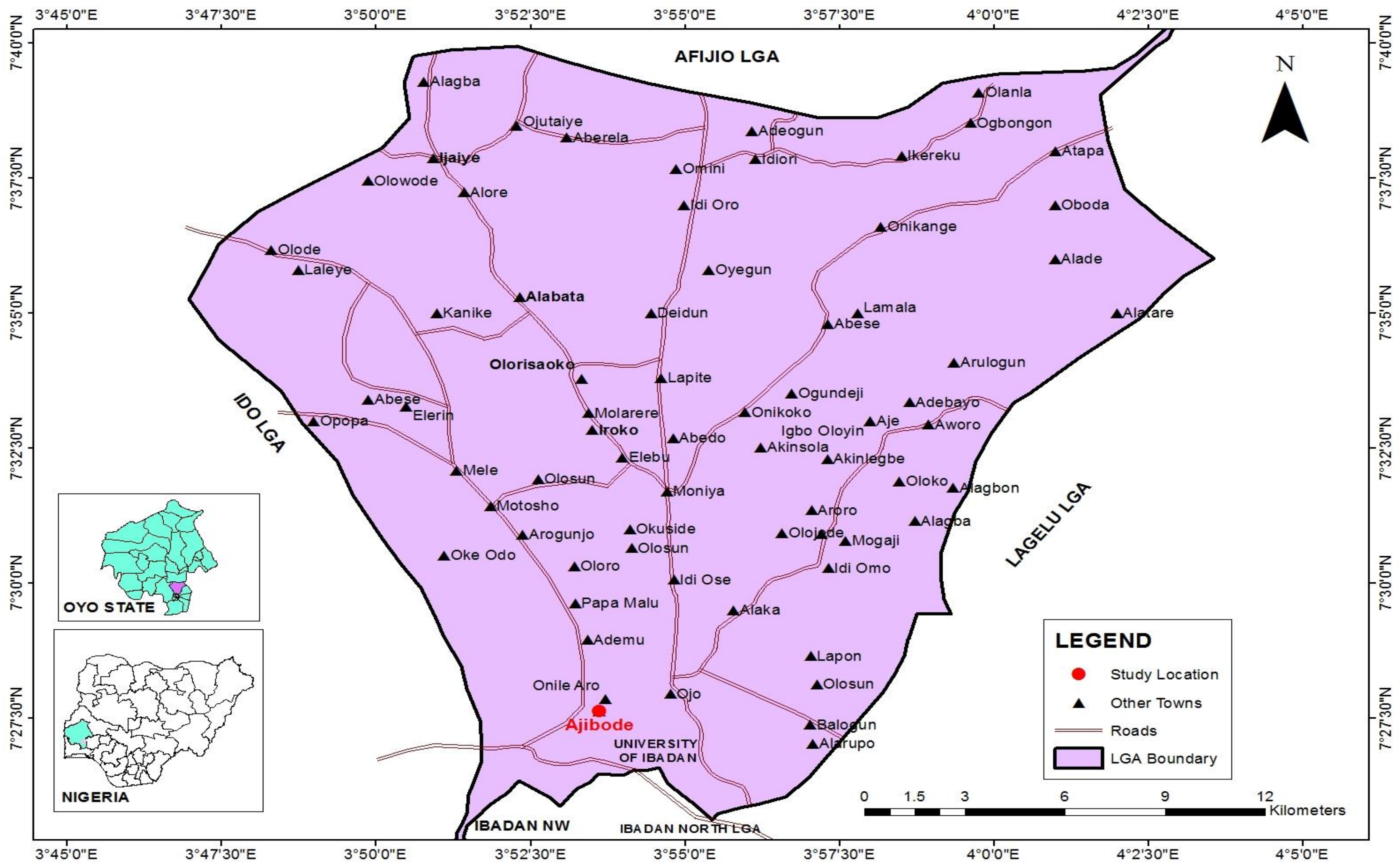


Fig. 3.1: Map of Akinyele L.G.A showing the sampling site (Ajibode)

conditioning chamber at 65% relative humidity (RH) and 20^{0}C until they attained EMC.

3.3 Procurement of fungi isolates

The fungal isolates used for bioincising the *Gmelina arborea* heartwood samples include:

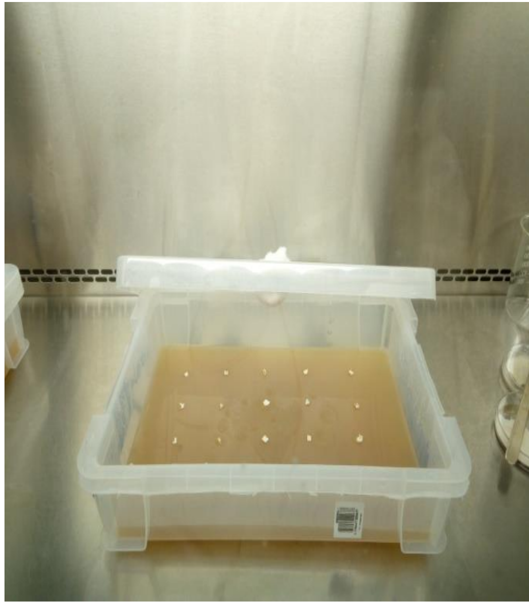
1. *Ganoderma adspersum* (Schulz.) Donk. (Isolate No. CBS109416), supplied by Westerdijk Fungal Biodiversity Institute, Netherlands;
2. *Inonotus dryophilus* (Berk.) Murr. (Isolate No. 999), supplied by the Culture Collection of Basidiomycetes, Czech Republic.

3.4 Growth Medium Preparation

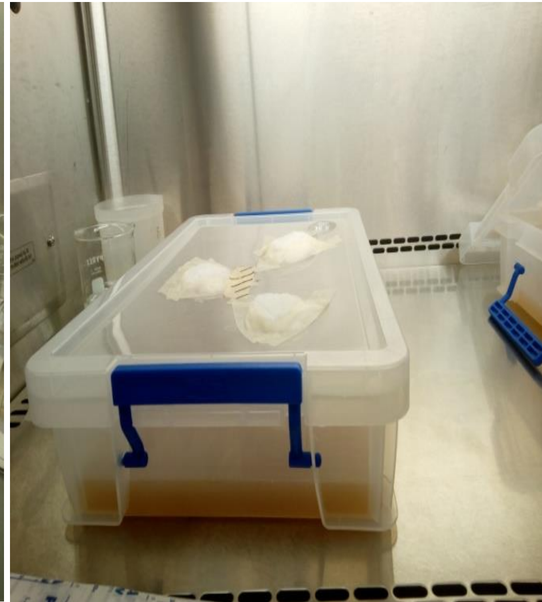
Inoculi of *Ganoderma adspersum* and *Inonotus dryophilus* were sub-cultured in a petri-dish containing 4% malt agar and incubated at 20^{0}C for 2 weeks. The two fungi species were subsequently sub-cultured in sterilized 500 mls squat jars containing 80mls of 4% malt agar extract and sterilized polypropylene boxes containing 1500 mls of 4% malt agar extract (Plates 3.1A – 3.1D). The polypropylene boxes containing the fungal inoculi were sealed round with parafilm to prevent comtamination from external sources. Both cultures in the squat jars and polypropylene boxes were all placed in an incubating room at 22^{0}C and 60% relative humidity (RH).

3.5 Bioincision of wood blocks

The exposure of the wood specimens to the bioincising fungi was in accordance with the EN 113 (1996) procedure. The growing fungus in each of the squat jars and polypropylene boxes were allowed to cover the entire surface of the agar before placing a sterilized (autoclaved) polypropylene mesh on the surface of the mycelia mat inside the jars and boxes. Prior to the exposure of the conditioned wood blocks to the fungi, they were autoclaved in empty squat jars and polypropylene boxes at 121^{0}C and 1.5 bars for 20 mins, and their tangential faces positioned directly on the polypropylene mesh inside the squat jars and polypropylene boxes which were laid over the fungal mycelia mats. This was done to prevent the leaching of the wood extractives into the nutrient media or diffusion of nutrients from the agar into the wood. Two wood blocks (6 cm x 2 cm x 2 cm) were placed in each squat jar, while ten wood samples (both samples with dimensions 30 cm x 2 cm x 2 cm and 15cm x 2 cm x



A: Fungus inoculi in agar



B: Sealed polypropylene boxes containing fungus inoculi

C: Fungus growing in squat jar

D: Fungus growing in polypropylene box

Plate 3.1: Culturing of bioincising fungi in squat jars and polypropylene boxes

0.6 cm) were incubated in each polypropylene box. For each fungus, ten replicates (n = 10) were used (two wood samples from individual trees), and were bioincised with both fungi for 3, 5, 7 and 9 weeks. Sets of sterile control were equally set up in squat jars and polypropylene boxes containing the malt agar extract without fungal inoculation for comparison with the bioincised samples. Half of the control samples were exposed for 3 weeks while the other half were exposed for nine weeks to represent the two extremes of the incubation period. The polypropylene boxes containing the fungal mycelia and wood samples were sealed round with parafilm to prevent comtamination from outside sources. At the end of each incubation period, the wood samples were extracted from each jar and polypropylene box; the fungal mycelia were meticulously scraped off the wood samples and weighed (W_2). The bioincised woods were subsequently dried at 105^0C for 24 hours, allowed to cool in a dessicator and weighed (W_3).

3.6 Moisture content determination

When the incubation periods had terminated, the bioincised wood samples were removed from squat jars and polypropylene boxes. The wood samples were carefully wiped to remove the adhering fungal mycelia and weighed. They were afterwards oven-dried at 105^0C for 24 hours, allowed to cool in a dessicator and weighed. The percentage wood moisture content was estimated gravimetrically using the formula below:

$$\boldsymbol{Moisture\ content}\ (\%) = \frac{\boldsymbol{W2-W3}}{\boldsymbol{W3}}\ \boldsymbol{x\ 100} \quad \text{..........................} \ (3.1)$$

Where, W_2 is the wet weight of the bioincised wood samples and W_3 is the oven-dried after bioincision.

3.7 Weight loss determination

Following the determination of moisture content of the samples, weight loss of the bioincised samples after oven-drying was estimated with the formula below:

$$\boldsymbol{Weight\ loss}\ (\%) = \frac{\boldsymbol{W1-W3}}{\boldsymbol{W1}}\ \boldsymbol{x\ 100} \quad \text{....................................} \ (3.2)$$

Where, W_1 is the oven-dry weight before bioincision and W_3 is the oven-dry weight after bioincision

3.8 Colour assessment

A visual assessment of the colour changes in the wood for both fungi before and after bioincision was undertaken.

3.9 Determination of absorption and retention of preservative

The bioincised samples and control specimens were impregnated with Tanalith (a water-borne preservative) by steeping, and by pressure treatment in a pressure impregnating vessel (Plate 3.2) using a full cell method with slight modification. A total number of 400 wood samples measuring 6 cm x 2 cm x 2cm (with 20 replicates for each incubation period and preservative application method) were used. Two hundred (200) wood specimens were totally immersed in a cold bath of the Tanalith preservative (5.5% concentration) for 24 hrs, after which the samples were drained and weighed, while the remaining 200 wood samples were pressure-treated with the preservative. For the pressure treatment, an initial vacuum was applied for 20 minutes, followed by the application of a pressure of 7 bars for 1 hour. A final vacuum was applied for 20 minutes and the preservative absorption and retention of the wood samples were determined as described below:

$$\boldsymbol{Absorption\ (Kg/m^3) = \frac{10^6\ x\ WCA}{1000\ x\ V}} \quad \text{.................................... (3.5)}$$

$$\boldsymbol{Retention\ (Kg/m^3) = \frac{10\ x\ C\ x\ WCA}{V}} \quad \text{.................................... (3.6)}$$

Where, WCA = Weight of preservative absorbed, V = Volume of oven-dry wood sample, and C = Concentration of treating solution

3.10 Determination of dye uptake and Penetration depth

Ten replicates per incubation period were used for determination of the chemical absorption and penetration depth. Both the bioincised samples and control samples were conditioned in the conditioning chamber at 20^0C and 65% RH until they attained a constant weight. The conditioned samples were coated with transparent water-borne lacquer in order to allow flow in the desired wood plane. Three layers of the lacquer were applied to all the wood blocks to ensure a complete sealing of the coated surfaces. In-between each layer of lacquer application, a period of 4 hrs was allowed for the curing of the lacquer. For the axial absorption and penetration, the wood blocks

Plate 3.2: Pressure impregnation vessel

were sealed at one end grain and the four sides (i.e. two tangential, two radial and one end grain). For the lateral absorption and penetration, the two end grains of the wood were sealed. All coated specimens were subsequently transferred to the conditioning chamber to stabilize at 20^0C and 65% RH and weighed (W_4). The specimens were impregnated with an aqueous blue dye (the dye used fixes to the wood when dry and allows for its traceability in the wood) dissolved in water (1:7) in a pressure impregnating apparatus using a full cell process, though with slight modification. The impregnating conditions were 20 mins of initial vacuum, followed by an application of pressure (7 bars) for 1 hour, and an application of a final vacuum of 20 mins. After impregnation, the wood specimens were extracted from the pressure apparatus, blotted with paper to remove the excess dye solution and weighed (W_5). The lateral and axial solution uptake for the wood samples was subsequently measured gravimetrically. The samples were allowed to air-dry before proceeding with the penetration tests. Wood blocks for the lateral penetration were cut transversely at the middle while those for the axial penetration were cut length-wise at 3 mm depth from the exposed tangential surface (sub-surface) and at the middle (10 mm) to expose a fresh section of the wood. The surfaces exposed were planed and the penetration depth of the wood samples in both wood directions was measured using a Leica DM6000 M microscope (Plate 3.3), with the images projected on a computer (Sint *et al.*, 2011).

3.11 Penetration area

The penetration area of the dye in the treated wood samples for both the bioincised and control samples was measured on the Leica DM6000 M microscope.

3.12 Anatomy of bioincised wood

Light microscopy of the control and bioincised wood samples was carried out using the Leica DM6000 M microscope. Wood blocks measuring 10 x 10 x 10 mm in dimension were cut from the exposed tangential surfaces of the control and bioincised samples (9 weeks bioincised samples) for both fungi. For the bioincised samples, the wood blocks were obtained from samples already treated with the blue dye in order to identify the cell types penetrated by the dye. The wood samples were soaked in water under vaccum pressure for 30 minutes until they became saturated. Thin sections of about 30 microns were obtained from the tangential, radial and transverse planes of the wood blocks with the aid of a microtome; the thin wood sections were mounted on

Plate 3.3: Leica DM6000 M Light Microscope

slides and covered with cover-slips before they were being examined under the microscope. The examination of the micro and ultrastructures of the cross-sections and transverse-radial sections of the control and bioincised samples were conducted using a JOEL JMS-840A scanning electron microscope (Plate 3.4) attached to an energy dispersive X-ray (EDS) INCA micro analytical system. Wood blocks measuring 10 x 10 x 10 mm in dimension were cut from the exposed tangential surfaces of the control and bioincised samples (9 weeks bioincised samples) for both fungi, and were soaked in water under vaccum pressure for 30 minutes. Thin slices were scaped off the surfaces of the wood blocks while saturated with water using a microtome in order to obtain a clean-cut surface and ensure a good view of the cells under the microscope. The blocks were subsequently oven-dried for 12 hrs and adhered unto aluminium stubs. The scanning electron microscopy was conducted under an accelerated voltage of 20 kv and probe current 45 nA.

3.13 Mechanical properties of bioincised wood

Impact bending strength and MOR are the commonly tested strength properties for bioincised wood as they are very sensitive wood mechanical properties (Pánek *et al.*, 2013). However, due to the potential degradation of lignin in the wood during bioincision, compression strength parallel to the grain, hardness, tensile strength parallel to grain and Modulus of elasticity (MOE) of the wood samples were also assessed. The strength properties of both the bio-incised and the control samples were determined. All the wood specimens were conditioned at 20^0C and 65% RH for 2 weeks before subjecting them to test.

3.13.1 Impact resistance

The impact resistance test was conducted using the Charpy Impact Testing Machine (Plate 3.5) following the British Standard Method BS 373 (1957) procedure. Standard test specimens measuring 100 mm x 10 mm x 5 mm (longitudinal x tangential x radial) were cut from the bioincised 20 x 20 x 300mm samples such that the samples obtained were taken from the tangential region in direct contact with the fungal mycelia. A 4-Juole pendulum was hoisted to a fixed height on the charpy testing machine and allowed to fall and strike the tangential face of the clamped specimen located at the bottom of the swing. The energy dissipated by the pendulum gives the impact energy

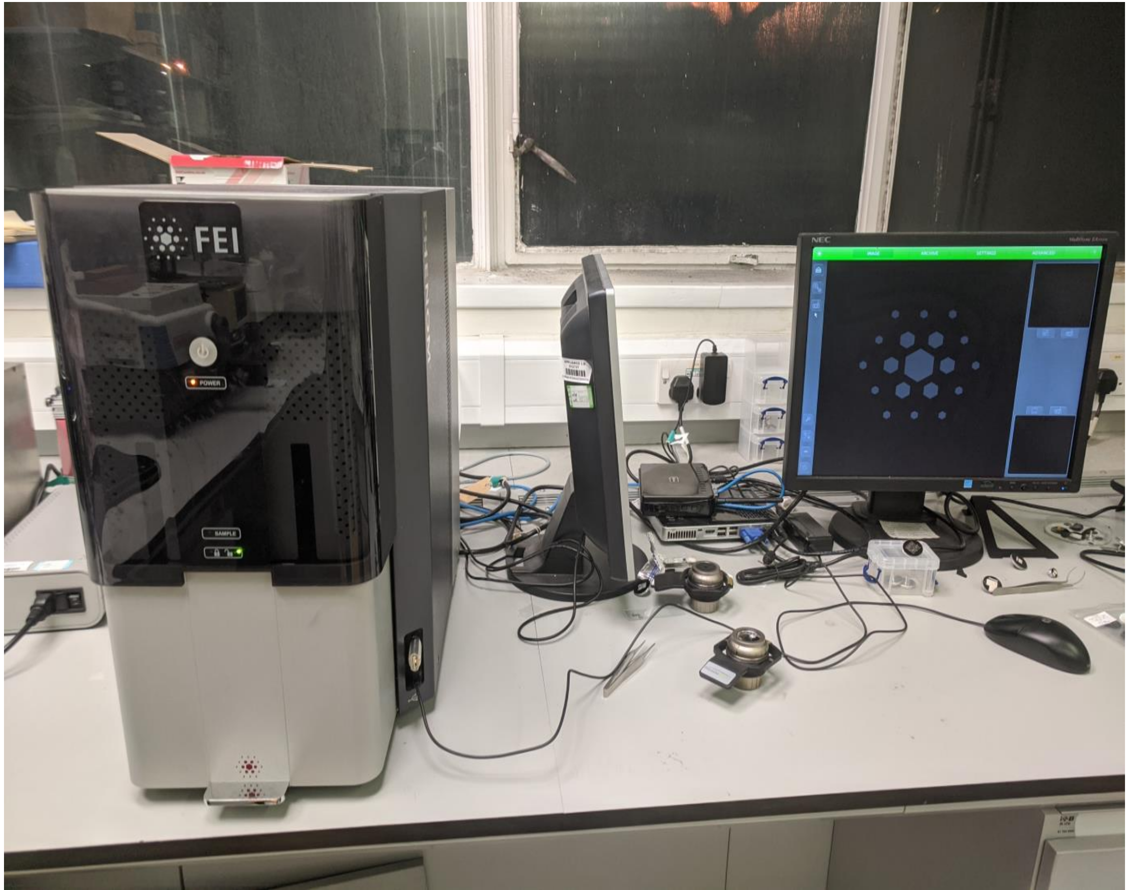


Plate 3.4: Scanning Electron Microscope (SEM)

Plate 3.5: The Charpy impact resistance tester

of the specimen. The impact resistance of the specimen was therefore estimated by the formula:

$$\textbf{Impact Resistance (J/cm}^2\textbf{)} = \frac{Impact\ energy}{cross-sectional\ area\ of\ specimen} \quad \text{.......................... (3.7)}$$

3.13.2 Modulus of Rupture (MOR) and Modulus of Elasticity (MOE)

The MOR and MOE were determined in accordance with British Standard Method BS 373 (1957). Standard test specimens of dimension 20 mm x 20 mm x 300 mm were prepared and tested on Instron 5500R model Universal Testing Machine (UTM) (Plate 3.6). The specimens were positioned on the instron machine such that the orientation of the growth rings lies parallel to the direction of the loading, with the distance between the points of support of the test specimen being 28 cm. Load application was at the rate of 6.604 mm/min with the specimens loaded on the radial face. The MOE and bending strength (MOR) of the specimens at the point of failure was determined from the machine, which was calculated using the formulae below:

$$\textbf{MOE (N/mm}^2\textbf{)} = \frac{PL^3}{4\Delta bd^3} \quad \text{................................ (3.8)}$$

Where, P = Load within the proportional limit of deflection in Newton (N)

L = Supporting span in mm

b = Width of test specimen in mm

d = Depth of test specimen in mm

Δ = deflection of the beam at mid-length

$$\textbf{MOR (N/mm}^2\textbf{)} = \frac{3PL}{2bd^2} \quad \text{................................ (3.9)}$$

Where, P = Maximum load at failure in Newton (N)

L = Supporting span in mm

b = Width of test specimen in mm

d = Depth of specimen in mm

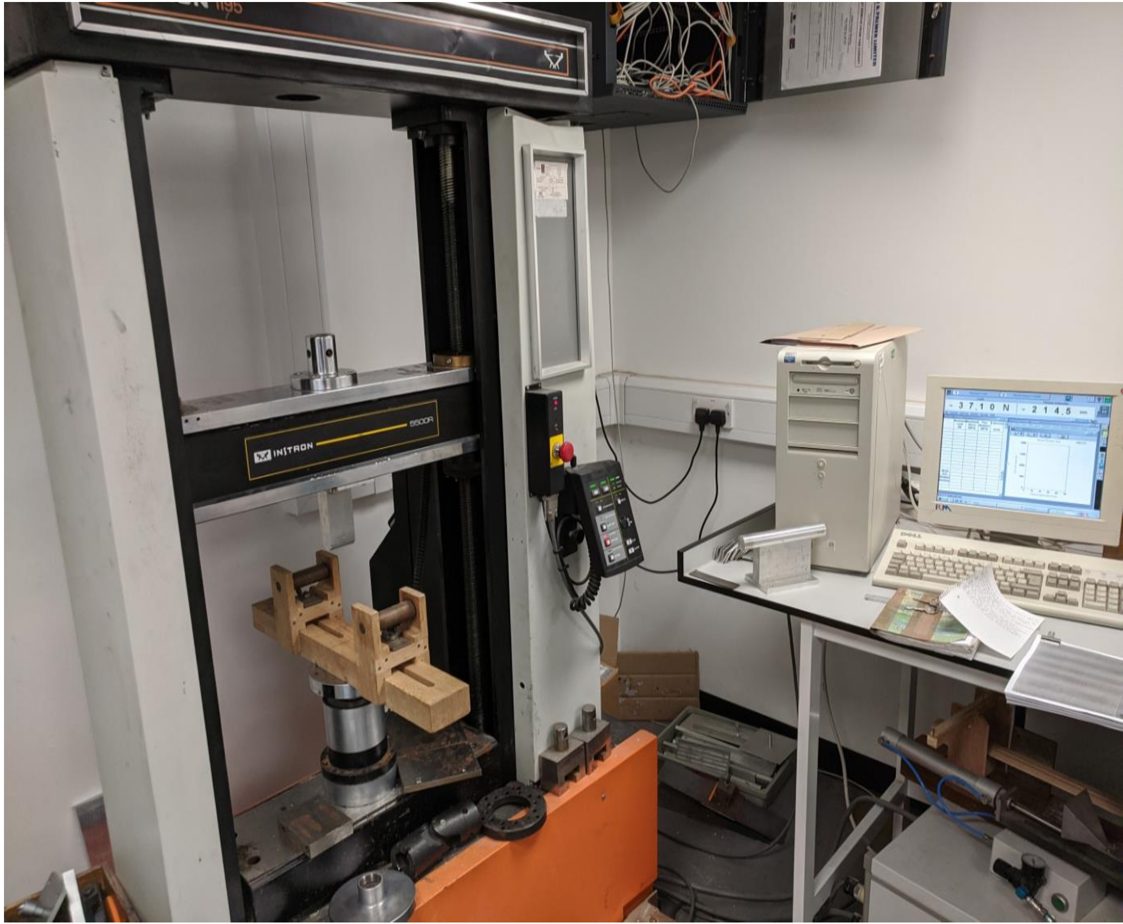


Plate 3.6: The Universal Testing Machine (UTM)

3.13.3 Maximum compressive strength parallel to the grain (MCS//)

Test specimens of dimension 20 mm x 20 mm x 60 mm was tested in accordance with BS 373 (1957) method of testing small clear specimen of timber. The specimens were tested on Instron 5500R model Universal Testing Machine (UTM), with the load applied in the direction parallel to the grain of the specimens at a constant speed of 0.635 mm/min. Maximum load at failure was recorded on the computer and MCS// was estimated using the formula below:

MCS// (N/mm²) $= \frac{P}{A}$................................ (3.10)

Where, P = Load (N)

A = Cross-sectional area of wood samples (mm^2)

3.13.4 Tensile strength parallel to the grain

Tensile strength parallel to the grain was determined in accordance with BS 373 (1957) method of testing small clear specimen of timber with slight modification with the aid of the Instron 5500R model Universal Testing Machine (UTM). The bioincised and control samples of dimension 150 mm x 20 mm x 6 mm were cut into a dog-bone shape using the CNC machine, with the width of the guage section being 5mm as shown in Figure 3.2 below. The dog-bone geometry was adopted so as to remove the stress concentrations in the grips, thereby providing a more accurate measurement of the wood tensile strength (Kretschmann, 2008). In order to avoid the slipping of the test specimens during testing, tensile clamps with large spikes were used to ensure a good grip on the samples. Cross-sectional dimensions of the guage section (length of the exposed portion during testing) of the wood specimens were measured with a calliper and recorded. The test specimen was placed in the upper grip of the moveable cross-head of the UTM, and the moveable cross-head was operated to move downwards in order to fix the lower portion of the specimen to the lower grip of the UTM. Load was applied continuously at the rate of 1.27 mm/min until failure occurred and the maximum stress at failure was recorded. The tensile strength was estimated using the formula below:

Tensile strength (N/mm²) $= \frac{P}{A}$................................ (3.11)

Where, P = Maximum stress at failure (N)

A = Cross-sectional area of wood samples (mm^2)

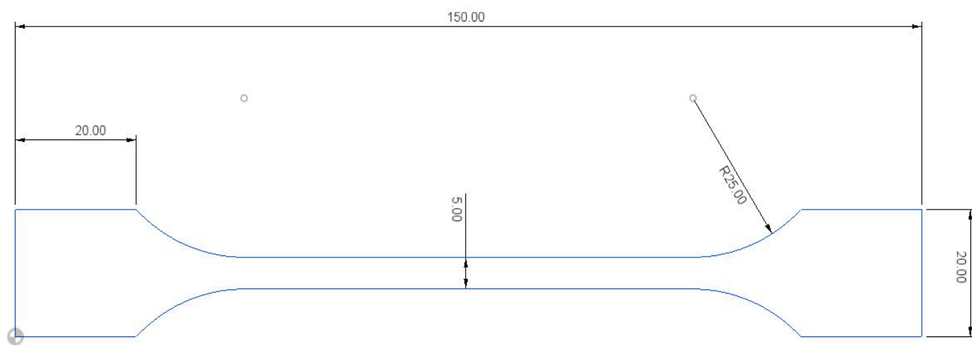


Fig. 3.2: Schematic illustration of the dog-bone shape tensile samples

3.13.5 Janka hardness

The hardness of the control and bioincised wood samples was evaluated using the Janka indentation test method following the British Standard BS 373 (1957) procedure, with the aid of the Instron 5500R model UTM. For this test, the hardeness value of the wood specimen is equivalent to the load needed to force the end of a hemispherical steel ball into the wood sample to a depth of half of its diameter, i.e. 0.222 inches or 5.64 mm, gives the hardeness value of the wood specimen. Load application was at the rate of 6.35 mm/min on each specimen on both the tangential (the side directly in contact with the fungal mycelia) and radial face, i.e a penetration was made on a tangential and radial face of the wood specimens. The corresponding hardness values were read from the computer. The hardness values obtained for the tangential and radial face of each specimen were pooled together to obtain the hardness value of individual wood specimen.

3.14 Fourier Transform Infra-red Spectroscopy

The infrared spectra of the control samples and the bioincised samples incubated for 9 weeks for both fungi were obtained using a Thermo Nicolet 8700 FTIR spectrometer in ATR (attenuated total reflectance) mode with GladiATR attachment (Plate 3.7) to detect the chemical changes that occurred in the wood samples after bioincision. Due to the slight to heavy pigmentation on the bioincised samples, spectra were obtained for both the inner and outer surfaces of the bioincised samples in order to delineate between spectral changes arising from the surface pigmentation and the wood component breakdown due to the fungal activities. Thin wood strips of about 1 mm thick were obtained from the tangential surfaces of the wood samples directly in contact with the fungal mycelia and the FTIR spectrometry was conducted directly on the surface of the wood strips using the ATR mode. For the control samples, five spectra were obtained from the outer surface of the wood strips which were taken from five specimens. For the bioincised samples, five spectra were obtained for the inner parts of the wood strips while ten spectra were obtained for the outer parts of the wood strips due to clear differences in pigmentation between the inner and the outer parts of the bioincised wood. Each spectrum consists of an average of 32 scans, with wavenumbers ranging from 600 to 4000 cm^{-1}. Background spectra were obtained using an empty collector after every three spectra to eliminate spectra noise and peaks arising from the build-up of CO_2 and water vapour as a result of environmental fluctuations.

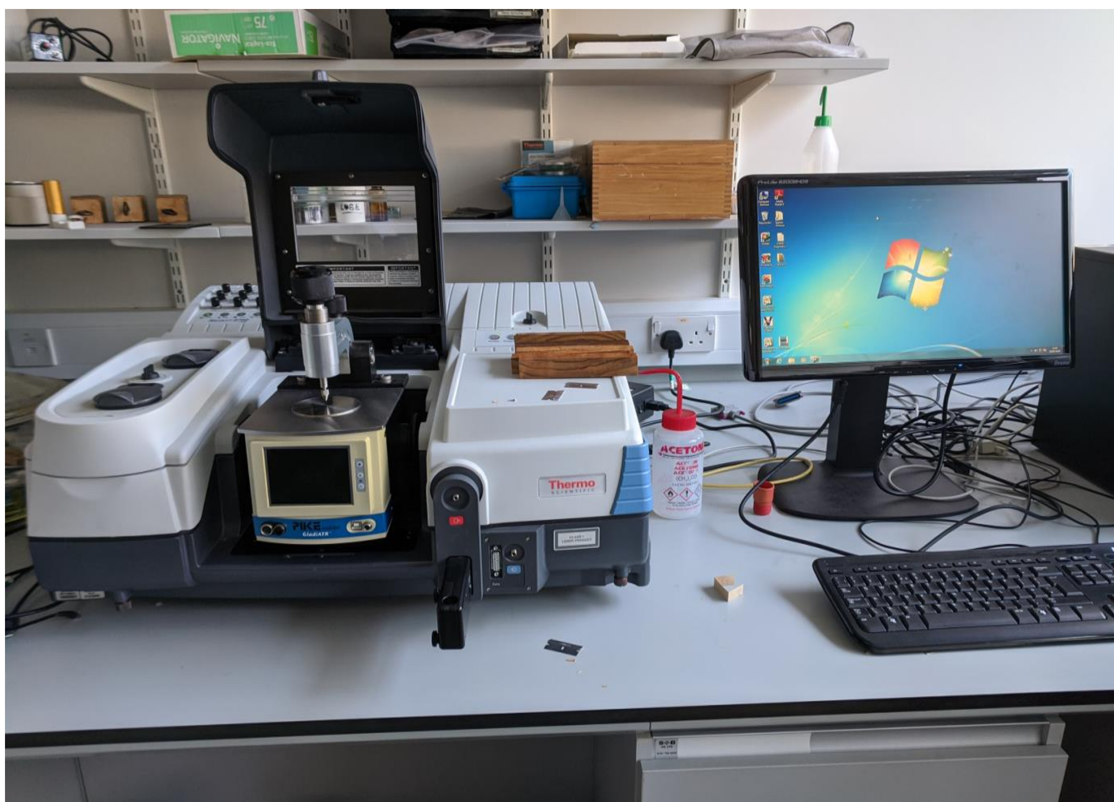


Plate 3.7: Fourier Transformed Infrared (FT-IR) Spectrometer

3.15 Resistance to fungal decay

The bioincised wood samples and control samples were treated with Tanalith preservative using the steeping method and pressure impregnation. For steeping, the wood specimens were totally immersed in a cold bath of the Tanalith preservative for 24hrs, after which the samples were drained and weighed, while the pressure-treated samples were treated in the pressure impregnating vessel with the preservative as described earlier. The treated wood blocks were allowed to drain of excess preservative, dried and conditioned in the conditioning chamber (20^0C and 65% RH), before they were exposed to actively growing cultures of a brown rot (*Coniophora puteana*) and a white rot (*Trametes versicolor*) and fungi for a period of 4, 8 and 20 weeks. They were incubated at 22^0C and 60% RH throughout the duration of the experiment. After the conclusion of each incubation period, the wood samples were extracted from the squat jars and the mycelia strands adhering to the wood surface were carefully brushed off and immediately weighed to estimate absorbed moisture. The test blocks were oven-dried at 105^0C for 24 hours, allowed to cool in a dessicator and re-weighed finally to estimate the weight loss using the formula below:

$$\boldsymbol{Weight\ loss}\ (\%) = \frac{\boldsymbol{W1-W2}}{\boldsymbol{W1}}\ \boldsymbol{x\ 100} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(vii)}$$

Where, W_1 is the oven-dry weight before exposure to fungus and W_2 is the oven-dry weight after exposure to fungus

3.16 Experimental Design and Data Analyses

The experimental data generated were subjected to both descriptive and inferential analyses. Tables, charts and well-anotated pictorial representations were used to present results of the descriptive analyses, while factorial experiment in Completely Randomized Design (CRD) was used for the inferential analyses. Factorial experiment was adopted as the experimental design in order to assess the interaction effects between the factors under consideration while Analysis of Variance (ANOVA) was utilized as the inferential analytical tool in order to check for significant differences among the means of the factors and among the interactions between the factors. Duncan Multiple Range Test was used for the post hoc tests where significant differences were observed among treatment means and their interactions.

CHAPTER FOUR

RESULTS

4.1 Physical properties

4.1.1 Weight loss

The average weight losses of the bioincised *Gmelina arborea* heartwood samples exposed to the two fungi at different incubation periods are shown in Table 4.1. Mean weight loss for the samples bioincised with *I. dryophilus* ranged from 0.09% to 0.48%, while those exposed to *G. adspersum* ranged from 0.02% to 0.8%. Exposure of the wood samples to both fungi resulted in a linear decrease in weight with increasing incubation time, except for those exposed to *I. dryophilus*, where there was a slight increase in weight after 9 weeks of exposure. Statistical analysis through Analysis of Variance revealed a significant effect ($p<0.05$) of the incubation period and interaction between the fungi and incubation period on the weight losses of the wood samples (Table 4.2). Wood samples exposed to *G. adspersum* for 9 weeks induced a considerably higher weight loss ($p<0.05$) than all the other bioincised wood samples (Table 4.1).

Table 4.3 shows the percentage mean weight loss for both fungi and for the different incubation periods. *G. adspersum* (0.32%) induced a higher weight loss on the wood samples than that of *I. dryophilus* (0.26%). Also, there was a linear decrease in weight loss with increasing exposure period of the wood samples to both fungi, which ranged from 0.05% to 0.61%. There were considerably higher ($p<0.05$) weight losses observed for samples incubated for 7 and 9 weeks than those incubated for 3 and 5 weeks.

Table 4.1: Mean weight loss for the bioincised wood samples of *G. arborea*

Fungus species	Incubation period (weeks)	Mean weight loss (%)
Inonotus dryophilus	3	0.09±0.03^{ad}
	5	0.33±0.03^{bc}
	7	0.48±0.04^{b}
	9	0.41±0.05^{bc}
Ganoderma adspersum	3	0.02±0.03^{a}
	5	0.25±0.05^{cd}
	7	0.51±0.04^{b}
	9	0.8±0.18^{e}
Control	---	0.00

Mean values with the same superscript are not significantly different at α = 0.05

Table 4.2: Analysis of Variance for the weight loss of bioincised *G. arborea* wood samples

Sources of Variation	Df	SS	MS	Fcal	p-value
Fungi	1	0.2293	0.2293	1.8254	0.178ns
Incubation period	4	17.0853	4.2713	33.9998	0.000*
Fungi*Incubation period	4	2.2399	0.56	4.4574	0.0016*
Error	290	36.4321	0.1256		
Total	299	55.9866			

Note: ‘*’ = significant at p<0.05; ‘ns’ = not significant at p<0.05

Table 4.3: Main effect analysis for the weight loss of the bioincised *G. arborea* wood samples

Parameters	Mean weight loss (%)
Fungi	
Inonotus dryophilus	0.26±0.02[a]
Ganoderma adspersum	0.32±0.05[a]
Incubation period	
3 weeks	0.05±0.02[a]
5 weeks	0.29±0.03[b]
7 weeks	0.49±0.03[c]
9 weeks	0.61±0.09[c]

Mean values with the same superscript are not significantly different at α = 0.05

4.1.2 Colour change

There were noticeable colour changes in the bioincised wood samples in comparison to the control samples (Plates 4.1 - 4.3). Samples bioincised with *I. dryophilus* exhibited colour changes, ranging from light brownish discolouration to deep brownish discolouration, which extended almost throughout the wood surface in some cases. For samples bioincised with *G. adspersum*, discolouration was less noticeable but there was slight brownish discolouration in some of the wood samples which was more or less localized on the wood surfaces.

4.1.3 Moisture content

The mean moisture content of the bioincised wood samples of *G. arborea* with both fungi is depicted in Table 4.4. Mean moisture content of the control samples was 26.48%, while that of the bioincised wood samples was between 29.49 - 36.69% and 36.56% - 53.08% for *I. dryophilus* and *G. adspersum*, respectively. For both fungi, the least moisture content of the bioincised wood samples was observed after 3 weeks of incubation while the highest was observed after 7 weeks of incubation. There was a slight decline in the moisture content for wood samples bioincised with *I. dryophilus* after 9 weeks, while the moisture content decline in samples bioincised with G. adspersum after 9 weeks was considerable. Samples bioincised with *G. adspersum* after 7 weeks of incubation had a significantly higher moisture content ($p<0.05$) than all the other bioincised wood samples. Statistical analysis shows a significant effect of fungi and incubation period on the moisture content of the bioincised samples. There was also a significant interaction effect of fungi and incubation period ($p<0.05$) on the of the bioincised samples' moisture content (Table 4.5).

As shown in Table 4.6, *G. arborea* wood samples bioincised with *G. adspersum* had a markedly higher average moisture content (39.77%) than those bioincised with *I. dryophilus* (31.82%) at ($p<0.05$). Based on the incubation period, samples incubated for 7 weeks had considerably higher mean moisture content (44.89%) than the other exposure periods, while the control samples had the least moisture content (26.48%). Mean moisture content of the bioincised samples tended to increase with increasing incubation period, with the exception of the decline observed at 9 weeks.

Plate 4.1: Colour of control samples of *G. arborea* wood

Plate 4.2: Colour changes in *G. arborea* wood samples bioincised with *I. dryophilus*

Plate 4.3: Colour changes in *G. arborea* wood samples bioincised with *G. adspersum*

Table 4.4: Mean Moisture content for the bioincised wood samples of *G. arborea*

Fungus species	Incubation period (weeks)	Moisture content (%)
Inonotus dryophilus	3	29.49±1.22^{ac}
	5	33.54±1.54^{bc}
	7	36.69±2.19^{bd}
	9	32.89±1.41^{bc}
Ganoderma adspersum	3	36.56±1.01^{bd}
	5	44.53±1.65^{e}
	7	53.08±1.90^{f}
	9	38.21±1.47^{d}
Control	---	26.48±0.75^{a}

Mean values with the same superscript are not significantly different at α = 0.05

Table 4.5: Analysis of Variance for the moisture content of bioincised *G. arborea* wood samples

Sources of Variation	**Df**	**SS**	**MS**	**Fcal**	**p-value**
Fungi	1	4746.1	4746.1	74.463	0.000*
Incubation period	4	11257.3	2814.3	44.154	0.000*
Fungi*Incubation period	4	2270.3	567.6	8.905	0.000*
Error	290	18484.1	63.7		
Total	299	36757.8			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.6: Main effect analysis for the moisture content of the bioincised *G. arborea* wood samples

Parameters	Moisture content (%)
Fungi	
Inonotus dryophilus	31.82±0.72[a]
Ganoderma adspersum	39.77±0.96[b]
Incubation period	
Control	26.48±0.53[a]
3 weeks	33.03±0.91[b]
5 weeks	39.04±1.33[c]
7 weeks	44.89±1.79[d]
9 weeks	35.55±1.07[b]

Mean values with the same superscript are not significantly different at α = 0.05

4.1.4 Preservative absorption and retention

The mean preservative absorption of the bioincised wood samples of *G. arborea* for both fungi at different exposure periods for the two different preservative application methods is presented in Table 4.7. For the steep-treated wood samples bioincised with *I. dryophilus*, maximum preservative absorption was observed in samples incubated for 7 weeks (102.47 Kg/m^3), while the least preservative absorption was observed in samples incubated for 3 weeks (91.89 Kg/m^3). Similarly, steep-treated wood samples bioincised with *G. adspersum* recorded a maximum preservative absorption after 7 weeks (106.59 Kg/m^3), while the least preservative absorption was observed in samples incubated for 5 weeks (101.23 Kg/m^3). The control (unincised) samples had a mean preservative absorption of 95.23 Kg/m^3. Overall, wood samples bioincised with *G. adspersum* for 7 weeks had the highest preservative absorption when the wood samples were steep-treated, though the absorption value was statistically similar ($p>0.05$) to the other steeped wood samples.

For the pressure-treated wood samples, the control samples had a mean preservative absorption of 112.02 Kg/m^3. Mean preservative absorption ranged between 106.72 - 134.90 Kg/m^3 and 123.10 – 139.97 Kg/m^3 for samples bioincised with *I. dryophilus* and *G. adspersum*, respectively. Highest preservative absorption for wood samples bioincised with *I. dryophilus* was observed in samples incubated for 7 weeks while the least preservative absorption was recorded in those incubated for 3 weeks. For the *G. adspersum* incised wood samples, maximum preservative absorption was observed in samples incubated for 5 weeks while the least preservative absorption was observed in samples incubated for 3 weeks. Overall, wood samples bioincised with *G. adspersum* for 5 weeks had the highest preservative absorption when the wood samples were pressure-treated, though the absorption value was statistically similar ($p>0.05$) to the other pressure-treated wood samples.

Furthermore, the mean preservative retention of the bioincised wood samples of *G. arborea* for both fungi at different exposure periods for the two different preservative application methods is presented in Table 4.8. For the steep-treated wood samples bioincised with *I. dryophilus*, maximum preservative retention was observed in samples incubated for 7 weeks (5.64 Kg/m^3), while the least preservative retention was observed in samples incubated for 3 weeks (5.05 Kg/m^3). Similarly, steep-treated

Table 4.7: Mean preservative absorption for the bioincised wood samples of *G. arborea* for the different method of preservation application

Fungus species	Incubation period (weeks)	Preservation absorption (Kg/m^3)	
		Steeping	Pressure
Inonotus dryophilus	3	91.89±3.48	106.72±6.94
	5	101.27±4.66	120.95±13.19
	7	102.47±3.74	134.90±14.55
	9	96.34±3.49	131.40±12.57
Ganoderma adspersum	3	105.72±3.75	123.10±7.79
	5	101.23±3.73	139.97±15.24
	7	106.59±4.22	130.18±12.69
	9	102.06±3.12	135.01±16.64
Control		95.23±4.13	112.02±8.46

Table 4.8: Mean preservative retention for the bioincised wood samples of *G. arborea* for the different method of preservation application

Fungus species	Incubation period (weeks)	Preservation retention (Kg/m^3)	
		Steeping	Pressure
Inonotus dryophilus	3	5.05±0.19	5.87±0.38
	5	5.57±0.26	6.65±0.73
	7	5.64±0.21	7.42±0.8
	9	5.30±0.19	7.23±0.69
Ganoderma adspersum	3	5.81±0.21	6.77±0.43
	5	5.57±0.21	7.70±0.84
	7	5.86±0.23	7.16±0.70
	9	5.61±0.17	7.43±0.92
Control		5.24±0.23	6.16±0.47

wood samples bioincised with *G. adspersum* recorded a maximum preservative retention after 7 weeks (5.86 Kg/m^3), while the least preservative retention was observed in samples incubated for 5 weeks (5.57 Kg/m^3). The control (unbioincised) samples had a mean preservative retention of 5.24 Kg/m^3. Overall, wood samples bioincised with *G. adspersum* for 7 weeks had the highest preservation retention when the wood samples were steep-treated, though the retention value was statistically similar ($p>0.05$) to the other steeped wood samples.

For the pressure-treated wood samples, the control samples had a mean preservative retention of 6.16 Kg/m^3. Mean preservative retention ranged between 5.87 - 7.42 Kg/m^3 and 6.77 – 7.70 Kg/m^3 for samples bioincised with *I. dryophilus* and *G. adspersum*, respectively. Highest preservative retention for wood samples bioincised with *I. dryophilus* was observed in samples incubated for 7 weeks while the least preservative retention was recorded in those incubated for 3 weeks. For the *G. adspersum* incised wood samples, maximum preservative retention was observed in samples incubated for 5 weeks while the least preservative retention was observed in samples incubated for 3 weeks. Overall, wood samples bioincised with *G. adspersum* for 5 weeks had the highest preservation retention when the wood samples were pressure-treated, though there was no significant difference ($p>0.05$) between the retention value and those of the other pressure-treated wood samples.

As shown in Table 4.9, statistical significance could not be established on the effect of fungi and incubation period nor their interaction on the preservative absorption and retention of the wood samples ($p>0.05$). However, the effect of the treatment method on both the preservative absorption and retention of the bioincised wood samples was significant ($p<0.05$).

The mean preservative absorption and retention of the wood samples based on fungi type, incubation period and treatment method is presented in Table 4.10. The mean preservative absorption and retention was higher in wood samples bioincised with *G. adspersum* (115.53 Kg/m^3 and 6.39 Kg/m^3) than those of *I. dryophilus* (108.90 Kg/m^3 and 5.99 Kg/m^3). For the incubation period, mean preservative absorption and retention was highest for bioincised samples incubated for 7 weeks (118.54 Kg/m^3 and 6.52 Kg/m^3) and the least was observed in the control samples (103.62 Kg/m^3 and 5.70 Kg/m^3). For the treatment method, significantly higher absorption and retention

Table 4.9: Analysis of Variance for the preservative absorption and retention of the bioincised wood samples of *G. arborea*

Sources of Variation	Df	SS	MS	Fcal	p-value
Preservative Absorption					
Fungi	1	4383	4383	2.705	0.100^{ns}
Incubation period (IP)	4	13733	3433	2.119	0.078^{ns}
Treatment method (TM)	1	61621	61621	38.037	0.000*
Fungi*Incubation period	4	2762	690	0.426	0.790^{ns}
Fungi* Treatment method	1	669	669	0.413	0.521^{ns}
IP*TM	4	5083	1271	0.784	0.536^{ns}
Fungi* IP*TM	4	2749	687	0.424	0.791^{ns}
Error	380	615608	1620		
Total	399	706606			
Preservative Retention					
Fungi	1	13.26	13.26	2.71	0.100^{ns}
Incubation period (IP)	4	41.54	10.39	2.12	0.078^{ns}
Treatment method (TM)	1	186.40	186.40	38.04	0.000*
Fungi*Incubation period	4	8.35	2.09	0.43	0.790^{ns}
Fungi* Treatment method	1	2.02	2.02	0.41	0.521^{ns}
IP*TM	4	15.38	3.84	0.78	0.536^{ns}
Fungi* IP*TM	4	8.32	2.08	0.42	0.791^{ns}
Error	380	1862.21	4.90		
Total	399	2137.48			

Note: ‘*’ = significant at p<0.05; ‘ns’ = not significant at p<0.05

Table 4.10: Main effect analysis for the preservative absorption and retention of the bioincised *G. arborea* wood samples

Parameters	Preservation absorption (Kg/m^3)	Preservation retention (Kg/m^3)
Fungi		
Inonotus dryophilus	108.90±2.81	5.99±0.15
Ganoderma adspersum	115.53±3.13	6.35±0.17
Incubation period		
Control	103.62±3.47	5.70±0.19
3 weeks	106.86±3.11	5.88±0.17
5 weeks	115.86±5.46	6.37±0.30
7 weeks	118.54±5.18	6.52±0.29
9 weeks	116.20±5.59	6.39±0.31
Treatment method		
Steeping	99.80±1.24[a]	5.49±0.07[a]
Pressure	124.63±3.83[b]	6.85±0.21[b]

Mean values with the same superscript within the same column are not significantly different at $\alpha = 0.05$

($p<0.05$) were observed in the pressure-treated bioincised wood samples (124.63 Kg/m^3 and 6.85 Kg/m^3) than the samples treated by steeping (99.80 Kg/m^3 and 5.49 Kg/m^3).

4.1.5 Axial and lateral absorption

The mean axial and lateral absorption of dye in the control and bioincised wood samples are presented graphically in Fig. 4.1 and Fig. 4.2. Mean axial absorption for the control samples is 31.38 Kg/m^3. For wood samples bioincised with *I. dryophilus*, the least axial absorption was observed for samples bioincised for 3 weeks (27.28 Kg/m^3), while the highest was recorded in samples bioincised for 5 weeks (67.05 Kg/m^3). For wood samples bioincised with *G. adspersum*, the least axial absorption was recorded in samples bioincised for 3 weeks (29.14 Kg/m^3), while maximum axial absorption was observed in samples bioincised for 9 weeks (97.11 Kg/m^3). For the lateral absorption, mean absorption for the control samples is 56.30 Kg/m^3, while maximum absorption (71.74 Kg/m^3 and 120.60 Kg/m^3) was observed in wood samples bioincised for 9 weeks for both *I. dryophilus* and *G. adspersum*, respectively. Furthermore, the lowest lateral absorption was observed in wood samples bioincised for 5 weeks for both *I. dryophilus* (28.10 Kg/m^3) and *G. adspersum* (33.72 Kg/m^3). Statistical analysis through ANOVA revealed a significant effect ($p<0.05$) of fungi, incubation period, wood axis, including interaction effects between fungi and incubation period, and incubation period and wood axis on the dye absorption of the wood samples (Table 4.11)

The effect of the fungi, incubation period and wood axis on the wood samples of *G. arborea* is presented in Table 4.12. For the fungi, bioincision of the wood samples with *G. adspersum* resulted in a significantly higher dye absorption (59.57 Kg/m^3) than those bioincised with *I. dryophilus* (47.84 Kg/m^3) at ($p<0.05$). For the incubation period, samples bioincised for 3 weeks gave the lowest dye absorption (41.22 Kg/m^3), while those bioincised for 9 weeks gave the highest dye absorption (84.79 Kg/m^3). The dye absorption of the incubated wood samples for 9 weeks gave a significantly higher absorption ($p<0.05$) than all the other incubated periods. Based on the wood axis, significantly higher dye absorption ($p<0.05$) was observed in the lateral plane (59.89 Kg/m^3) than the axial plane (47.53 Kg/m^3).

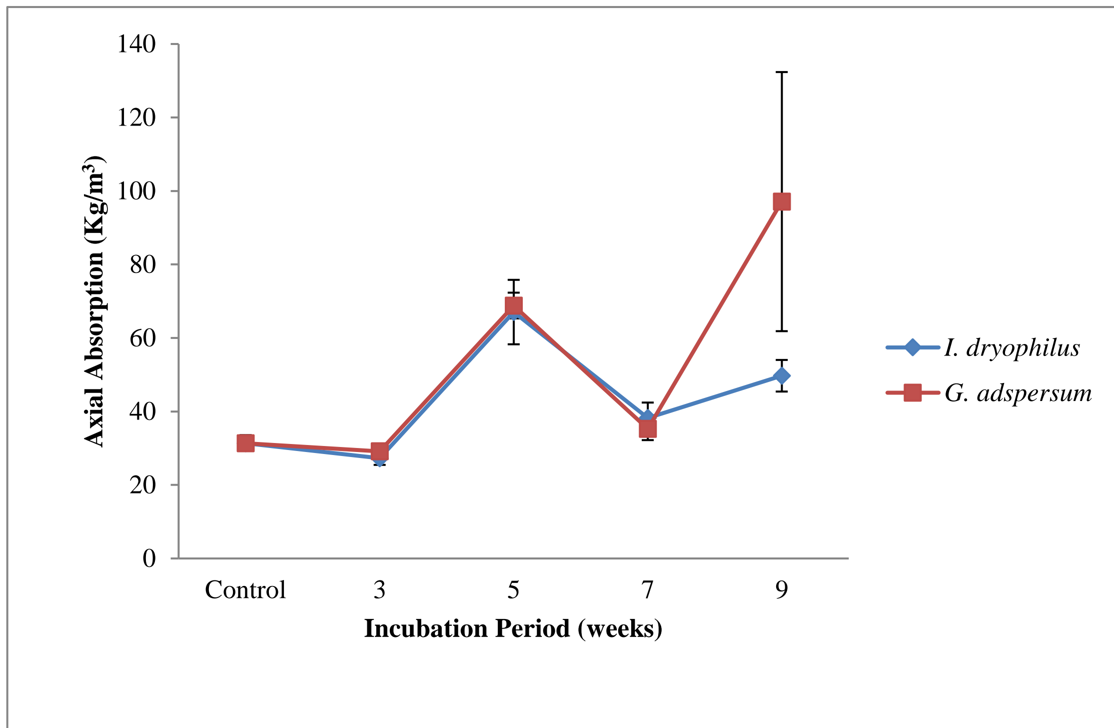


Fig 4.1: Axial absorption of chemical dye in bioincised wood of *G. arborea* (error bars = mean±SE; n=90)

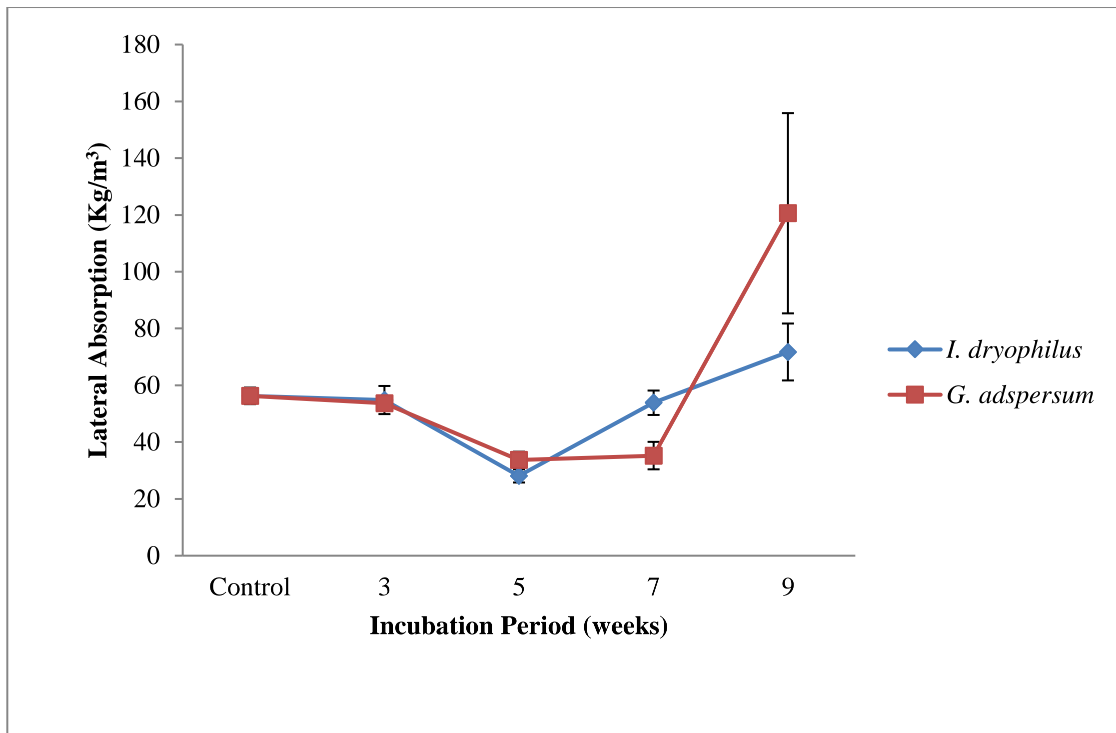


Fig 4.2: Lateral absorption of chemical dye in bioincised wood of *G. arborea* (error bars = mean±SE; n=90)

Table 4.11: Analysis of Variance for the dye absorption of the bioincised wood samples of *G. arborea*

Sources of Variation	df	SS	MS	Fcal	p-value
Fungi	1	6880.8	6880.8	4.7883	0.030*
Incubation period (IP)	4	50298.5	12574.6	8.7507	0.000*
Wood axis (WA)	1	7630.2	7630.2	5.3099	0.022*
Fungi*Incubation period	4	16840.1	4210.0	2.9298	0.022*
Fungi* Wood axis	1	222.8	222.8	0.1551	0.694^{ns}
IP*WA	4	30538.0	7634.5	5.3129	0.000*
Fungi* IP*WA	4	730.8	182.7	0.1271	0.972^{ns}
Error	180	258657.8	1437.0		
Total	199	371799.1			

Note: '*' = significant at $p<0.05$; 'ns' = not significant at $p<0.05$

Table 4.12: Main effect analysis for the dye absorption of the bioincised *G. arborea* wood samples

Parameters	Dye absorption (Kg/m^3)
Fungi	
Inonotus dryophilus	47.84±2.81[a]
Ganoderma adspersum	59.57±5.38[b]
Incubation period	
Control	43.84±2.34[a]
3 weeks	41.22±2.54[a]
5 weeks	49.42±3.85[a]
7 weeks	49.27±2.98[a]
9 weeks	84.79±13.06[b]
Wood axis	
Axial	47.53±4.22[a]
Lateral	59.89±4.35[b]

Mean values with the same superscript are not significantly different at α = 0.05

Pairwise comparisons of the interaction effects of fungi-incubation period and wood axis-incubation period are presented in Tables 4.13 and 4.14, respectively. Wood samples bioincised with *G. adspersum* for 9 weeks gave a significantly higher dye absorption ($p<0.05$) than both the control samples and the other bioincised samples (Table 4.13). On the other hand, Table 4.14 shows that the axial dye absorptions were both similar and significantly higher after bioincision for 5 weeks (67.94 Kg/m^3) and 7 weeks (73.41 Kg/m^3), while a significantly high lateral absorption ($p<0.05$) was observed in wood samples bioincised for 9 weeks (96.17 Kg/m^3).

4.1.6 Axial penetration depth and penetration area

The mean axial penetration depth of dye in the control samples and bioincised wood samples at the sub-surface and middle sections are presented graphically in Fig. 4.3, while dye penetration at the middle and sub-surface sections in a sectioned bioincised sample is shown in Plate 4.4. The least axial penetration depth of the dye was observed in the control samples, with penetration depths of 5.39 mm and 8.55 mm obtained at the sub-surface and middle sections of the wood samples, respectively. Sub-surface sections of the wood samples bioincised with *I. dryophilus* had axial penetration which increased from 3 weeks of exposure (12.54 mm) to 7 weeks of exposure (20.76 mm), but decreased at 9 weeks of exposure (15.46 mm). Middle sections of the wood samples bioincised with *I. dryophilus* showed a dip in axial penetration from 3 weeks of incubation (11.29 mm) to 5 weeks of incubation (9.96 mm), but continually increased with prolonged incubation period, with the maximum axial penetration observed at 9 weeks of exposure (22.96 mm). For the sub-surface sections of the wood samples bioincised with *G. adspersum*, axial penetration depth ranged from 9.42 mm to 28.28 mm at 7 weeks and 9 weeks of incubation, respectively, while the middle sections of the wood samples ranged from 8.95 mm (7 weeks) to 34.18 mm (9 weeks).

The mean axial penetration area of dye in the control samples and bioincised wood samples at the sub-surface and middle sections are presented graphically in Fig. 4.4. The control samples had the least axial penetration area at both the the sub-surface (0.07%) and middle sections (0.07%) of the wood samples. Sub-surface sections of the wood samples bioincised with *I. dryophilus* had axial penetration area which steadily increased from 3 weeks of exposure (0.12%) to 9 weeks of exposure (2.68%).

Table 4.13: Effect of Fungi*Incubation period on dye absorption of bioincised *G. arborea* wood

Fungus species	Incubation period (weeks)	Dye absorption (Kg/m^3)
Inonotus dryophilus	3	41.05±4.07[a]
	5	47.57±6.28[a]
	7	46.02±3.44[a]
	9	60.73±10.72[a]
Ganoderma adspersum	3	41.39±3.14[a]
	5	51.27±4.60[a]
	7	52.52±4.84[a]
	9	108.85±22.89[b]
Control	---	43.84±3.36[a]

Mean values with the same superscript are not significantly different at α = 0.05

Table 4.14: Effect of Wood axis*Incubation period on dye absorption of bio-incised *G. arborea* wood

Incubation period (weeks)	Dye absorption (Kg/m^3)	
	Axial	**Lateral**
Control	31.38 ± 1.47^{a}	56.30 ± 2.00^{ab}
3	28.21 ± 1.17^{a}	54.23 ± 2.69^{ab}
5	67.94 ± 4.60^{b}	30.91 ± 1.93^{a}
7	36.72±2.56a	61.82 ± 3.64^{b}
9	73.41 ± 18.34^{b}	96.17 ± 18.70^{c}

Mean values with the same superscript within the same column are not significantly different at α = 0.05

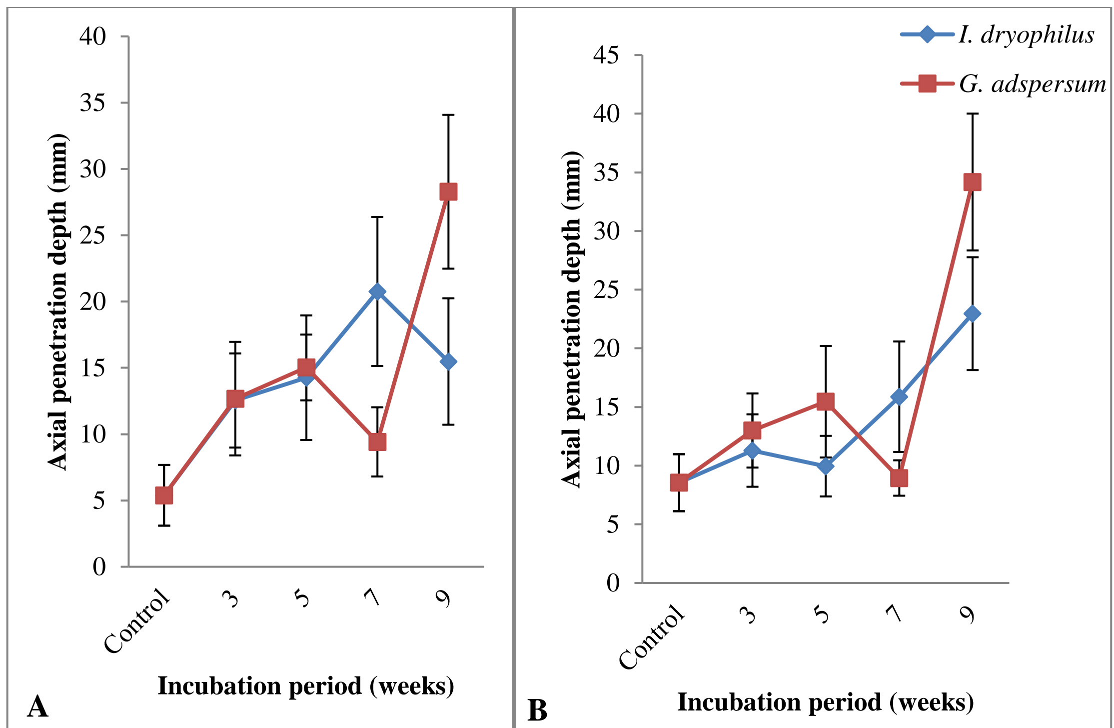


Fig 4.3: Axial dye penetration depth of the control and bioincised samples at the (A) sub-surface and (B) middle sections of *G. arborea* wood (error bars = mean±SE; n=90)

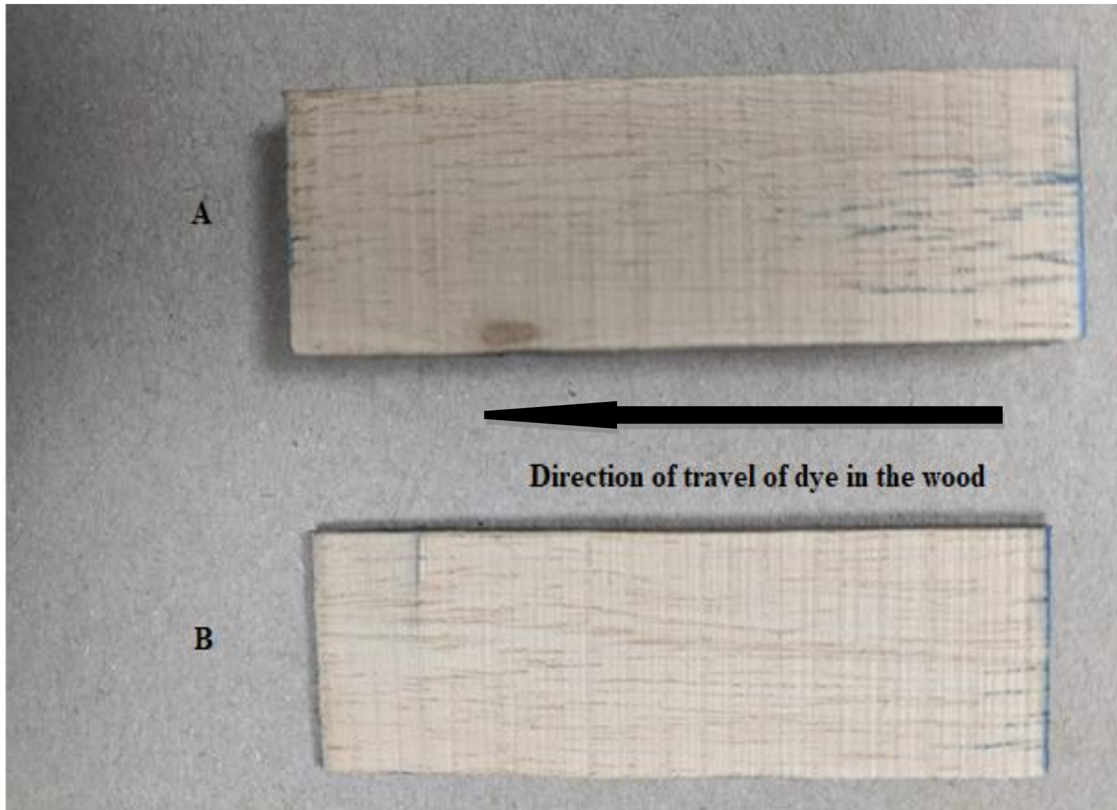


Plate 4.4: Dye penetration in a sample of the bioincised wood showing (A) dye penetration at the middle section (B) dye penetration at the sub-surface section

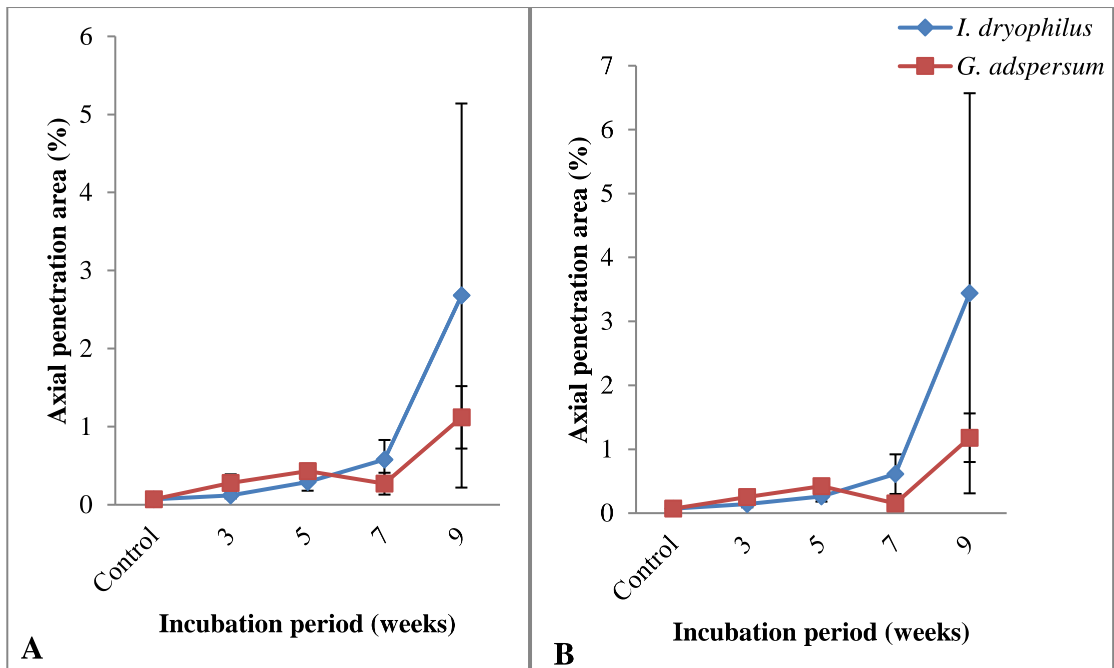


Fig. 4.4: Axial dye penetration area of the control and bioincised samples at the (A) sub-surface and (B) middle sections of *G. arborea* wood (error bars = mean±SE; n=90)

Similarly, middle sections of the wood samples bioincised with *I. dryophilus* showed a steady increase in axial penetration area from 3 weeks of incubation (0.14%) to 9 weeks of incubation (3.44%). For the sub-surface and middle sections of the wood samples bioincised with *G. adspersum*, similar trends of axial penetration area were observed. Axial penetration area tended to increase from samples exposed for 3 weeks (0.28% and 0.25%) to samples exposed for 9 weeks of incubation (1.12% and 1.18%), for both the sub-surface and middle sections, respectively, but a decline was observed at 7 weeks of exposure (0.27% and 0.15%) for both the sub-surface and middle sections, respectively. Statistical analysis using ANOVA showed a significant effect ($p<0.05$) of incubation period and interaction effect of fungi and incubation period on the axial penetration depth of dye in the wood samples, while the axial penetration area of the dye in the wood samples was significantly influenced ($p<0.05$) by the incubation period (Table 4.15).

The main effect for axial dye penetration depth and penetration area in the bioincised samples of *G. arborea* wood is presented in Table 4.16. The mean axial penetration depth of the dye in samples bioincised with *G. adspersum* (15.09 mm) was higher than those bioincised with *I. dryophilus* (13.71 mm). On the contrary, higher axial penetration area was observed in the *I. dryophilus* bioincised samples (0.83%) than those bioincised with *G. adspersum* (0.42%). For the incubation period, axial penetration depth and penetration area increased with increasing incubation period. The least penetration depth and penetration area were observed in the control samples (6.97 mm and 0.07%), while significantly higher penetration depth and penetration area ($p<0.05$) were observed in the samples incubated for 9 weeks (25.23 mm and 2.11%) when compared with the control and the other bioincised wood samples. Based on the section depth, though the axial penetration depth and penetration area at the middle section (14.88 mm and 0.66%) were higher than those obtained at the sub-surface (13.92 mm and 0.59%), a significant difference could not be established between them ($p>0.05$).

Pairwise comparison of the interaction effects of fungi-incubation period is presented in Tables 4.17. Wood samples bioincised with *G. adspersum* for 9 weeks gave a significantly higher axial dye penetration ($p<0.05$) than both the control samples and the other bioincised samples.

Table 4.15: Analysis of Variance for Axial penetration depth and penetration area

Sources of Variation	df	SS	MS	Fcal	p-value
Axial penetration depth					
Fungi	1	96.09	96.09	0.63	0.430^{ns}
Incubation period (IP)	4	7096.08	1774.02	11.57	0.000*
Section depth (SD)	1	45.68	45.68	0.30	0.586^{ns}
Fungi*Incubation period	4	2287.79	571.95	3.73	0.006*
Fungi* Section depth	1	41.58	41.58	0.27	0.603^{ns}
IP*SD	4	613.92	153.48	1.00	0.408^{ns}
Fungi* IP*SD	4	75.27	18.82	0.12	0.974^{ns}
Error	180	27591.70	153.29		
Total	199	37848.11			
Axial penetration area					
Fungi	1	8.10	8.10	0.99	0.321^{ns}
Incubation period (IP)	4	112.40	28.10	3.43	0.009*
Section depth (SD)	1	0.23	0.23	0.03	0. 867^{ns}
Fungi*Incubation period	4	30.28	7.57	0.92	0.452^{ns}
Fungi* Section depth	1	0.39	0.39	0.05	0.828^{ns}
IP*SD	4	1.43	0.36	0.04	0.996^{ns}
Fungi* IP*SD	4	0.91	0.23	0.03	0.999^{ns}
Error	180	1475.89	8.20		
Total	199	1629.62			

Note: ‘*’ = significant at p<0.05; ‘ns’ = not significant at p<0.05

Table 4.16: Main effect for axial dye penetration depth and penetration area in bioincised samples of *G. arborea* wood.

Parameters	Penetration depth (mm)	Penetration area (%)
Fungi		
Inonotus dryophilus	13.71±1.32	0.83±0.40
Ganoderma adspersum	15.09±1.44	0.42±0.07
Incubation period		
Control	6.97±1.16[a]	0.07±0.02[a]
3 weeks	12.38±1.71[ab]	0.20±0.04[ab]
5 weeks	13.67±1.86[b]	0.35±0.05[b]
7 weeks	13.75±2.06[b]	0.40±0.11[b]
9 weeks	25.23±2.79[c]	2.11±0.98[c]
Section depth		
Sub-surface	13.92±1.39	0.59±0.25
Middle	14.88±1.38	0.66±0.32

Mean values with the same superscript within the same column are not significantly different at α = 0.05

Table 4.17: Effect of Fungi*Incubation period on Axial penetration depth of dye in bio-incised *G. arborea* wood

Fungus species	Incubation period (weeks)	Penetration depth (mm)
Inonotus dryophilus	3	11.92±2.29^{ab}
	5	12.11±2.66^{ab}
	7	18.32±3.61^{b}
	9	19.22±3.41^{b}
Ganoderma adspersum	3	12.84±2.59^{ab}
	5	15.24±2.61^{ab}
	7	9.18±1.47^{a}
	9	31.23±4.06^{c}
Control	---	6.97±1.66^{a}

Mean values with the same superscript within the same column are not significantly different at α = 0.05

4.1.7 Lateral penetration depth and penetration area

The mean radial and tangential penetration depth of dye in the control samples and bioincised wood samples are presented graphically in Fig. 4.5. For the radial penetration, mean radial penetration depth of the dye for the control samples is 0.08 mm. For wood samples bioincised with *I. dryophilus*, the least radial penetration was observed for samples bioincised for 7 weeks (0.86 mm), while the highest was recorded in samples bioincised for 5 weeks (3.48 mm). For wood samples bioincised with *G. adspersum*, the least radial penetration was recorded in samples bioincised for 3 weeks (0.12 mm), while maximum radial penetration depth was observed in samples bioincised for 9 weeks (4.81 mm). For the tangential penetration, mean tangential penetration depth for the control samples is 0.38 mm, while maximum tangential penetration depth (0.63 mm and 3.03 mm) was observed in wood samples bioincised for 9 weeks for both *I. dryophilus* and *G. adspersum*, respectively. Furthermore, the lowest tangential penetration depth was observed in wood samples bioincised for 5 weeks for both *I. dryophilus* (0.49 mm), while that of the *G. adspersum* incised wood samples was observed at 3 weeks of exposure (0.36 mm). Statistical analysis through ANOVA revealed a significant effect ($p<0.05$) of incubation period and wood axis on the lateral penetration depth of the dye in the wood samples (Table 4.18).

The mean lateral penetration area of the dye in the control samples and bioincised wood samples are presented graphically in Fig. 4.6. The mean lateral penetration area of the dye for the control samples is 0.001%. For wood samples bioincised with *I. dryophilus*, the lowest lateral penetration area was observed in samples bioincised for 7 weeks (0.77%), while the highest was recorded in samples bioincised for 5 weeks (4.55%). For wood samples bioincised with *G. adspersum*, the least lateral penetration area was recorded in samples bioincised for 5 weeks (0.003%), while maximum tangential penetration was observed in samples bioincised for 9 weeks (16.98%). Statistical analysis through ANOVA revealed a significant effect ($p<0.05$) of incubation period on the lateral penetration area of the dye in the wood samples (Table 4.18).

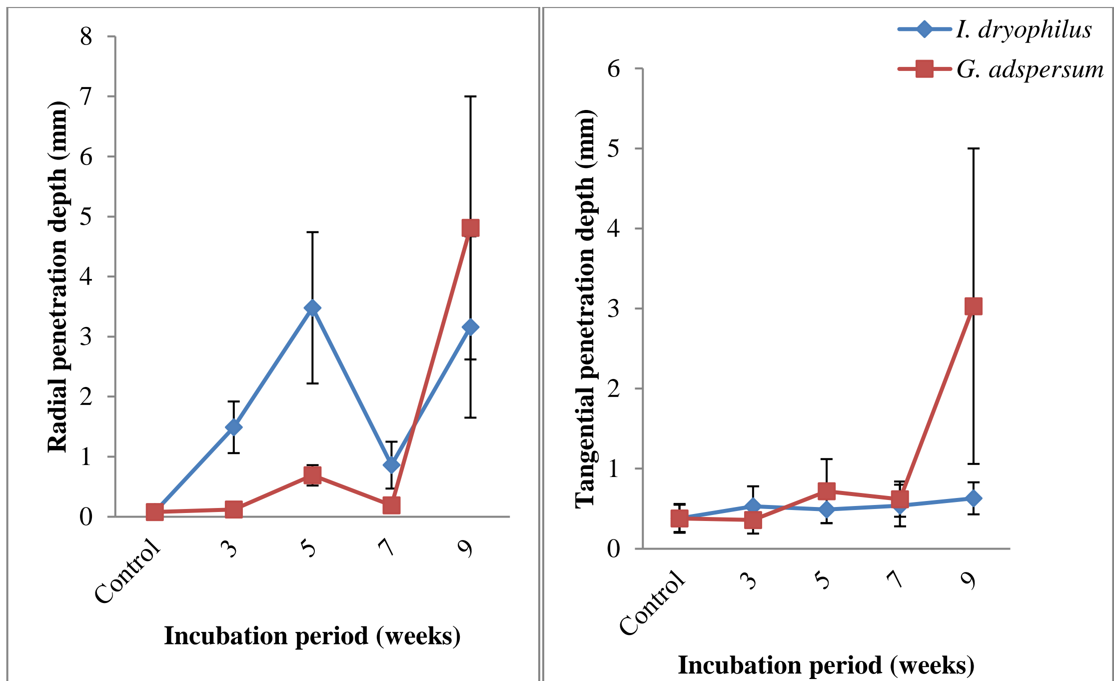


Fig 4.5: Radial and Tangential dye penetration depth of the control and bioincised samples of *G. arborea* wood (error bars = mean±SE; n=90)

Table 4.18: Analysis of Variance for Lateral penetration depth and area

Sources of Variation	df	SS	MS	Fcal	p-value
Lateral penetration depth					
Fungi	1	0.21	0.21	0.03	0.859^{ns}
Incubation period (IP)	4	184.18	46.04	6.87	0.000*
Wood axis (WA)	1	26.59	26.59	3.97	0.048*
Fungi*Incubation period	4	63.75	15.94	2.38	0.054^{ns}
Fungi* Wood axis	1	16.28	16.28	2.43	0.121^{ns}
IP*WA	4	43.95	10.99	1.64	0.166^{ns}
Fungi* IP*WA	4	12.85	3.21	0.48	0.751^{ns}
Error	180	1206.87	6.71		
Total	199	1554.66			
Lateral penetration area					
Fungi	1	42.49	42.49	0.41	0.525^{ns}
Incubation period	4	1633.18	408.29	3.91	0.006*
Fungi*Incubation period	4	860.66	215.17	2.06	0.093^{ns}
Error	90	9395.49	104.39		
Total	99	11931.81			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

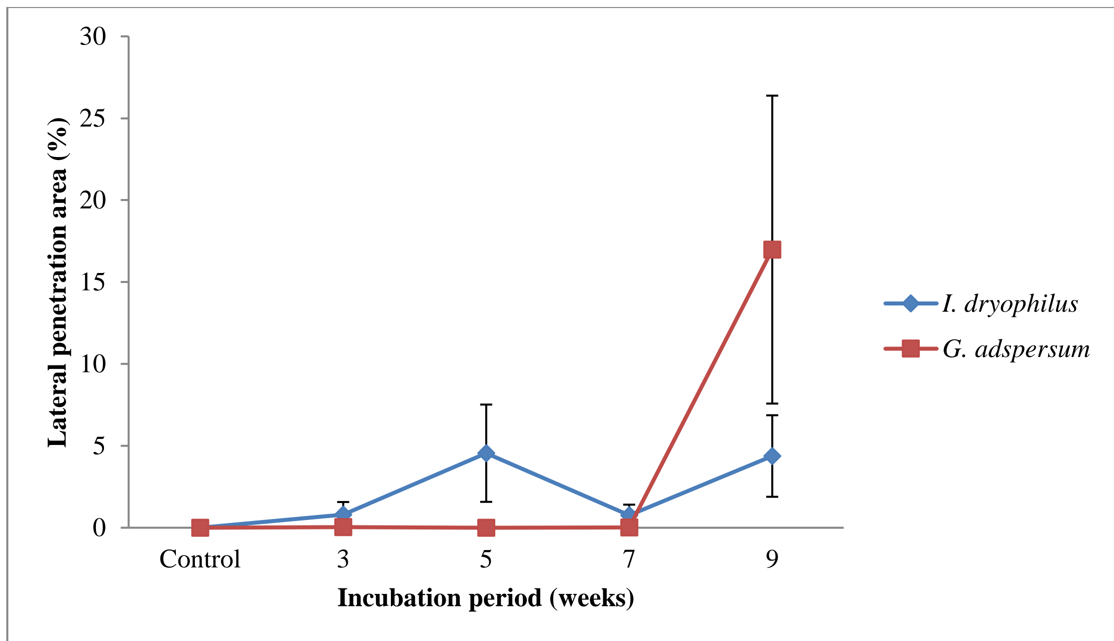


Fig 4.6: Lateral dye penetration area of the control and bioincised samples of *G. arborea* wood (error bars = mean±SE; n=90)

The main effect for the lateral dye penetration depth and penetration area in the bioincised samples of *G. arborea* wood is presented in Table 4.19. The mean lateral penetration depth of the dye in samples bioincised with *G. adspersum* (3.40 mm) was higher than those bioincised with *I. dryophilus* (1.16 mm). On the contrary, higher lateral penetration area was observed in the *I. dryophilus* bioincised samples (2.10%) than those bioincised with *G. adspersum* (0.42%). Based on the incubation period, lateral penetration depth tended to increase with increasing incubation period, with the exception at 7 weeks where a decline was observed. The least lateral penetration depth and penetration area were observed in the control samples (0.23 mm and 0.001%), while significantly higher penetration depth and penetration area ($p<0.05$) were observed in the samples incubated for 9 weeks (2.91 mm and 10.68%) when compared with the control and the other bioincised wood samples. For the wood axis, radial penetration depth was almost twice as much (1.50 mm) and significantly higher ($p<0.05$) than the tangential penetration depth (0.77 mm). Conversely, tangential penetration area (0.66%) was higher than that of the radial penetration area (0.59%).

4.2 Anatomical properties

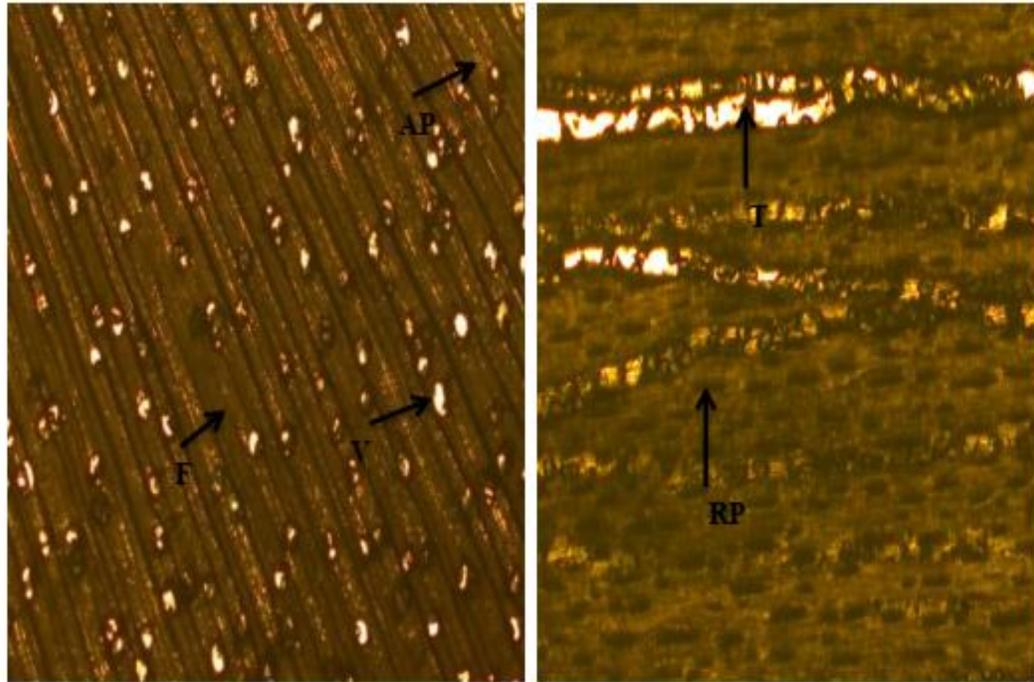
4.2.1 Light microscopy

The results of the light microscopy are presented in Plates 4.5 – 4.10. Plate 4.5A shows the cross-section (C/S) of the control samples while Plate 4.5B, 4.5C represents tangential (TLS) and radial sections (RLS) of the control samples, respectively. Plates 4.6A and 4.6C shows the cross-section of the wood samples bioincised with *G. adspersum* for 9 weeks while Plate 4.6B represents tangential sections of the samples bioincised with *G. adspersum* for 9 weeks. Plates 4.7A, 4.7B, 4.7C depicts the cross-section, tangential and radial sections, respectively, of the wood samples bioincised with *I. dryophilus* for 9 weeks. For the control samples, it can be seen that the fibres, axial and ray parenchyma are heavily occluded by extraneous materials deposited during the heartwood formation. Similarly, the vessels contain numerous tylosic occlusions in their lumina, which either renders the vessels partially or completely occluded (Plates 4.5A - 4.5C). For the samples bioincised with *G. adspersum* (Plates 4.6A - 4.6C), it can be seen that there are apparent traces of blue dye in the vessels, axial and ray parenchyma, and fibre.

Table 4.19: Main effect for Lateral penetration depth and penetration area

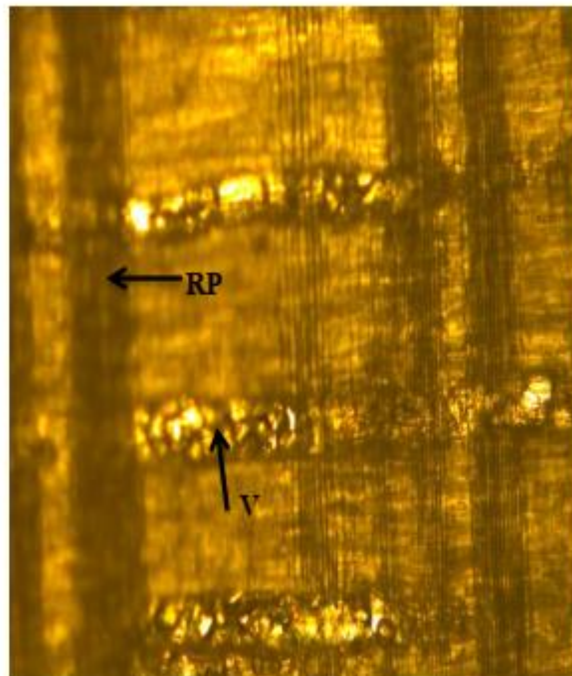
Parameters	Penetration depth (mm)	Penetration area (%)
Fungi		
Inonotus dryophilus	1.16±0.23	2.10±0.82
Ganoderma adspersum	3.40±2.05	0.42±0.07
Incubation period		
Control	0.23±0.07[a]	0.001±0.00[a]
3 weeks	0.62±0.15[ab]	0.42±0.39[ab]
5 weeks	1.35±0.38[b]	2.28±1.54[b]
7 weeks	0.55±0.13[b]	0.39±0.32[b]
9 weeks	2.91±0.83[c]	10.68±4.95[c]
Wood axis		
Radial	1.50±0.33[a]	0.59±0.25
Tangential	0.77±0.21[b]	0.66±0.32

Mean values with the same superscript within the same column are not significantly different at $\alpha = 0.05$



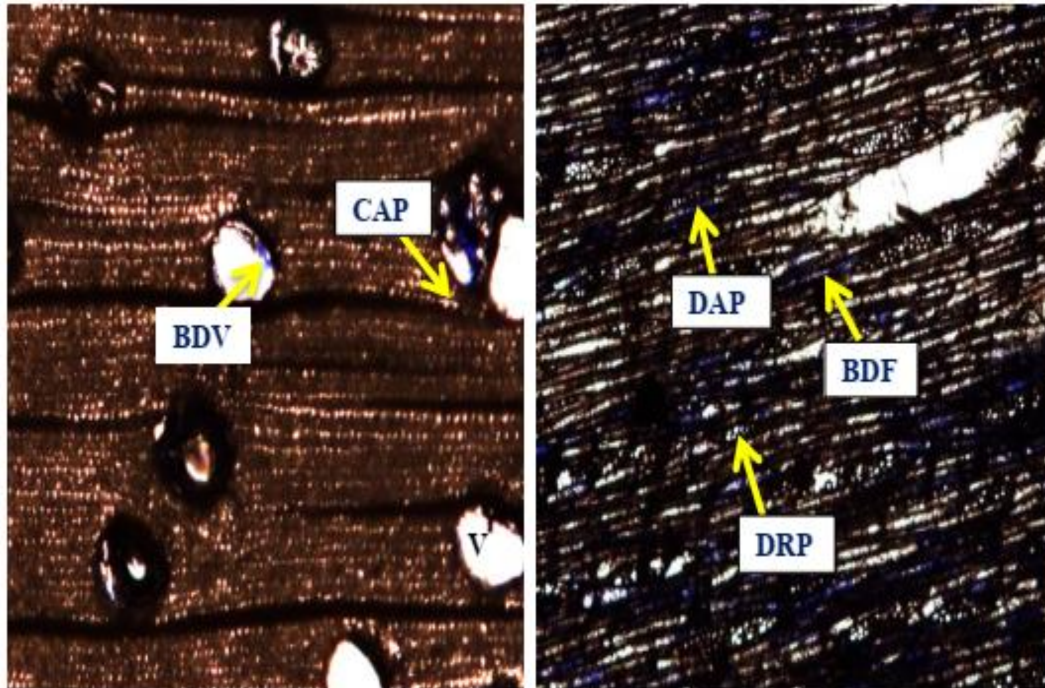
A: C/S of control samples
Mag x20

B: TLS of control samples
Mag x20



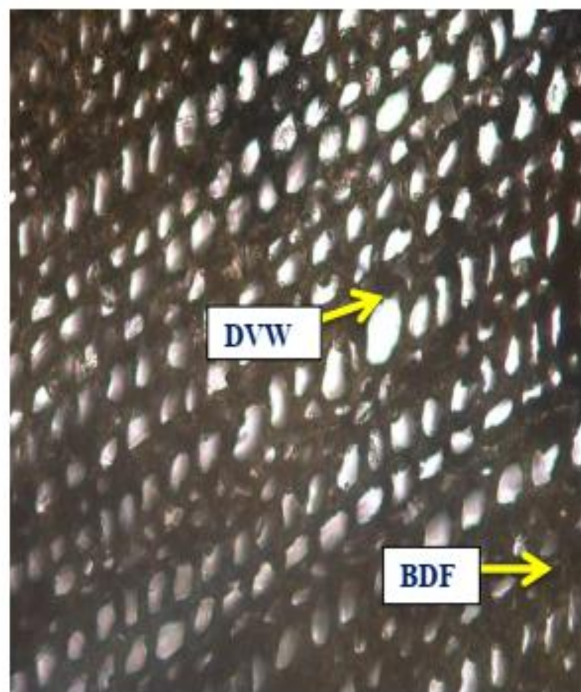
C: RLS of control samples
Mag x20

Plate 4.5: Light microscopy sections of control samples: V – Vessels; AP – Axial Parenchyma; F – Fibre; T – Tyloses; RP – Ray Parenchyma



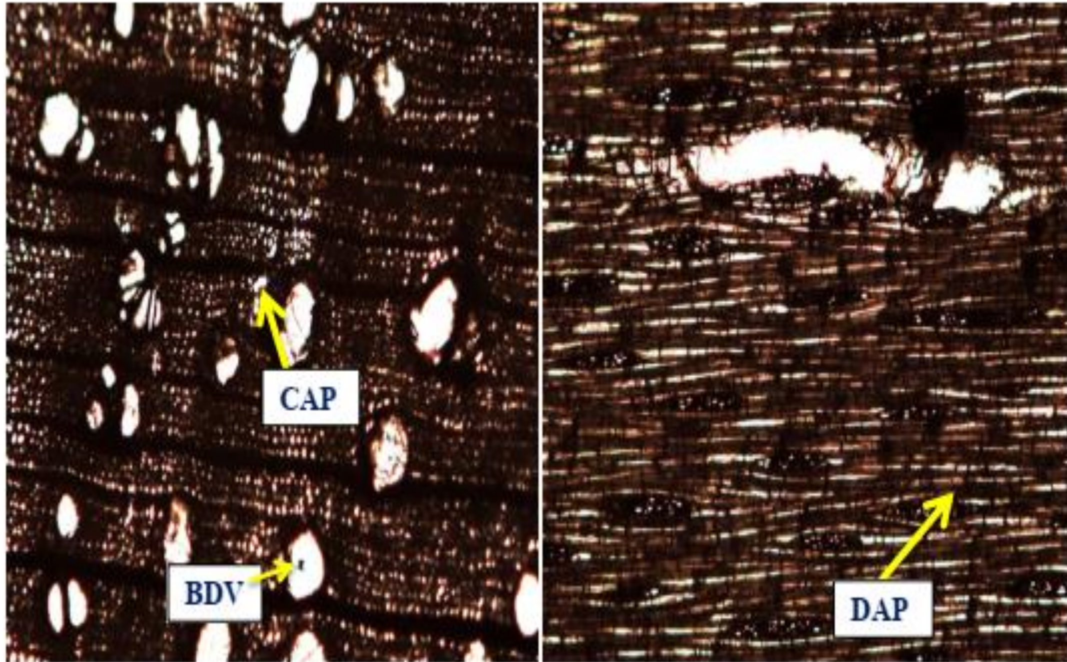
A: C/S of *G. adspersum* treated samples Mag x20

B: TLS of *G. adspersum* treated samples Mag x20



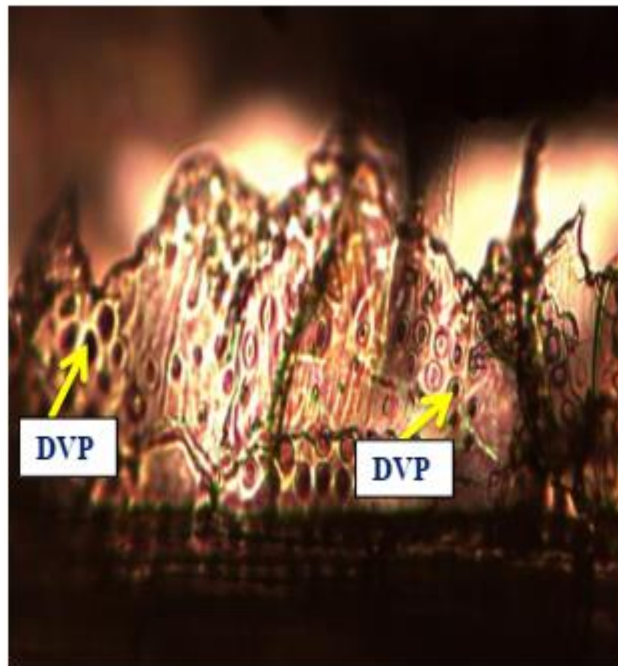
C: C/S of *G. adspersum* treated samples (Mag x30)

Plate 4.6: Light microscopy sections of *G. adspersum* incised samples: V – Vessels; CAP – Coalesced Axial Parenchyma; BDV – Blue dye in vessel; BDF – Blue dye in fibre; DVW – Delignification of vessel wall; DAP – Blue dye in axial parenchyma; DRP – Blue dye in ray parenchyma



A: C/S of *I. dryophilus* treated samples
Mag x20

B: TLS of *I. dryophilus* treated samples
Mag x20



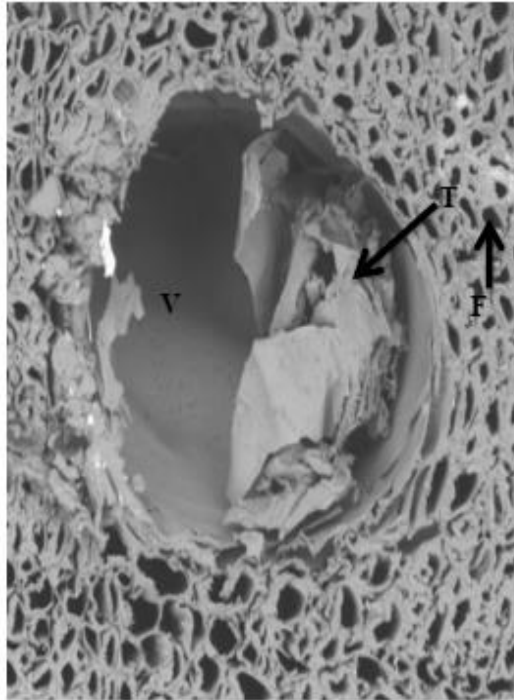
C: RLS of *I. dryophilus* treated samples (Mag x20)

Plate 4.7: Light microscopy sections of *I. dryophilus* incised samples: CAP – Coalesced Axial Parenchyma; BDV – Blue dye in vessel; BDF – Blue dye in fibre; DVP – Blue dye in vessel pit; DAP – Blue dye in axial parenchyma

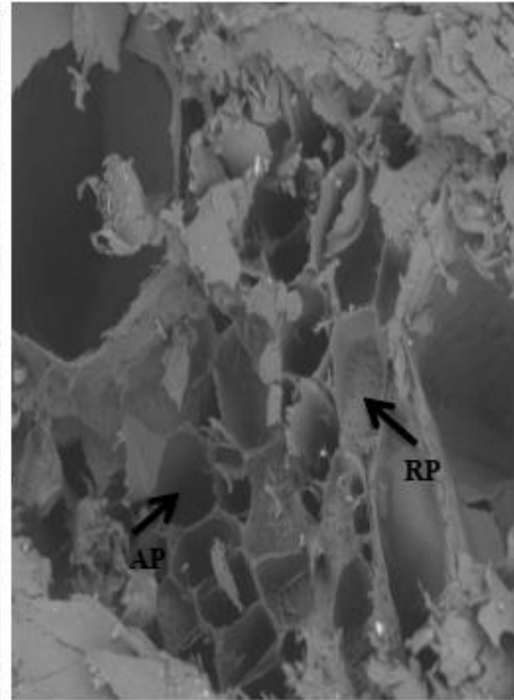
The cell walls of two axial parenchyma cells appear degraded, coupled with the delignification of the middle lamella, leading to a coalescence of the two parenchyma cells (Plate 4.6A). There is also evidence of delignification of the vessel wall by the *G. adspersum* hyphae from the lumina outwards, and the presence of the blue dye in a possibly unoccluded fibre cell (Plate 4.6C). Similarly, the *I. dyophilus* bioincised samples (Plates 4.7A - 4.7C) showed similar attributes to that of the samples bioincised with *G. adspersum*; however, the presence of the blue dye in the vessels and ray parenchyma was not too obvious. Coalescence of axial parenchyma cells due to possible delignification and degradation of their cell walls was also noticeable (Plate 4.7A). There was also the presence of the blue dye within the vessel pits (Plate 4.7C). For both bioincised samples, the blue dye appears to be virtually absent in the occluded fibre cells and axial parenchyma. Also, no major damages appear to have been induced on the structural components of the bioincised wood samples, as all the cells remained somewhat intact when compared with those of the control samples.

4.2.2 Scanning electron microscopy

The results of the scanning electron micrographs are presented in Plates 4.8 - 4.10. Plates 4.8A and 4.8B show the cross-section of the sound wood (control samples) of *Gmelina arborea* heartwood. Plates 4.9A - 4.9D represent the tangential section of the wood samples bioincised with *G. adspersum* for 9 weeks. Plate 4.10A and 4.10D represent the tangential section of the wood samples bioincised with *I. dryophilus* for 9 weeks, while Plates 4.10B and 4.10C represent the cross-section of the wood samples bioincised with *I. dryophilus* for 9 weeks. Micrographs from the control samples show the vessels plugged with tyloses, as well as sound cells of fibres, ray and axial parenchyma. Micrographs from the wood samples bioincised with *G. adspersum* show the fungal hyphae within and around the ray parenchyma (Plate 4.9A), as well as growing in a fibre cell that is devoid of occlusion (Plate 4.9B). Penetration of the tyloses by the advancing hyphae in the vessel was also noticeable (Plate 4.9C). Also, the fungal hyphae can be seen on the tangential vessel wall in a network-like fashion, with slight enlargement of the vessel pits on the radial wall due to the coalescing of pits also observed (Plate 4.9D). Erosion troughs are also visible on the vessel wall (Plate 4.9D). The fungal hyphae are also observed to move from the vessel lumina via the vessel pits into adjoining cells, possibly the ray parenchyma cells or vice-versa (Plate 4.9D). No significant damage to the wood cells was observed, except for the

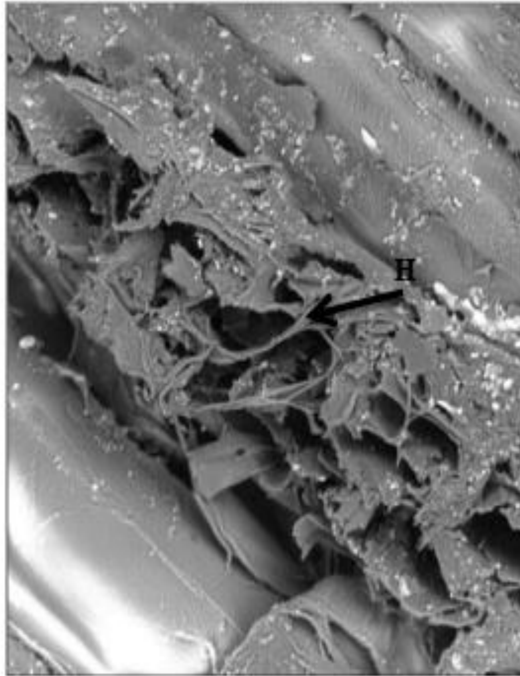


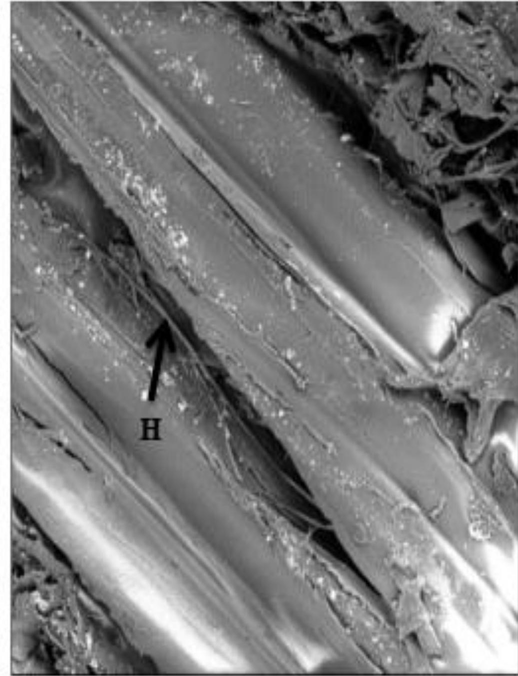
A: C/S of control samples
Mag x525



B: C/S of control samples
Mag x990

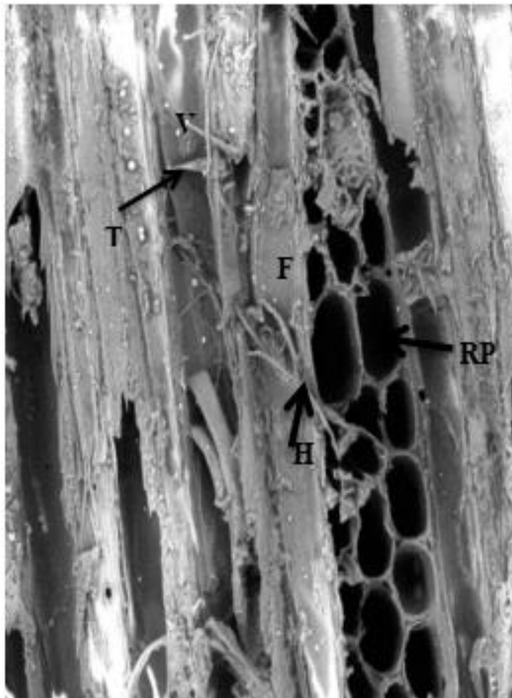
Plate 4.8: SEM of control samples: V – Vessels; AP – Axial Parenchyma; F – Fibre; T – Tyloses; RP – Ray Parenchyma

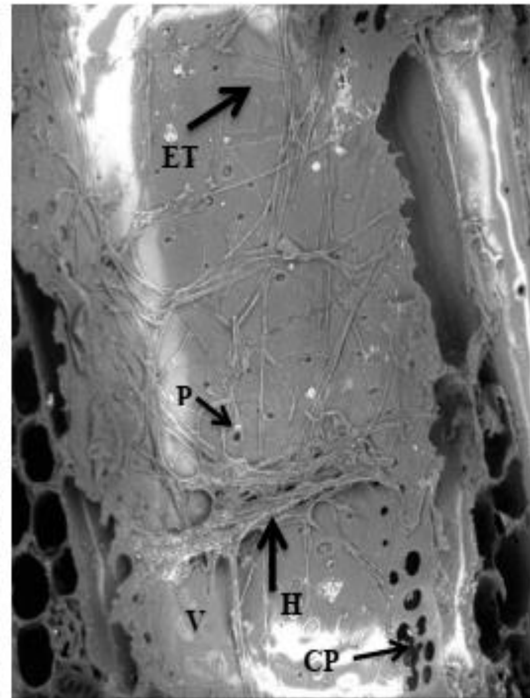




A: TLS of *G. adspersum* bioincised sample Mag x1725

B: TLS of *G. adspersum* bioincised samples Mag x1800

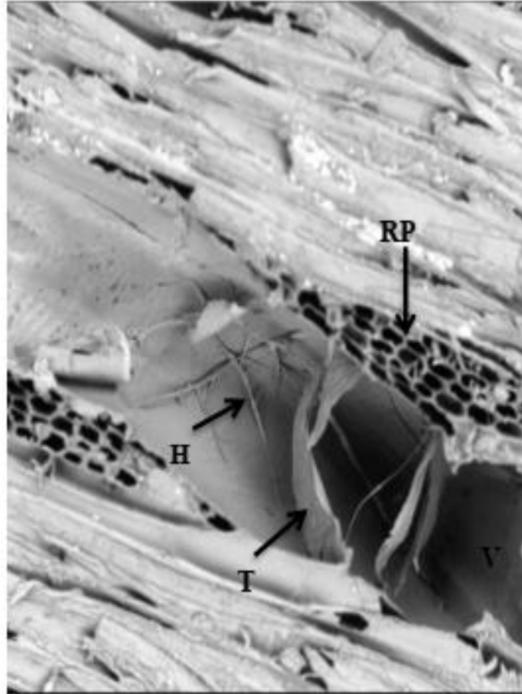




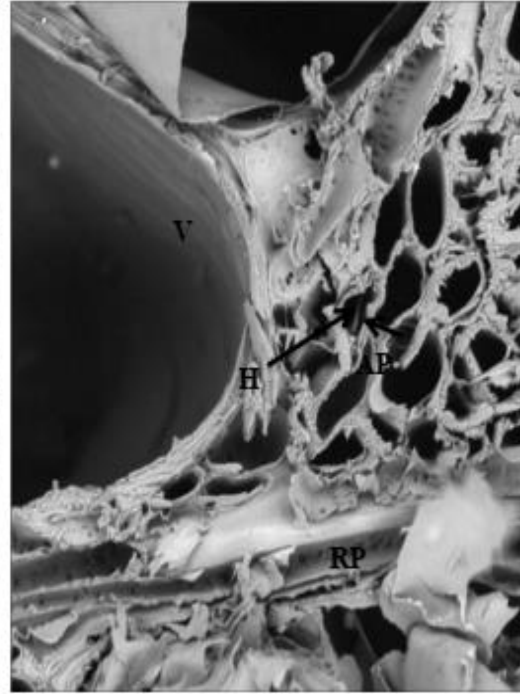
C: TLS of *G. adspersum* bioincised samples Mag x1600

D: TLS of *G. adspersum* bioincised samples Mag x1360

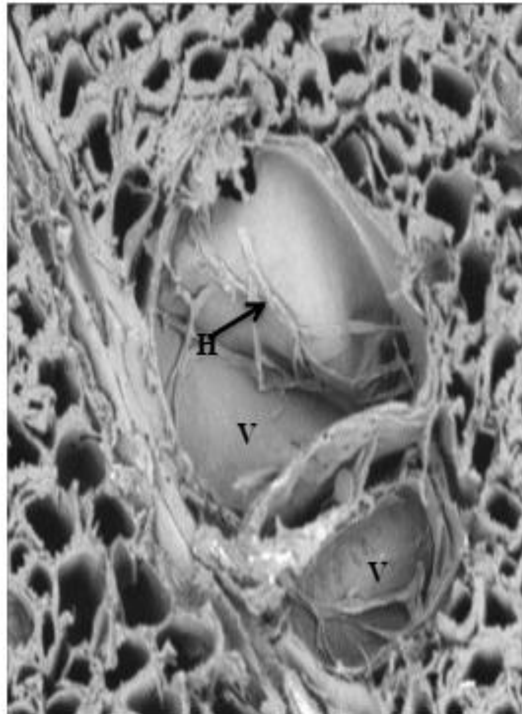
Plate 4.9: SEM of *G. adspersum* incised samples: V – Vessels; H – Fungal hyphae; F – Fibre; T – Tyloses; RP – Ray Parenchyma; ET – Erosion trough; CP – Coalesced pit; P – Pit



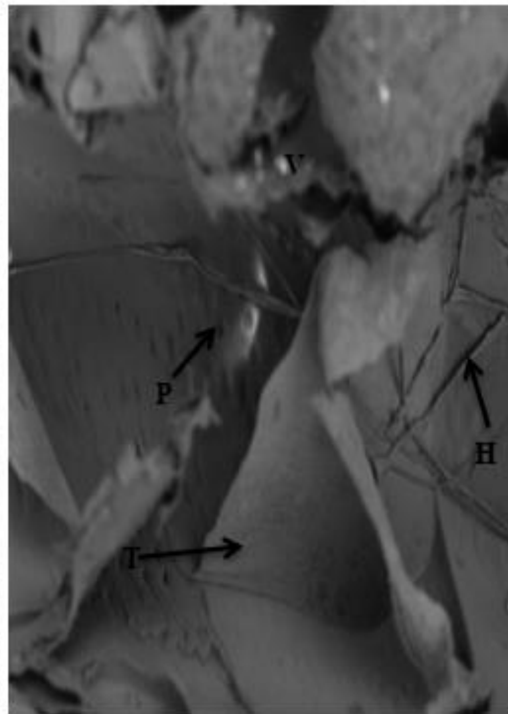
A: TLS of *I. dryophilus* bioincised samples
Mag x605



B: C/S of *I. dryophilus* bioincised samples
Mag x1300



C: C/S of *I. dryophilus* bioincised samples
Mag x995



D: TLS of *I. dryophilus* bioincised samples
Mag x1040

Plate 4.10: SEM of *I. dryophilus* incised samples: V – Vessels; H – Fungal hyphae; T – Tyloses; RP – Ray Parenchyma; P – Pit; AP – Ray Parenchyma

enlargement of the vessel pits and erosion of the vessel wall. Micrographs from the wood samples bioincised with *I. dryophilus* show the fungal hyphae penetrating the tyloses in a vessel (Plate 4.10A). The fungal mycelia can also be seen within the axial parenchyma (Plate 4.10B). Micrographs from the transverse sections of the bioincised wood samples also show the fungal mycelia within a multiple pore (Plate 4.10C), and moving through the tyloses in the vessel (pore). The vessel pits can be seen to be sealed, possibly due to the incrustration of the pits by extractives or aspiration of the pits and the fungal hyphae is observed to move along the vessel wall rather than moving through the vessel pits into adjoining cells (Plate 4.10D). Similarly, obvious damages to wood cells were not observed in the micrographs.

4.3 Mechanical properties

4.3.1 Impact resistance

The mean impact resistance of the control samples and bioincised wood samples for both fungi is presented graphically in Fig. 4.7. The mean impact resistance of the control samples was 0.91 J/cm^2. For wood samples bioincised with *I. dryophilus*, mean impact resistance values ranged from 1.08 J/cm^2 to 1.19 J/cm^2. There was an inconsistent increase in impact resistance of the samples with increasing incubation period, with the impact resistance increasing from samples bioincised for 3 weeks (1.08 J/cm^2) to those bioincised for 7 weeks (1.19 J/cm^2) and then decreasing at 9 weeks (1.08 J/cm^2), at which the least impact resistance was observed. For the wood samples bioincised with *G. adspersum*, mean impact resistance values ranged from 0.78 J/cm^2 to 1.11 J/cm^2. Here, the impact resistance more or less decreased with increasing incubation period, contrary to those bioincised with *I. dryophilus*. The impact resistance of the bioincised wood samples was considerably influenced ($p<0.05$) by the fungi species as revealed by the statistical analysis using ANOVA (Table 4.20).

The main effect for the impact resistance of the bioincised samples of *G. arborea* wood is presented in Table 4.21. The mean impact resistance of the samples bioincised with *I. dryophilus* (1.07 J/cm^2) was considerably higher ($p<0.05$) than those bioincised with *G. adspersum* (0.91 J/cm^2). Based on the incubation period, an inconsistent trend in the impact resistance of the samples was observed. The least impact resistance was observed in the control samples (0.91 J/cm^2), while the highest was observed in the

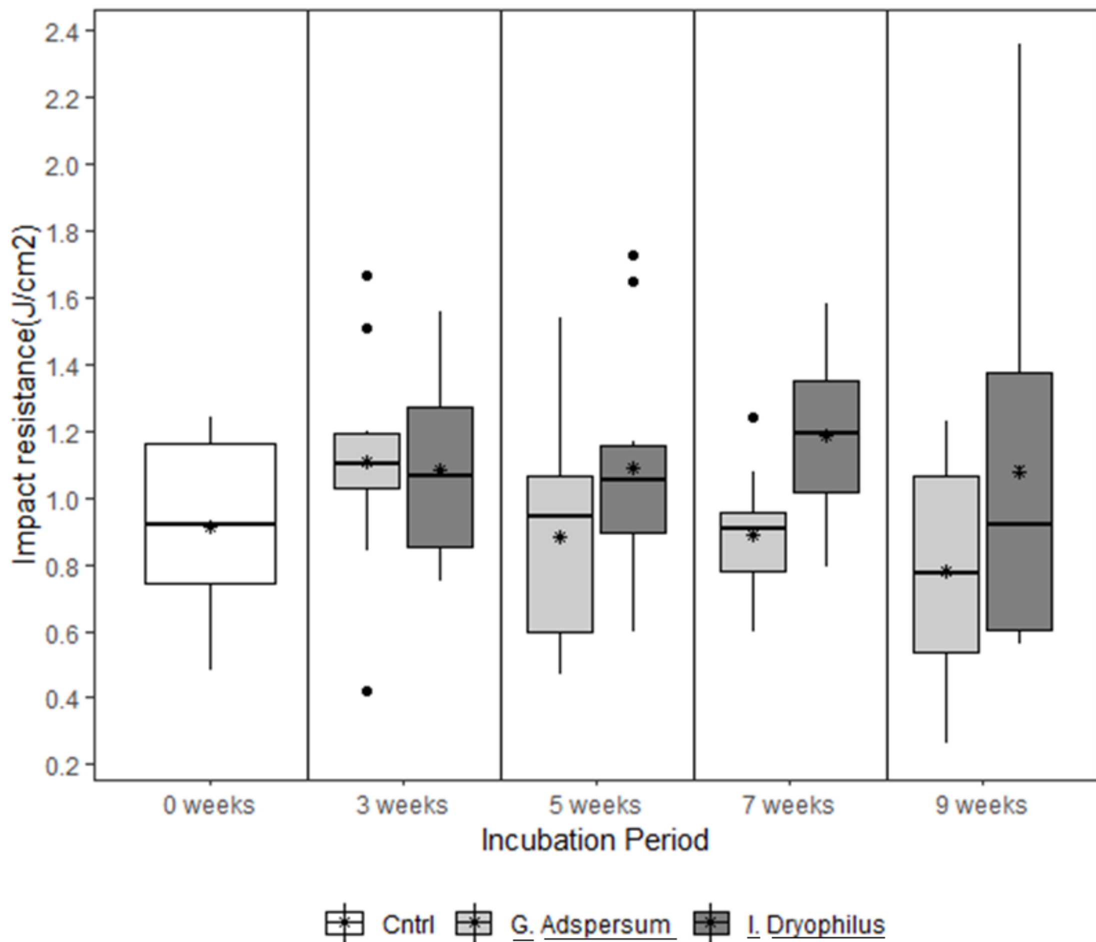


Fig. 4.7: Boxplot showing the Impact resistance of the bioincised samples at varying incubation periods

Table 4.20: Analysis of Variance for Impact resistance

Sources of Variation	Df	SS	MS	Fcal	p-value
Fungi	1	0.6134	0.6134	5.483	0.021*
Incubation period	4	0.4494	0.1123	1.004	0.410^{ns}
Fungi*Incubation period	4	0.5035	0.1259	1.125	0.350^{ns}
Error	90	10.0684	0.1119		
Total	99	11.6346			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.21: Main effect for Impact resistance of the bioincised wood samples

Parameters	Impact resistance (J/cm^2)
Fungi	
Inonotus dryophilus	1.07 ± 0.05^{a}
Ganoderma adspersum	0.91 ± 0.04^{b}
Incubation period	
Control	0.91±0.06
3 weeks	1.10±0.07
5 weeks	0.99±0.08
7 weeks	1.04±0.06
9 weeks	0.93±0.11

Mean values with the same superscript are not significantly different at α = 0.05

samples incubated for 3 weeks (1.10 J/cm^2). Overall, the impact resistance of the wood samples increased after bioincision as they all had higher impact resistance than the control samples.

4.3.2 MOE and MOR

The mean MOE and MOR of the control samples and bioincised wood samples for both fungi are presented graphically in Fig. 4.8 and 4.9, respectively. The mean MOE of the control samples was 7771.06 MPa. For wood samples bioincised with *I. dryophilus*, mean MOE values ranged from 7318.75 MPa to 7608.29 MPa. The highest MOE was observed in the samples bioincised for 5 weeks, while the least was observed in samples bioincised for 7 weeks. For the wood samples bioincised with *G. adspersum*, mean MOE values ranged from 6919.73 MPa to 7553.44 MPa. The MOE of the bioincised samples decreased consistently from 3 weeks of incubation (7553.44 MPa) to 7 weeks of incubation (6919.73 MPa), and slightly increased at 9 weeks of incubation (7305.03 MPa).

The mean MOR of the control samples is 77.90 MPa. For wood samples bioincised with *I. dryophilus*, mean MOR values ranged from 74.02 MPa to 75.54 MPa. There was a more or less decreasing trend in MOR of the samples with increasing incubation period, with the MOR decreasing from samples bioincised for 3 weeks (75.54 MPa) to those bioincised for 7 weeks (74.02 MPa), but with a slight increase at 9 weeks (75.22 MPa). For the wood samples bioincised with *G. adspersum*, mean MOR ranged from 52.64 MPa to 74.41 MPa. Similar to the observation in the samples bioincised with *I. dryophilus*, a more or less decreasing trend in MOR of the samples with increasing incubation period was noted, with the MOR decreasing from samples bioincised for 3 weeks (74.41 MPa) to those bioincised for 7 weeks (52.64 MPa), but with a sharp increase at 9 weeks (70.80 MPa). According to the statistical analysis in Table 4.22, the fungi species and incubation period had no significant effect ($p>0.05$) on the MOE of the bioincised samples, but the MOR was considerably influenced ($p<0.05$) by both factors.

The main effect for the MOE and MOR of the bioincised samples of *G. arborea* wood is presented in Table 4.23. The mean MOE of the samples bioincised with *I. dryophilus* was 7519.09 MPa, while that bioincised with *G. adspersum* was 7371.94 MPa. The MOR of samples bioincised with *I. dryophilus* (75.62 MPa) was

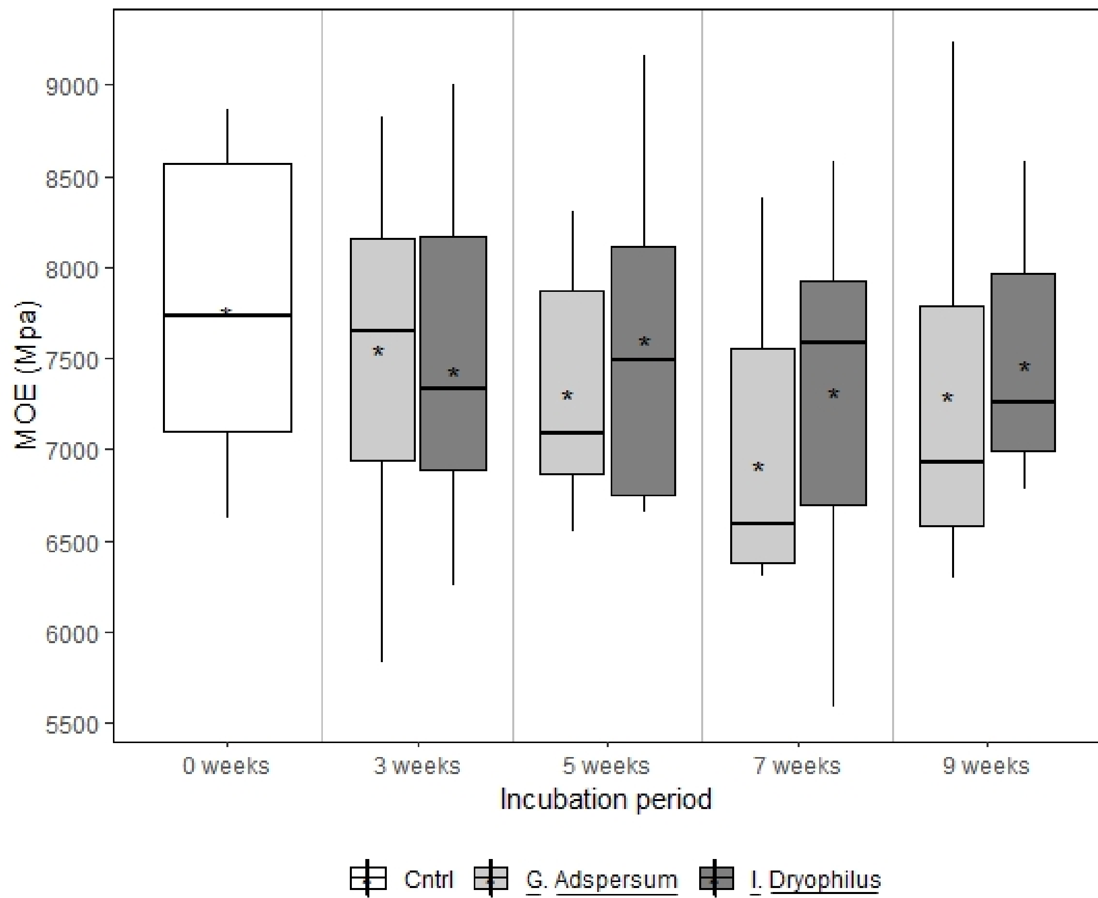


Fig. 4.8: Boxplot showing the Modulus of Elasticity (MOE) of the bioincised samples at varying incubation periods

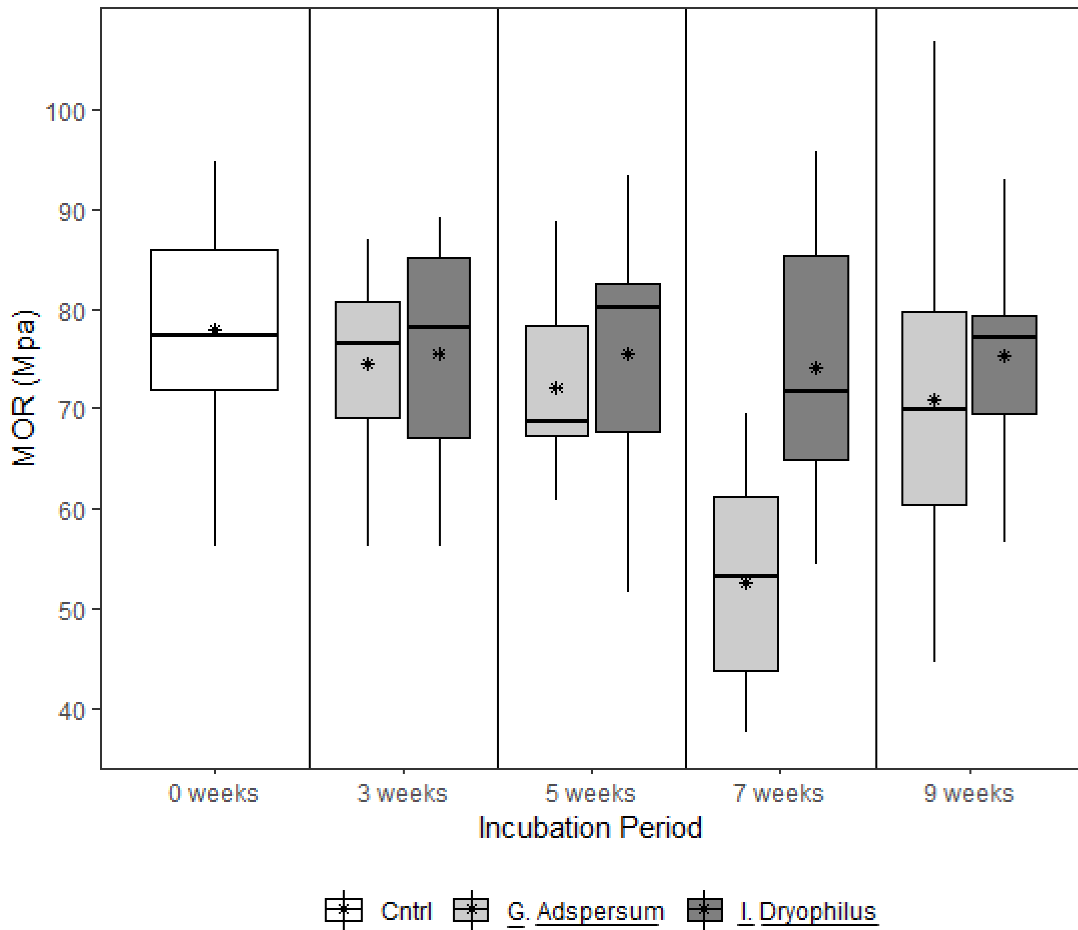


Fig. 4.9: Boxplot showing the Modulus of Rupture (MOR) of the bioincised samples at varying incubation periods

Table 4.22: Analysis of Variance for MOE and MOR

Sources of Variation	Df	SS	MS	Fcal	p-value
Modulus of Elasticity					
Fungi	1	$5.41x10^5$	$5.41x10^5$	0.794	0.375^{ns}
Incubation period (IP)	4	$4.37x10^6$	$1.09x10^6$	1.603	0.180^{ns}
Fungi*Incubation period	4	$8.98x10^5$	$2.24x10^5$	0.329	0.858^{ns}
Error	90	$6.14x10^7$	$6.82x10^5$		
Total	99	$6.72x10^7$			
Modulus of Rupture					
Fungi	1	913.8	913.8	5.591	0.020*
Incubation period (IP)	4	2424.0	606.0	3.708	0.008*
Fungi*Incubation period	4	1531.7	382.9	2.343	0.061^{ns}
Error	90	14708.9	163.4		
Total	99	19578.4			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.23: Main effect for MOE and MOR of the bioincised wood samples

Parameters	MOE (MPa)	MOR (MPa)
Fungi		
Inonotus dryophilus	7519.09±116.19	75.62±1.72[a]
Ganoderma adspersum	7371.94±117.04	69.58±2.16[b]
Incubation period		
Control	7771.06±176.17	77.90±2.71[a]
3 weeks	7493.25±190.36	74.97±2.33[a]
5 weeks	7459.37±180.55	73.78±2.69[a]
7 weeks	7119.24±186.90	63.33±3.69[b]
9 weeks	7384.67±174.58	73.01±3.39[a]

Mean values with the same superscript within the same column are not significantly different at $\alpha = 0.05$

considerably higher ($p<0.05$) than those bioincised with *G. adspersum* (69.58 MPa). Based on the incubation period, both the MOE and MOR of the wood samples followed a similar trend. The MOE and MOR reduced with increasing incubation time, except for the 9 weeks of exposure where a slight increase was noticed. The highest MOE and MOR values were observed in the control samples (7771.06 MPa and 77.09 MPa), while the least MOE and MOR values were observed in the samples incubated for 7 weeks (7119.24 MPa and 63.33 MPa). The mean MOR of the samples bioincised for 7 weeks was significantly lower ($p<0.05$) than those of the other incubation periods.

4.3.3 Maximum compressive strength (//)

The mean maximum compressive strength of the control samples and bioincised wood samples for both fungi is presented graphically in Fig. 4.10. The average MCS of the control samples was 43.13 N/mm^2. For wood samples bioincised with *I. dryophilus*, mean MCS values ranged from 40.29 N/mm^2 to 42.38 N/mm^2. There was a more or less decreasing trend in MOR of the samples with prolonged exposure period, with the MCS decreasing from samples bioincised for 3 weeks (41.21 N/mm^2) to those bioincised for 7 weeks (40.29 N/mm^2), but with an increase at 9 weeks (42.38 N/mm^2). For the wood samples bioincised with *G. adspersum*, mean MCS ranged from 38.51 N/mm^2 to 42.17 N/mm^2. Here, an inconsistent trend in MCS of the bioincised samples with increasing incubation period was observed, with the highest MCS observed in samples bioincised for 9 weeks (42.17 N/mm^2), while the least MCS was observed in samples bioincised for 5 weeks (38.51 N/mm^2). Statistical analysis through ANOVA (Table 4.24) revealed that the fungi species and incubation period had no significant effect ($p>0.05$) on the MCS of the bioincised samples.

Table 4.25 shows the main effect for the MCS of the bioincised samples of *G. arborea* wood. The mean MCS of the samples bioincised with *I. dryophilus* was 41.58 N/mm^2, which was higher than those bioincised with *G. adspersum* (41.09 N/mm^2). Based on the incubation period, there was an inconsistent trend in the MCS of the wood samples with increasing incubation period. The highest MCS was observed in the control samples (43.13 N/mm^2), while the least MCS was observed in samples bioincised for 5 weeks (39.70 N/mm^2).

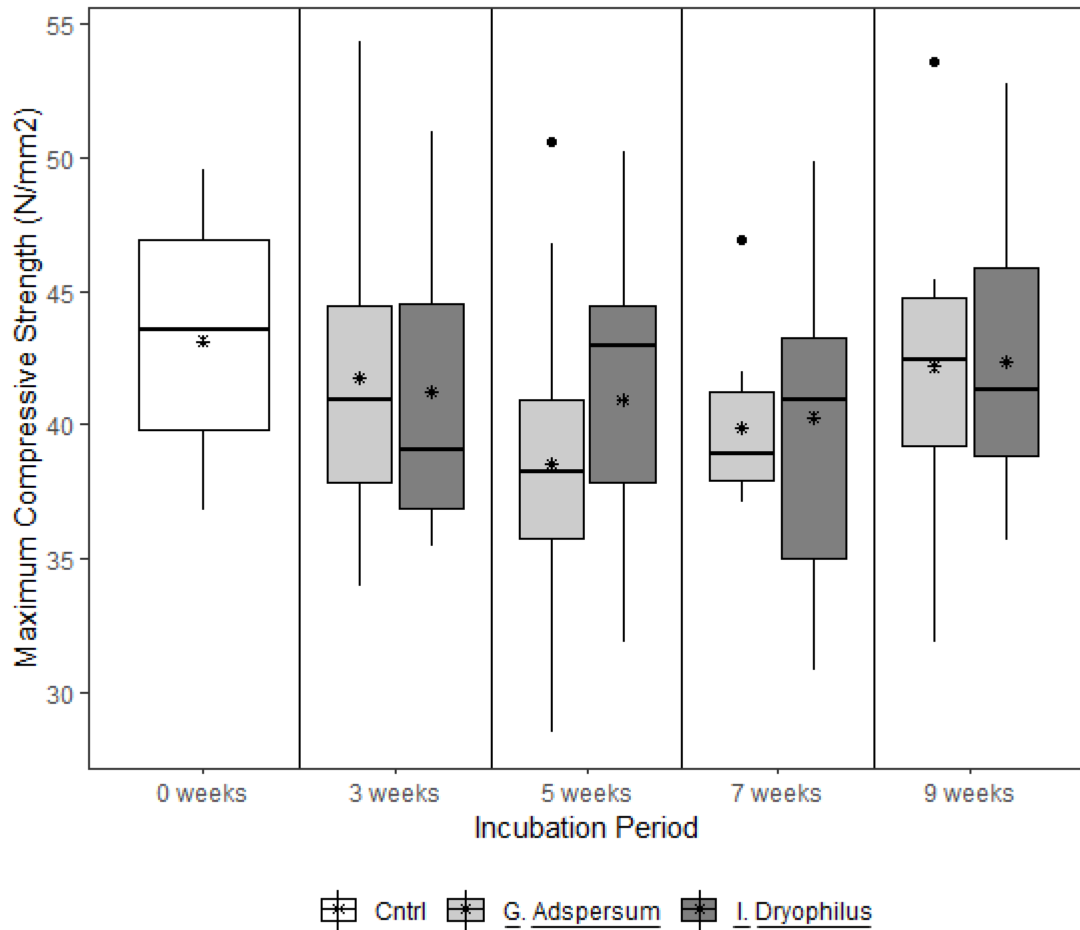


Fig. 4.10: Boxplot showing the Maximum Compressive Strength (MCS//) of the bioincised samples at varying incubation periods

Table 4.24: Analysis of Variance for Maximum compressive strength

Sources of Variation	Df	SS	MS	Fcal	p-value
Fungi	1	6.0	6.0	0.198	0.658^{ns}
Incubation period	4	167.0	41.7	1.367	0.252^{ns}
Fungi*Incubation period	4	25.0	6.2	0.204	0.935^{ns}
Error	90	2748.7	30.5		
Total	99	2946.7			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.25: Main effect for Maximum compressive strength of the bioincised wood samples

Parameters	Maximum compressive strength (N/mm^2)
Fungi	
Inonotus dryophilus	41.58±0.77
Ganoderma adspersum	41.09±0.77
Incubation period	
Control	43.13±0.10
3 weeks	41.48±1.30
5 weeks	39.70±1.41
7 weeks	40.09±1.09
9 weeks	42.27±1.20

4.3.4 Tensile strength (//)

The mean tensile strength of the control samples and bioincised wood samples for both fungi is presented graphically in Fig. 4.11. The mean tensile strength of the control samples was 46.81 MPa. For wood samples bioincised with *I. dryophilus*, mean tensile strength values ranged from 43.86 MPa to 52.49 MPa. There was no defined trend in the tensile strength of the samples with increasing incubation period, with the highest tensile strength observed in the samples bioincised for 5 weeks, while the least was observed in the samples bioincised for 7 weeks. For the wood samples bioincised with *G. adspersum*, mean tensile strength ranged from 36.80 MPa to 49.50 MPa. Similar to the samples bioincised with *I. dryophilus*, an inconsistent trend in the tensile strength of the bioincised samples with increasing incubation period was observed, with the highest tensile strength observed in samples bioincised for 9 weeks, while the least was observed in samples bioincised for 5 weeks. Statistical analysis through ANOVA (Table 4.26) revealed that the fungi species and incubation period had no pronounced effect ($p>0.05$) on the bioincised wood samples' tensile strength.

The main effect for the tensile strength of the bioincised samples of *G. arborea* wood is presented in Table 4.27. The mean tensile strength of the samples bioincised with *I. dryophilus* (47.16 MPa) was higher than those bioincised with *G. adspersum* (43.88 MPa). Based on the incubation period, the tensile strength of the samples more or less reduced with increasing incubation time, except for the 9 weeks of exposure where a sharp increase was noticed. The highest tensile strength was observed in the samples bioincised for 9 weeks (48.70 MPa), while the least tensile strength was observed in the samples incubated for 7 weeks (40.81 MPa).

4.3.5 Janka hardness

The average hardness of the control samples and bioincised wood samples for both fungi are presented graphically in Fig. 4.12. The mean hardness of the control samples was 2241.05 N. For wood samples bioincised with *I. dryophilus*, mean hardness values ranged from 2255.00 N to 2460.90 N. There was a more or less decreasing trend in the hardness of the samples with increasing incubation period, with the hardness decreasing from samples bioincised for 3 weeks (2460.90 N) to those bioincised for 7 weeks (2255.00 N), but with a slight increase at 9 weeks of exposure (2269.15 N). For the wood samples bioincised with *G. adspersum*, mean hardness lay in the range of

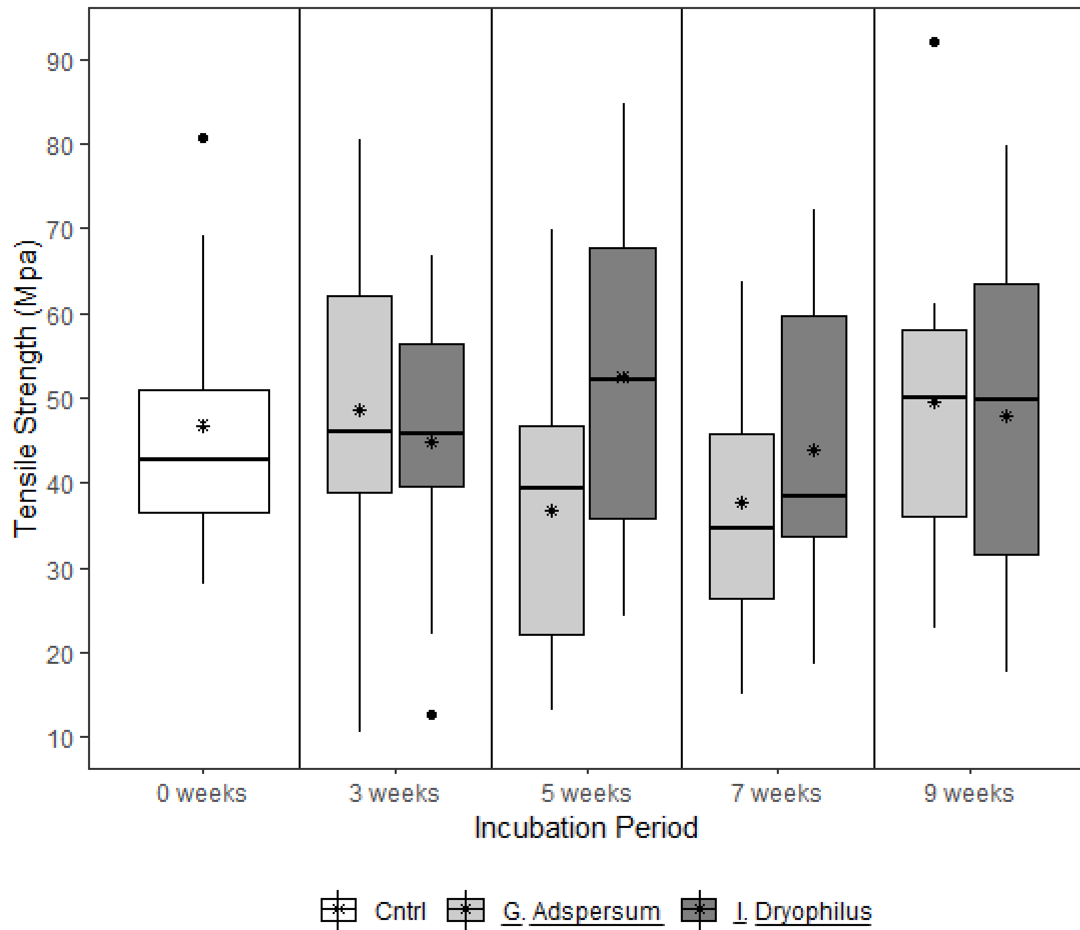


Fig. 4.11: Boxplot showing the Tensile strength // of the bioincised samples at varying incubation periods

Table 4.26: Analysis of Variance for Tensile strength

Sources of Variation	**Df**	**SS**	**MS**	**Fcal**	**p-value**
Fungi	1	268.6	268.6	0.761	0.385^{ns}
Incubation period	4	718.5	179.6	0.509	0.729^{ns}
Fungi*Incubation period	4	1233.5	308.4	0.874	0.483^{ns}
Error	90	31760.7	352.9		
Total	99	33981.3			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.27: Main effect for Tensile strength of the bioincised wood samples

Parameters	Tensile strength (MPa)
Fungi	
Inonotus dryophilus	47.16±2.62
Ganoderma adspersum	43.88±2.62
Incubation period	
Control	46.81±3.61
3 weeks	46.64±4.33
5 weeks	44.64±4.62
7 weeks	40.81±3.81
9 weeks	48.70±4.45

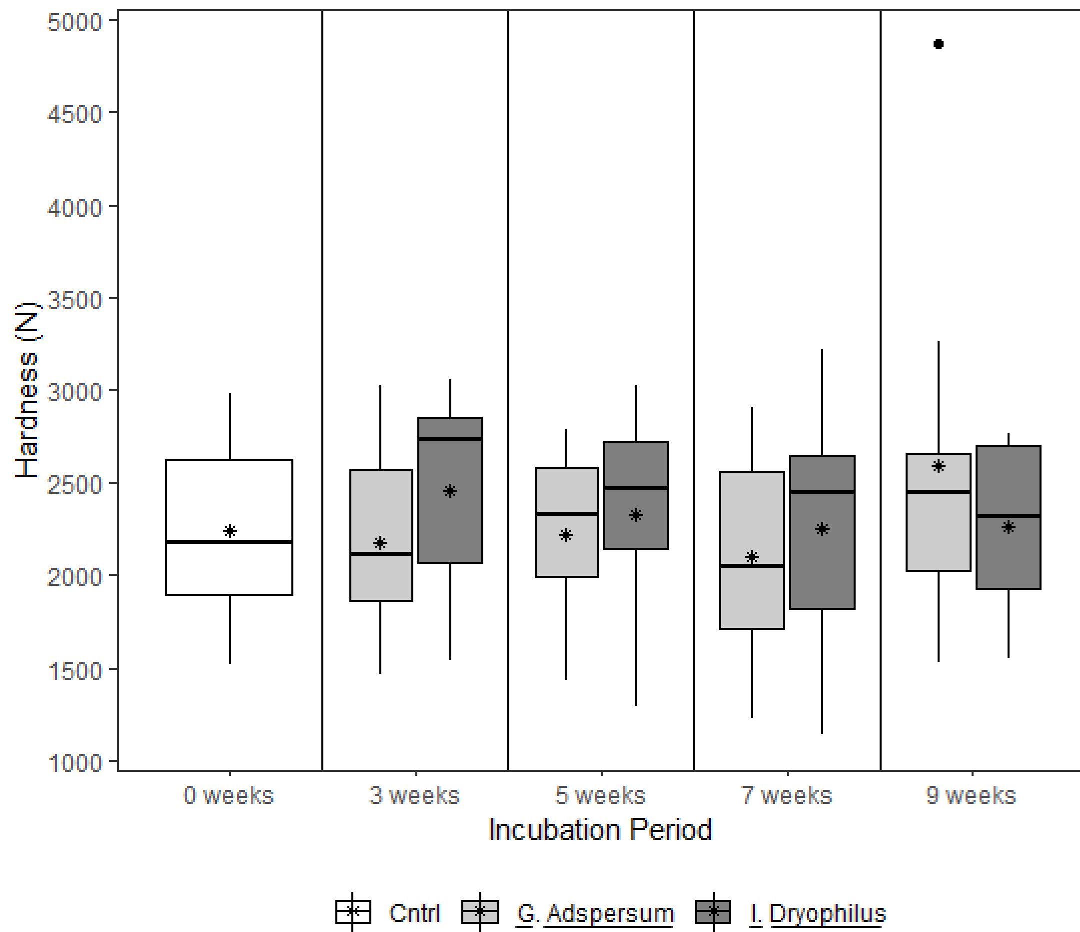


Fig. 4.12: Hardness change of the wood across the incubation periods

2104.58 N to 2593.08 N. Here, an inconsistent trend in the average hardness of the bioincised samples with increasing incubation period was observed, with the highest hardness observed in the samples bioincised for 9 weeks, while the least hardness was observed in the samples bioincised for 7 weeks.

The mean radial and tangential hardness of the control samples and bioincised wood samples for both fungi are presented graphically in Fig. 4.13. The mean radial and tangential hardness of the control samples were 2178.67 N and 2303.43 N, respectively. For wood samples bioincised with *I. dryophilus*, mean radial hardness lay in the range of 2071.50 N to 2364.50 N, while mean tangential hardness was in the range of 2374.44 N to 2557.30 N. There was an initial increase in the tangential and radial hardness of the wood specimens after bioincising for 3 weeks, but both the radial and tangential hardness decreased with increasing incubation time, except for the tangential hardness, where a slight increase was observed at 9 weeks of bioincision (2466.81 N). For the wood samples bioincised with *G. adspersum*, mean radial hardness ranged from 2048.06 N to 2455.49 N, while mean tangential hardness was in the range of 2307.02 N to 2730.68 N. An inconsistent trend in the radial hardness of the bioincised samples with increasing exposure period was observed, with the highest radial hardness observed in the samples bioincised for 9 weeks, while the least radial hardness was observed in the samples bioincised for 3 weeks. The tangential hardness, on the other hand, decreased with prolonged exposure period, except for a sharp increase observed in the samples bioincised for 9 weeks (2730.68 N). Statistical analysis through ANOVA (Table 4.28) revealed that the fungi species and incubation period had no significant effect ($p>0.05$) on the hardness of the bioincised samples, but the hardness of the bioincised samples was considerably influenced ($p<0.05$) by the wood axis.

Table 4.29 shows the main effect for the hardness of the bioincised samples of *G. arborea* wood. The mean hardness of the samples bioincised with *I. dryophilus* was 2311.09 N, which was higher than those bioincised with *G. adspersum* (2267.99 N). Based on the incubation period, there was an inconsistent trend in the hardness of the wood samples with increasing exposure period. The highest hardness was observed in the samples bioincised for 9 weeks (2431.12 N), while the least hardness was observed in samples bioincised for 7 weeks (2179.79 N). Based on the wood axis, the tangential

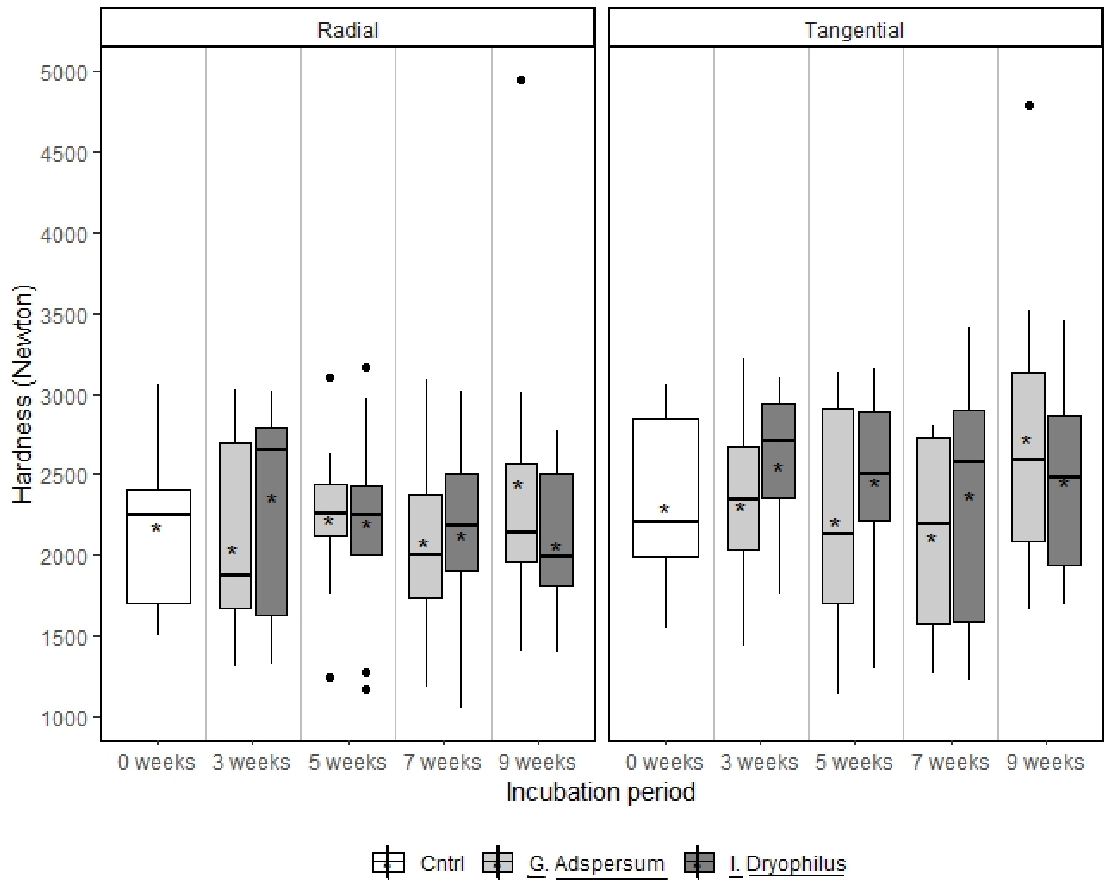


Fig. 4.13: Boxplot showing the radial and tangential hardness of the bioincised samples at varying incubation periods

Table 4.28: Analysis of Variance for Janka hardness

Sources of Variation	Df	SS	MS	Fcal	p-value
Fungi	1	9.29×10^4	9.29×10^4	0.240	0.624^{ns}
Incubation period (IP)	4	1.42×10^6	3.55×10^5	0.919	0.454^{ns}
Wood axis (WA)	1	1.79×10^6	1.79×10^6	4.641	0.033*
Fungi*Incubation period	4	2.10×10^6	5.24×10^5	1.358	0.251^{ns}
Fungi* Wood axis	1	1.38×10^5	1.38×10^5	0.356	0.551^{ns}
IP*WA	4	3.39×10^5	8.47×10^4	0.219	0.927^{ns}
Fungi* IP*WA	4	1.92×10^5	4.81×10^4	0.124	0.974^{ns}
Error	180	6.95×10^7	3.86×10^5		
Total	199	7.56×10^7			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.29: Main effect for Janka hardness of the bioincised wood samples

Parameters	Janka hardness (N)
Fungi	
Inonotus dryophilus	2311.09±57.22
Ganoderma adspersum	2267.99±65.97
Incubation period	
Control	2241.05±80.62
3 weeks	2319.22±90.54
5 weeks	2276.52±91.90
7 weeks	2179.79±91.90
9 weeks	2431.12±122.21
Wood axis	
Tangential	2384.21±61.85^{a}
Radial	2194.87±60.23^{b}

Mean values with the same superscript are not significantly different at α = 0.05

hardness (2348.21 N) was significantly higher ($p<0.05$) than the radial hardness (2194.87 N).

4.4 Fourier Transform Infrared (FT-IR) Spectroscopy

Determining chemical changes in wood after fungal degradation through analytical chemical procedures is complex, as a result of concomitant degradation processes. This makes it difficult to establish percentage changes in the individual components of the wood (Pandey, 1999), especially when the chemical changes are small. FT-IR offers the chance to analyse multiple changes in the full chemical mix present in wood; detecting small changes; higher replicability; ease of sample preparation, coupled with the fact that it is an almost non-destructive testing method. Bioincision, by design, usually yields minimal chemical changes in the wood, hence FT-IR was therefore used to analyse the chemical alterations in the Gmelina wood samples bioincised with both fungi by making comparisons between the spectra obtained for the control samples and bioincised samples. Assignments of main infra-red bands for solid wood are presented in Table 4.30, and Fig. 4.14 shows the labels for identified peaks for *G. arborea* unincised heartwood specimen.

The spectral bands of the control samples is shown in Fig 4.15 while wood samples bioincised with *G. adspersum* and *I. dryophilus* for 9 weeks are presented in Figures 4.16 – 4.20. The average control spectrum obtained from the mean of five spectra (Fig. 4.15), obtained from five control wood samples was used as the basis of comparison with the bioincised samples in order to identify the changes in the chemical constituents of the bioincised samples. A strong OH intramolecular and intermolecular stretching absorption was observed at 3335 cm^{-1}, which signifies an interaction of the cellulose, hemicellulose and lignin hydroxyl groups with moisture, while a characteristic symmetric CH stretching (2915 cm^{-1}) and asymmetric CH stretching (2850 cm^{-1}) absorption in aromatic methoxyl, methyl and methylene groups of lignin side chains were noticed. In the fingerprint region, which falls between 1800 cm^{-1} and 600 cm^{-1}, many well-defined peaks were also observed. The peaks identified in this region were assigned as unconjugated hemicellulose ketone/aldehyde C=O stretch at 1734 cm^{-1}, water associated with cellulose or lignin at 1650 cm^{-1}, C=C stretch of the syringyl unit of lignin aromatic ring at 1593 cm^{-1}, C=C stretch of the guaiacyl unit of lignin aromatic ring at 1504 cm^{-1}, C–H asymmetric deformation in $–OCH_3$, CH_2 in

Table 4.30: IR bands assignments of solid wood

Wavenumber (cm^{-1})	Band Assignment
3580–3550	Free OH(6) and OH(2), weakly absorbed water
3460–3405	O(2)H...O(6) intramolecular hydrogen bonds
3375–3340	O(3)H...O(5) intramolecular hydrogen bonds in cellulose
3310–3230	O(6)H...O(3) intermolecular hydrogen bonds in cellulose
3175	–OH stretching intramolecular hydrogen bonds in cellulose
2938–2920	Symmetric CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains
2840–2835	Asymmetric CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains
1770–1760	C=O stretching in conjugated ketones
1740–1720	C=O stretch in unconjugated ketones
1650–1640	Water associated with lignin or cellulose
1610–1590	C=C stretching of the aromatic ring (S)
1515–1505	C=C stretching of the aromatic ring
1470–1455	C–H asymmetric deformation in $–OCH_3$, CH_2 in pyran ring symmetric scissoring
1430–1422	C–H asymmetric deformation in $–OCH_3$
1375–1365	CH bending in cellulose I and cellulose II and hemicellulose
1335–1320	C1–O vibrations in S derivatives, CH in-plane bending in cellulose I and cellulose II
1315	CH_2 wagging in cellulose I and cellulose II
1282–1277	CH deformation in cellulose I and cellulose II
1268	Guaiacyl ring breathing, C–O linkage in guaiacyl aromatic methoxyl groups
1235–1230	Syringyl ring breathing and C–O stretching in lignin and xylan
1205–1200	OH in-plane bending in cellulose I and cellulose II
1162–1125	C–O–C asymmetric stretching in cellulose I and cellulose II
1140	Aromatic C–H in-plane deformation; typical for G units, where G condensed > G etherified
1128–1110	Aromatic C–H in-plane deformation (typical for S units), C=O stretch
1086–1075	C–O deformation in secondary alcohols and aliphatic ethers
1060–1015	C–O valence vibration mainly from C(3)–O(3)H
1047–1004	C–O stretching in cellulose I and cellulose II
996–985	C–O valence vibration
970	=CH out-of-plane deformation (trans)
930–915	Aromatic C–H out-of-plane deformations, pyran ring vibration

Note: S: Syringyl; G: Guaiacyl.
Source: Popescu *et al.* (2007).

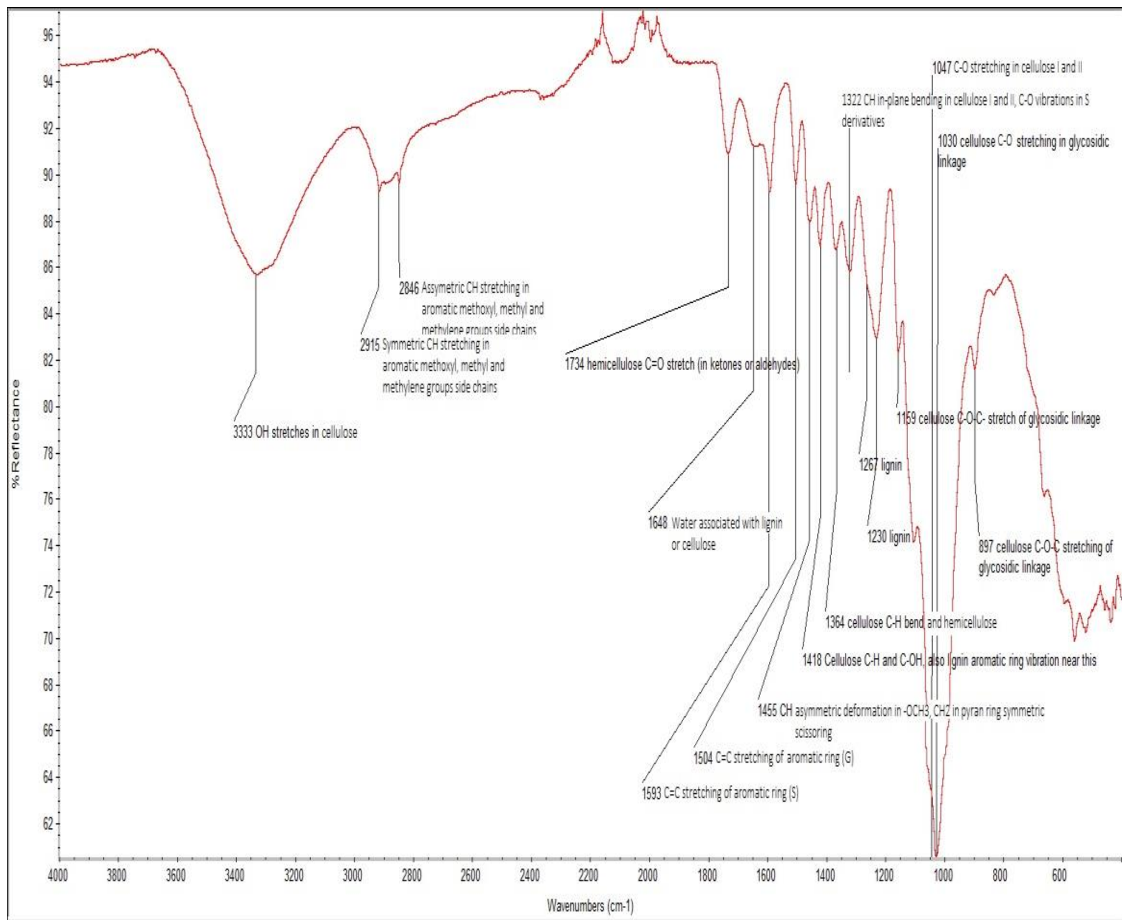


Fig. 4.14: FTIR Spectra for the *Gmelina arborea* control samples with labels

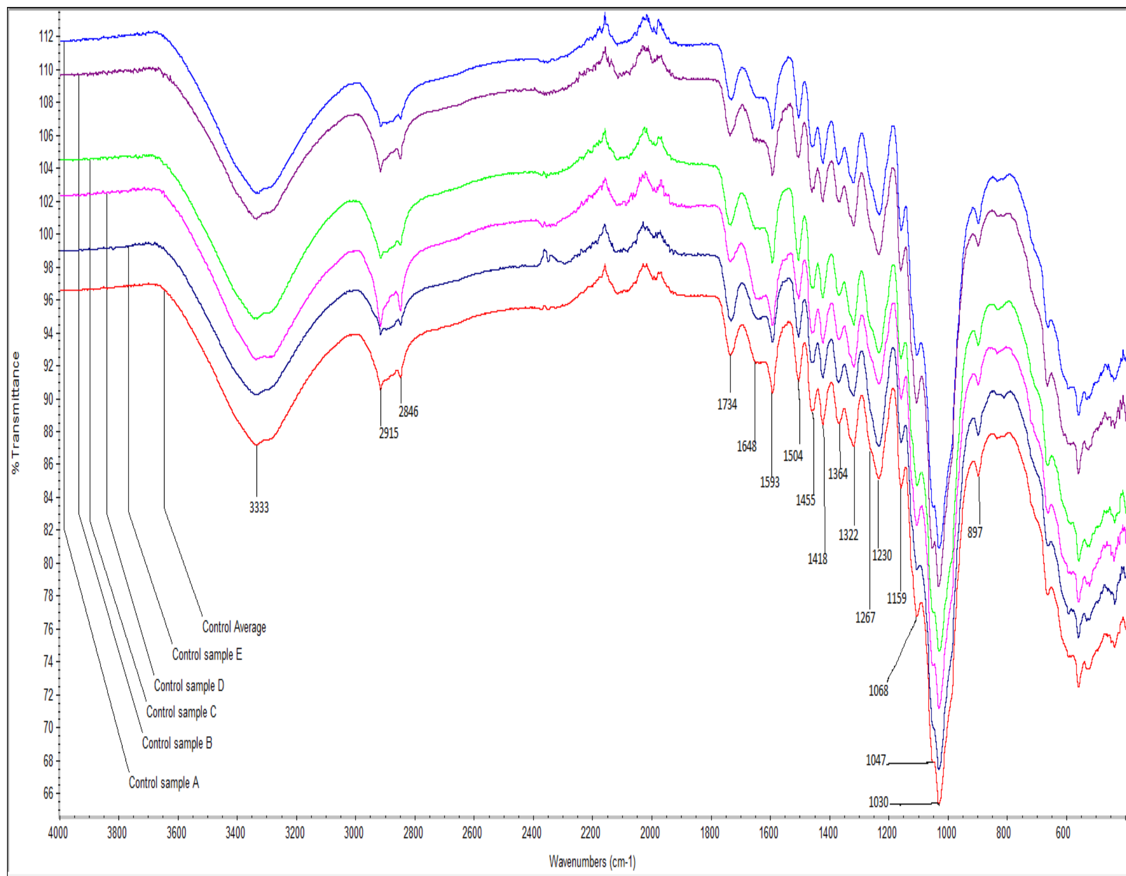


Fig. 4.15: FTIR Spectra for the *Gmelina arborea* control samples (n = 5)

pyran ring symmetric scissoring at 1455 cm^{-1}, C–H asymmetric deformation in $–OCH_3$ at 1418 cm^{-1}, cellulose and hemicellulose C-H bending at 1364 cm^{-1}, C-O vibrations in syringyl derivatives of lignin and cellulose CH in-plane bend at 1322 cm^{-1}, breathing in lignin guaiacyl ring with C-O linkage in guaiacyl aromatic methoxy groups at 1267 cm^{-1}, breathing in lignin syringyl ring and C-O stretch in xylan and lignin at 1230 cm^{-1}, cellulose C-O-C assymetric stretching of cellulose glycosidic link at 1159 cm^{-1}, cellulose C-O vibration at C3 at 1068 cm^{-1}, C-O stretching in cellulose I and II at 1047 cm^{-1} and 1030 cm^{-1} and, cellulose C-O-C stretching of glycosidic link at 897 cm^{-1}.

Similar to the control samples, an average spectrum for the inner part of the excised samples from five spectra was equally obtained for each of the fungi species. All spectra for *G adspersum* inner were very similar (Fig. 4.16), so an average spectrum was produced using five sampled spectra. Similarly, all *I. dryophilus* samples were very similar and five spectra were combined to give an average (Fig. 4.17). These average spectra for both fungi were compared with that of the control spectrum. For the *G. adspersum* and *I. dryophilus* incised inner samples, changes observed between their spectra and that of the control spectra were negligible.

FT-IR was also conducted on the outer surfaces of the excised samples due to the varying degree of pigmentation observed on the wood surfaces of the bioincised samples. Ten spectra were obtained from ten bioncised wood samples for each of the fungus species to better investigate and represent this variability. For the wood samples bioincised with *G. adspersum*, the ten spectra were grouped into four different categories based on the strength of the peaks at 1595 cm^{-1} and 1322 cm^{-1}, and the gradual disappearance of the shoulder at 1620 cm^{-1} - 1648 cm^{-1} as we move from Type 4 to Type 1, which is obvious from the gradual sharpening of this peak from Type 4 to Type 1 (Fig. 4.18). Thus, Type 1 had strong peaks at 1595 cm^{-1} and 1322 cm^{-1}, but had a weaker absorption at the 2915 cm^{-1}, 2846 cm^{-1}, 1734 cm^{-1}, 1230 cm^{-1} and 1038 cm^{-1} region, together with a broad and weak absorption at the 3333 cm^{-1} region. There was also a strong absorption at approximately 793cm^{-1} and a complete disappearance of the 1648 cm^{-1} peak. Type 2 had a broad peak and weaker absorption at the 1595 cm^{-1} region, but absorption in the 1038 cm^{-1} region was clearly visible. Type 3 was a hybrid, consisting of a slightly weaker peak at 1595 cm^{-1} than Type 1 and Type 2, but with more prominent absorption intensities at 2846 cm^{-1} and 2915 cm^{-1}, and the

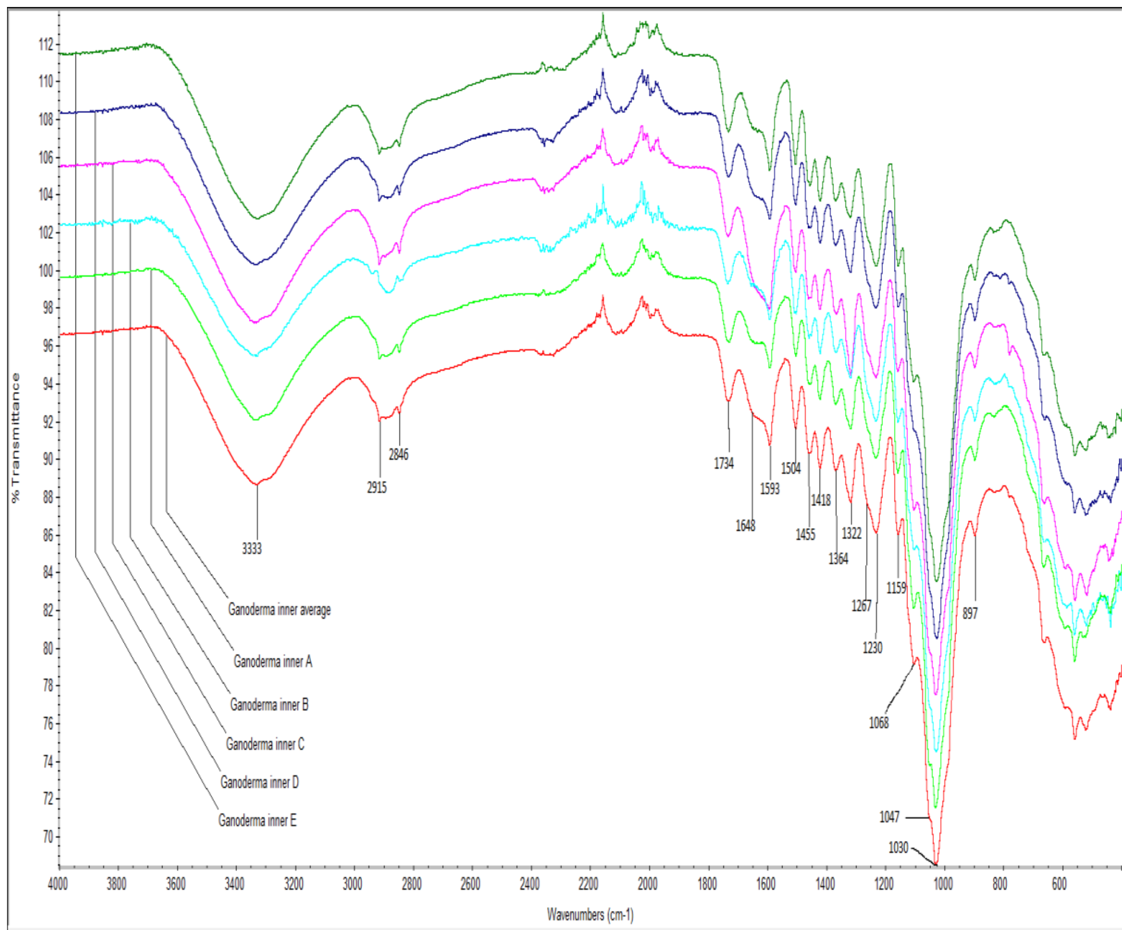


Fig 4.16: FTIR Spectra for the *Ganoderma adspersum* incised inner samples after 9 weeks (n = 5)

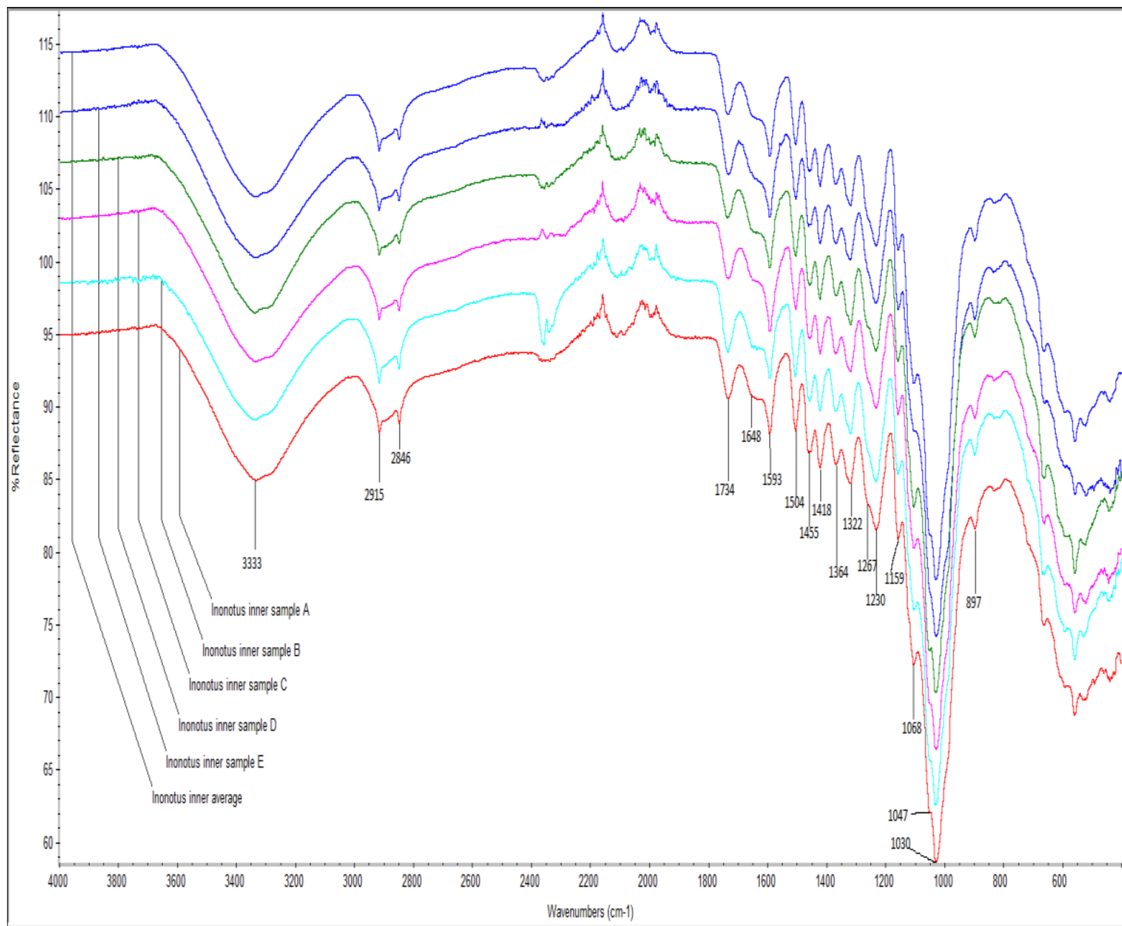


Fig 4.17: FTIR Spectra for the *Inonotus dryophilus* incised inner samples after 9 weeks (n = 5)

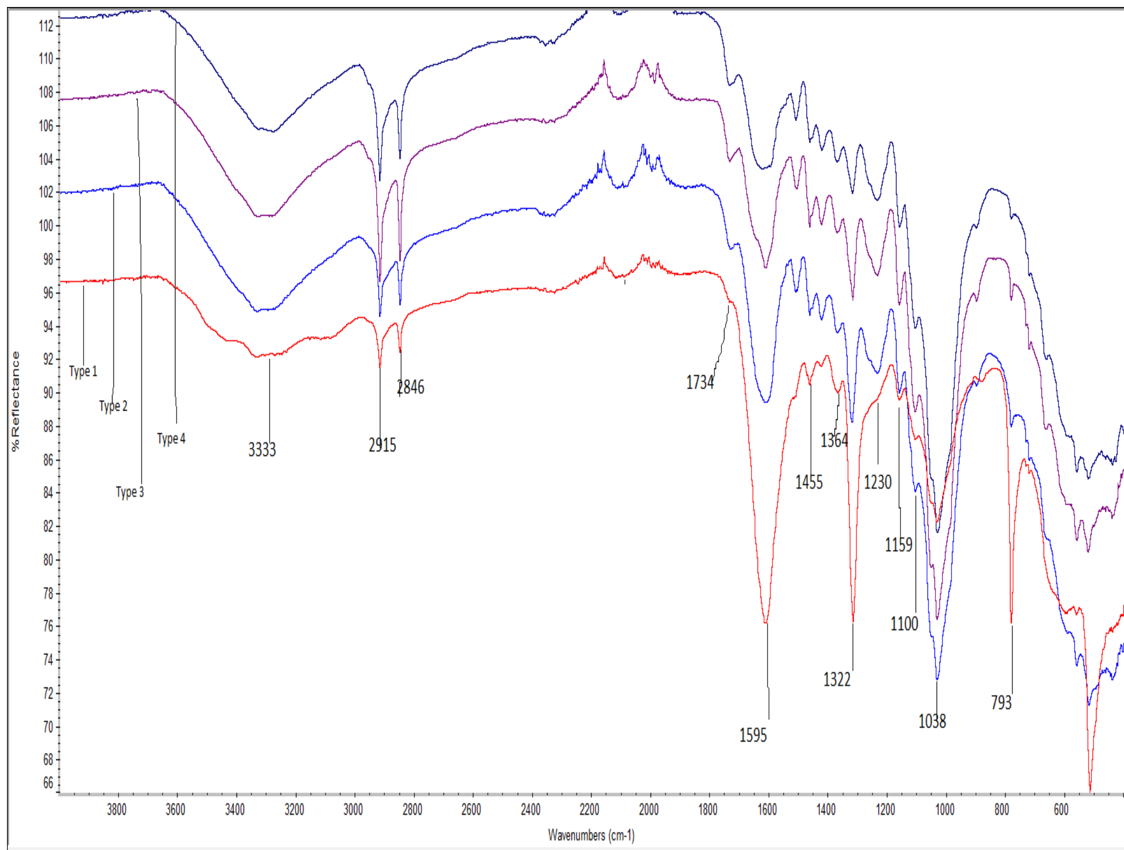


Fig 4.18: FTIR Spectra for the *Ganoderma adspersum* incised outer samples after 9 weeks (n = 10)

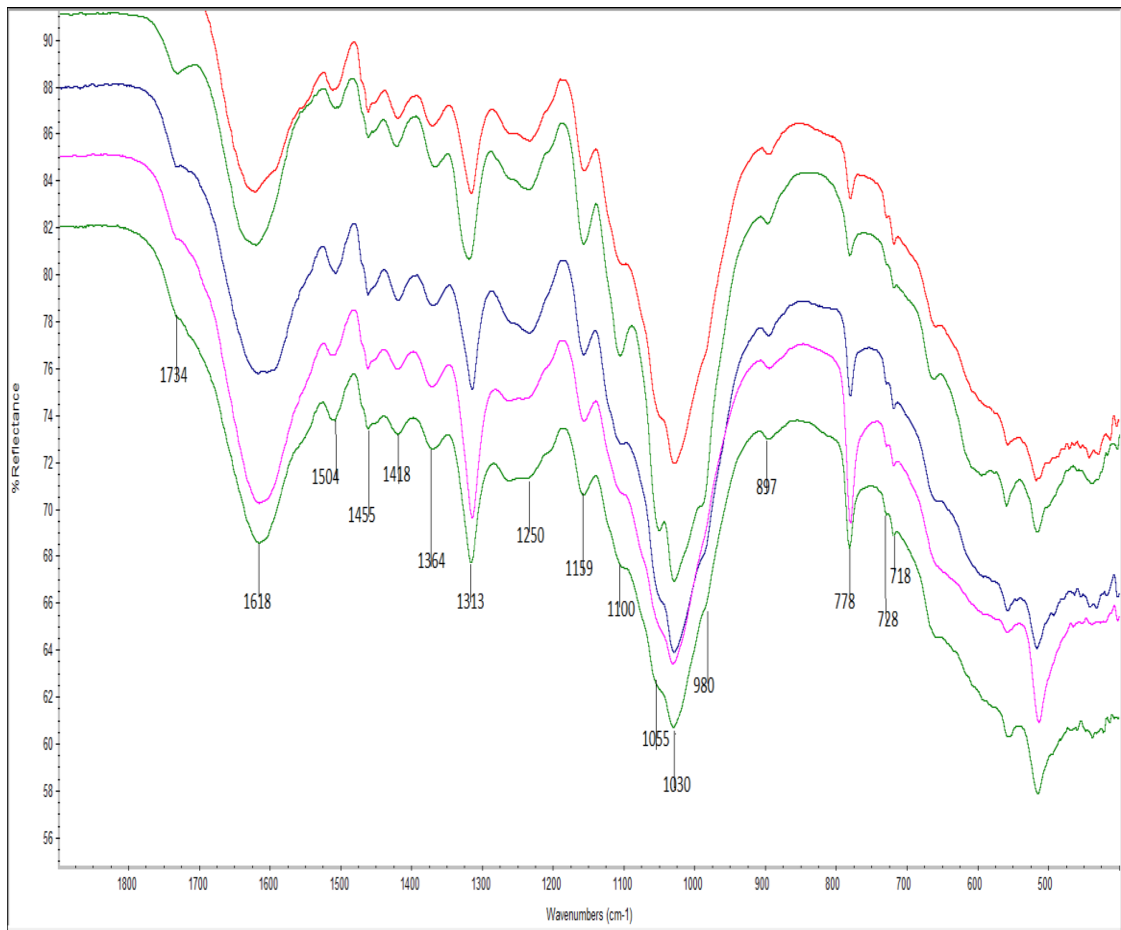


Fig. 4.19: FTIR Spectra for the *Inonotus dryophilus* incised outer samples (Type 1) after 9 weeks showing only the fingerprint region (n = 10).

appearance of a weak absorption at 1648 cm^{-1}. Type 4 was rather wider and flatter in the 1595 cm^{-1} region, i.e. all peaks were indistinct between 1590 cm^{-1} – 1648 cm^{-1} possibly due to an equal magnitude of the absorption at both locations which combined together to give a broad and indistinct peak, while the absorption at 793 cm^{-1} was very weak (i.e. different to Type 1, 2 and 3).

For the wood samples bioincised with *I. dryophilus*, the ten spectra were a bit complex and more difficult to group, but they were eventually segregated into two main types (Fig. 4.19 and 4.20). For these two spectral groups, there was a steady transition between one type and the next, which made it harder to draw absolute boundaries between the spectra. Type 1 (Fig. 4.19), which exhibited higher variations among its spectra, had a single strong peak at 1618 cm^{-1} and a decrease in the absorption intensity at 1030 cm^{-1}. These spectra were obtained from the bioincised samples whose outer surfaces appeared to be highly pigmented. However, the sugar regions (1313 cm^{-1}, 1100 cm^{-1}, 1055 cm^{-1}, 1030 cm^{-1}, 778 cm^{-1}) and lignin region (1250 cm^{-1}) appeared to differ slightly. Type 2A to Type 2E on the other hand (Fig. 4.20), were gradually transitioning from having a strong peak at 1629 cm^{-1}, with a shoulder nearby at 1593 cm^{-1} (Type 2) to having a strong peak at 1593 cm^{-1}, with little evidence of the 1629 cm^{-1} peak (Control). However, all the Type 2 spectra together with the control had strong peaks at 1030 cm^{-1}. All the Type 2 spectra were obtained from bioincised samples with lighter pigmentation in comparison to those of the Type 1 category.

4.5 Resistance of treated bioincised *G. arborea* wood to fungi attack

The mean percentage weight loss of the bioincised *G.arborea* heartwood samples treated with tanalith preservative is shown in Table 4.31. Samples bioincised with *I. dryophilus* had a lower average weight loss (4.14%) than those bioincised with *G. adspersum* (4.37%). On the basis of incubation period, the percentage mean weight loss ranged from 3.81 – 4.59%; the control samples having the highest mean weight loss (4.59%), while the least was observed in samples bioincised for 9 weeks (3.81%). The percentage weight loss showed an inconsistent trend with increasing incubation period. Based on treatment type, the pressure-treated wood samples had a lower mean weight loss (4.17%) than the steep-treated samples (4.33%).

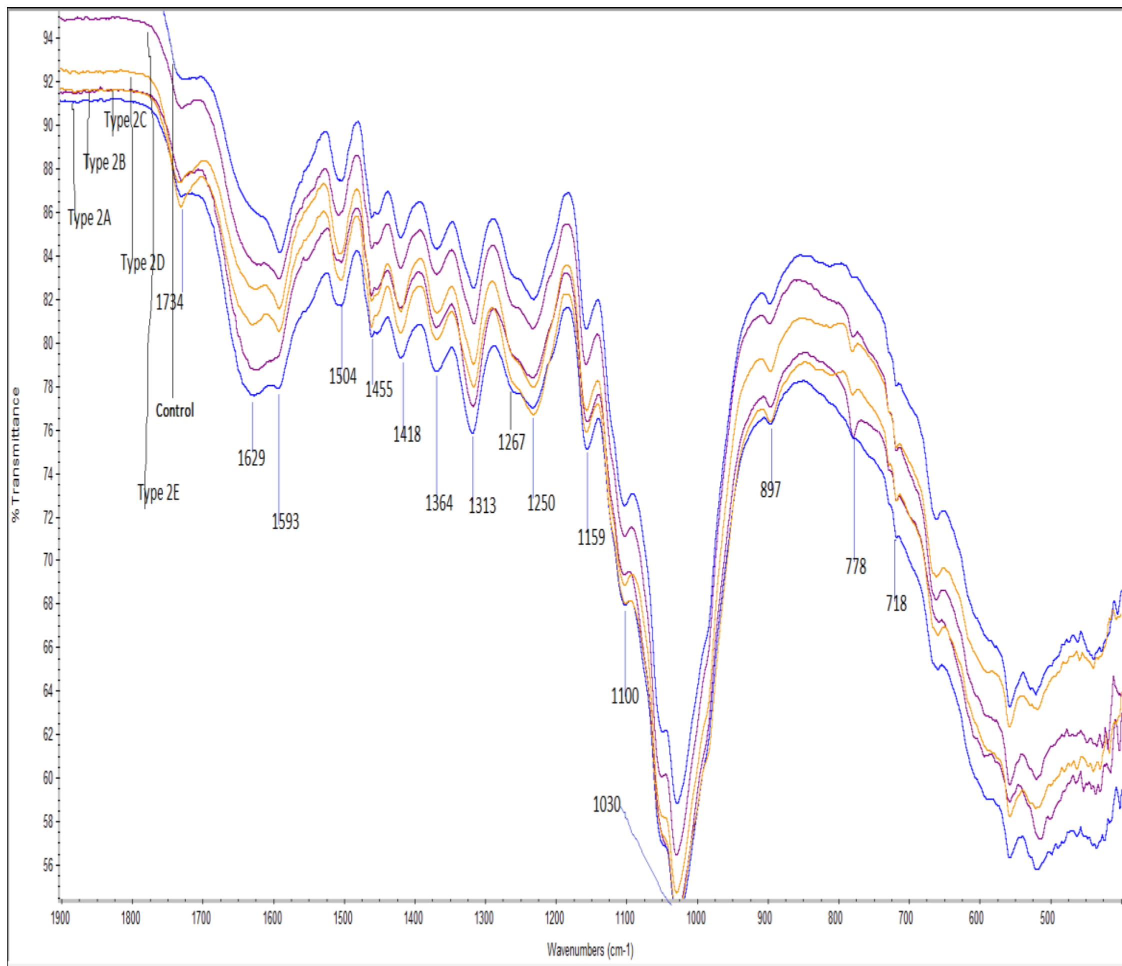


Fig 4.20: FTIR Spectra for the *Inonotus dryophilus* incised outer samples (Type 2) after 9 weeks showing only the fingerprint region (n = 10).

Based on the decay fungi, the white rot fungus (*T. vesicolor*) induced a higher weight loss (4.34%) than those exposed to the brown rot fungus (*C. puteana*) (4.17%). Based on the exposure period of the treated samples to the decay fungi, the weight loss ranged from 4.09 – 4.35%. The general trend of weight loss based on the exposure period was an increasing weight loss with increasing exposure period. Hence, samples exposed for 4 weeks had the least weight loss (4.09%), while those exposed for 20 weeks had the highest weight loss (4.35%).

Statistical analysis through ANOVA revealed that the bioincising incubation period had a significant effect ($p<0.05$) on the weight loss of the treated bioincised samples (Table 4.32). Follow-up analysis of the effect of incubation period on the weight loss of the treated bioincised samples is shown in Table (4.33). The samples bioincised for 9 weeks had a significantly lower ($p<0.05$) mean weight loss (3.81%) than the control samples and samples exposed for the other incubation periods.

Table 4.31: Main effect of weight loss of treated bioincised *G. arborea* wood after exposure to fungi attack

Parameters	Weight loss (%)
Bioincising fungi	
Inonotus dryophilus	4.14±0.05
Ganoderma adspersum	4.37±0.17
Incubation period	
Control	4.59±0.29
3 weeks	4.49±0.08
5 weeks	3.99±0.1
7 weeks	4.40±0.27
9 weeks	3.81±0.11
Treatment type	
Steeping	4.33±0.12
Pressure	4.17±0.13
Decay fungi	
Trametes versicolor (white rot)	4.34±0.13
Coniophora puteana (brown rot)	4.17±0.12
Exposure period	
4 weeks	4.09±0.07
8 weeks	4.32±0.18
20 weeks	4.35±0.18

Table 4.32: Analysis of Variance for the weight loss of the treated bioincised *G. arborea* after exposure to fungi attack

Sources of Variation	df	SS	MS	Fcal	p-value
Bioincising fungi (BF)	1	3.12	3.12	1.75	0.19^{ns}
Incubation period (IP)	4	21.89	5.47	3.06	0.02*
Treatment type (TT)	1	1.65	1.65	0.92	0.34^{ns}
Decay fungi (DF)	1	1.90	1.90	1.06	0.31^{ns}
Exposure period (EP)	2	3.52	1.76	0.98	0.38^{ns}
BF*IP	4	7.92	1.98	1.11	0.36^{ns}
BF*TT	1	0.07	0.07	0.04	0.84^{ns}
IP* TT	4	9.97	2.49	1.39	0.24^{ns}
BF*DF	1	0.83	0.83	0.46	0.50^{ns}
IP*DF	4	12.16	3.04	1.70	0.15^{ns}
TT*DF	1	0.63	0.63	0.35	0.55^{ns}
BF*EP	2	0.99	0.50	0.28	0.76^{ns}
IP*EP	8	14.73	1.84	1.03	0.42^{ns}
TT*EP	2	0.56	0.28	0.16	0.86^{ns}
DF*EP	2	5.56	2.78	1.55	0.22^{ns}
BF* IP*TT	4	4.03	1.01	0.56	0.69^{ns}
BF* IP*DF	4	8.32	2.08	1.16	0.33^{ns}
BF* TT*DF	1	0.10	0.10	0.06	0.81^{ns}
IP* TT*DF	4	6.63	1.66	0.93	0.45^{ns}
BF* IP*EP	8	25.22	3.15	1.76	0.09^{ns}
BF* TT*EP	2	1.08	0.54	0.30	0.74^{ns}
IP* TT*EP	8	12.87	1.61	0.90	0.52^{ns}
BF* DF*EP	2	6.63	3.31	1.85	0.16^{ns}
IP* DF*EP	8	9.82	1.23	0.69	0.70^{ns}
TT* DF*EP	2	3.25	1.63	0.91	0.41^{ns}
BF* IP*TT*DF	4	6.96	1.74	0.97	0.43^{ns}
BF* IP*TT*EP	8	6.36	0.79	0.44	0.89^{ns}
BF* IP*DF*EP	8	17.77	2.22	1.24	0.28^{ns}
BF*TT*DF*EP	2	1.32	0.66	0.37	0.69^{ns}
IP* TT*DF*EP	8	19.30	2.41	1.35	0.23^{ns}
BF*IP* TT*DF*EP	8	9.97	1.25	0.70	0.69^{ns}
Error	119	22.94	1.79		
Total	238	439.44			

Note: '*' = significant at p<0.05; 'ns' = not significant at p<0.05

Table 4.33: Effect of Incubation period on the weight loss of the treated bioincised *G. arborea* wood exposed to fungi attack

Incubation period	Weight loss (%)
Control	4.59 ± 0.29^{a}
3 weeks	4.49 ± 0.08^{ab}
5 weeks	3.99 ± 0.1^{bc}
7 weeks	4.40 ± 0.27^{ab}
9 weeks	3.81 ± 0.11^{c}

Mean values with the same superscript are not significantly different at $\alpha = 0.05$

CHAPTER FIVE

DISCUSSION

5.1 Physical properties

5.1.1 Weight loss

The depletion of cell wall components of the wood during decay by fungi can be assessed by estimating the percentage loss in weight of the decayed wood. The range of weight losses of the bioincised wood samples observed for both fungi fall below 1%, indicating that a relatively insignificant proportion of the structural cell wall components was degraded. This infers that most of the activities of the fungi species occurred on the non-structural components of the wood such as extractives and tyloses. According to Lehringer *et al.* (2010), negligible weight loss of wood, coupled with an appreciable increase in wood permeability are the main goals of a successful wood bioincision. The low fungal activity within the wood, perhaps, might be as a result of the inhibiting effect of the wood extractives. The poor fungal activity in the heartwood of wood species is well documented by several studies. Schwarze *et al.* (2006) reported a mass loss of less than 1% for heartwood of *Abies alba* and *Picea abies* incubated with *Physisporinus vitreus* fungus after 6 weeks. Panek *et al.* (2013) discovered poor activity of the fungus *Trichoderma viride* in the heartwood zone of spruce wood after 9 weeks of exposure. Martínez-Iñigo *et al.* (1999) reported a weight loss below 1% in Scot pine heartwood exposed to the white rot fungus *Funalia trogii* after 6 weeks, but recorded an appreciable weight loss in the extractive-free heartwood and sapwood of the wood species exposed to the same fungus species after 6 weeks. From the result of this study, *Ganoderma adspersum*, however, proved to be more tolerant of the wood extractives than *Inonotus dryophilus*, as evidenced by the significantly higher weight loss of the wood at higher incubation period (9 weeks). The increase in weight loss with increasing incubation period observed for both fungi indicates an increase in fungal activity within the wood and consequently, the increase in wood component degradation. This observation is consistent with reports from

several related studies (e.g. Thaler *et al.*, 2012; Koyani and Rajput, 2015; Emaminasab *et al.*, 2015).

5.1.2 Colour change

Discolouration of wood by fungi occurs due to the deposition of the secretions from the fungal hyphae on the wood during the growth of the fungi (Zabel and Morell, 1992). The degree of discolouration, therefore, may indicate the extent of fungal activities on the wood. The discolouration observed in the wood bioincised with *I. dryophilus* ranged from a slight to heavy brownish discolouration, while that observed in the wood samples bioincised with *G. adspersum* was not too obvious. In both cases, the discolouration occurred in patches on the wood surfaces, which implies that the wood colonization by both fungi was rather heterogeneous (Lehringer, 2011). Wood discolouration negatively affects the aesthetic quality of wood, and hence, such wood may not be suitable in applications where aesthetic value is of major importance. Thus, *G. arborea* wood bioincised with *I. dryophilus* may not be suitable in applications where wood aesthetic property is one of the major wood characteristics under consideration.

5.1.3 Moisture content

The moisture content of wood plays a very important role in wood degradation by fungi. The moisture content of the control samples incubated on sterile agar almost attained fibre saturation point (usually between 27 to 30%) by absorbing moisture from the surface of the agar, which may be sufficient to initiate fungal decay by the bioincising fungi. However, the relatively low moisture content level in the control wood samples, which may be caused by the presence of hydrophobic substances in the wood such as the extractives, may not be enough to support the optimal growth of the invading fungus. Brischke *et al.* (2017) however reported that some basidiomycetes are capable of causing considerable decay at moisture contents significantly below the fibre saturation point. For both bioincising fungi in this study, moisture content of the wood specimens increased steadily with increasing incubation period, except for those incubated for 9 weeks. This increase in moisture content can be attributed to the increasing fungal activity in the wood, brought about by respiration of the wood cell constituents and the translocation of water by the fungi mycelia (Theden, 1941; Peterson and Cowling, 1973). The moisture content reduction observed after 9 weeks

of incubation may be as a result of decreasing fungal activity in the wood due to the inhibiting effect of the wood extractives, coupled with the fact that some moisture may probably have been lost from the wood blocks and incubation chamber during wood incubation (Smith and Shortle, 1991). Increase in moisture content of decayed wood with increasing incubation period was also reported by Smith and Shortle (1991), where they observed increasing moisture content in heartwood species of Redcedar, Black cherry, Red pine and Red Oak exposed to the fungi *Postia placenta* and *Trametes versicolor* for 7, 14 and 84 days. Thaler *et al.* (2012) also recorded increase in moisture content of Norway spruce with increasing incubation period after bioincising with three different fungi species. Wood samples bioincised with *G.adspersum* had significantly higher moisture content than those bioincised with *I. dryophilus*, which connotes higher fungal activity in the wood and hence, may further explain the higher weight loss observed in the *G.adspersum* bioincised wood samples.

5.1.4 Absorption and retention

A reasonable amount of absorption and retention of wood preservative or wood modifying chemicals in wood after impregnation is essential for an effective and optimal performance of the chemical in the wood. (FAO, 1986). Apparently, the bioincision of the wood by both fungi species improved the preservative uptake and retention by the wood for both preservative treatment methods. Minimum preservative uptake and retention were observed in wood samples bioincised for 3 weeks for both fungi while maximum preservative uptake and retention were observed in those bioincised for 7 weeks for both fungi. The initial reduction in preservative uptake and retention of the bioincised wood at 3 weeks is possibly due to the partial blockage of the flow paths or effective pore openings in the wood structure by the fungal hyphae, particularly the vessels, as the fungi began to establish itself in the wood, with virtually insignificant or no degradation of cell components or cell occlusions occurring at this stage. Subsequent increase in the absorption and retention of the preservative in the bioincised wood with increasing incubation period reflects the creation of new pathways in the wood as a result of the increasing fungal activities on the wood cell structures and occlusions present within the wood cells. A further decline in the preservative absorption and retention of the bioincised wood at the latter stage (9 weeks of incubation) could be as a result of the increase in degradation products which could have led to the obstruction of some new or existing openings within the wood

structure (Thaler *et al.*, 2012) or perhaps, increase in the fungal hyphal strands in the wood, coupled with the reduced activities on the wood cells and occlusions due to the inhibiting effect of the wood extractives. Consequently, the increased fungal mycelia in the wood possibly led to the obstruction of some of the cell openings in the wood structure, thereby impairing further ingress of the preservative into the wood. Reduction in the permeability of normal and tension wood of Poplar bioincised with *Physisporineus vitreus* and *Xylaria longipes* with increasing incubation period to safranin impregnation was equally reported by Emaminasab *et al.* (2015) due to the obstruction of the vessel lumina by the fungal hyphae of both bioincising fungi.

While bioincision did enhance the uptake and retention of the preservative in the wood, the improvement was rather insignificant when compared to the control samples. Also, the *G. adspersum* fungus improved the preservative absorption and retention of the wood samples than the *I. dryophilus* fungus, though the improvement was statistically insignificant. A maximum improvement of about 17% in preservative uptake was obtained in the wood samples bioincised with each of the fungus species after pressure impregnation. This is far below the approximately 50% improvement in hydrophobisation substance uptake reported by Lehringer *et al.* (2011) in the heartwood of Norway spruce bioincised with *Physisporineus vitreus*. This wide difference can be attributed to the differences in wood species, and difference in the nature of occlusions in the wood species. Hardwoods are composed of more diverse cells than softwoods, making the activities of the bioincising fungus on each individual cell type of *G. arborea* wood highly variable in comparison to that of the Norway spruce, which is a softwood. Also, the preservative uptake improvement observed in this study is equally lower than the 26% improvement in CCA uptake reported by Owoyemi and Kayode (2008) for mechanically incised *G. arborea* heartwood, implying that the mechanical incision was more effective in opening up the wood structure of the species than the biological pre-treatment of the wood species. However, average preservative retention observed for the wood in this study is slightly higher than the 4.1% retention reported by Moya and Berrocal (2015) for *Gmelina arborea* heartwood treated with Wolmanit CX-10® preservative.

Expectedly, the treatment method showed statistical difference in the preservative uptake and retention in the wood samples, with the pressure-treated wood samples having a significantly higher preservative uptake and loadings than the steep-treated

wood samples. Pressure impregnation of wood has generally been reported to yield better chemical uptake and penetration results than non-pressure treatment methods (Lehringer *et al.*, 2009a; Schubert *et al.*, 2011; Thaler *et al.*, 2012). According to Lehringer (2011), the permeability improvement as a result of bioincision is such that the bioincised wood requires the use of pressure treatment for chemical impregnation as substance uptake with non-pressure treatments is considerably low to ensure a deep and uniform chemical penetration and hence, an optimal performance of the chemical agent in the wood.

5.1.5 Axial and lateral absorption

Permeability variation is not only observed between wood species, but it is also noticeable along the principal directions within wood (Comstock, 1970; Dinwoodie, 2000; Hansmann *et al.* 2002). The observed trend in axial absorption of the dye in the wood was inconsistent with increasing incubation period, but overall, tended to increase with increasing incubation period for both fungi. This inconsistency in axial absorption with increasing incubation time may be due to the inherent variability within the wood in terms of the extractive content and their micro-distribution (Mangindaan *et al.*, 2017), anatomical variations, as well as variations in the distribution of the various infiltrations in the wood. For instance, Zabel and Morell (1992) stated that there is an extreme variability in the extractive contents along the radial and axial axis within the heartwood of species. Also, Wong *et al.* (2005) stressed that there is variation in the natural durability of wood radially from pith to bark, with the inner heartwood closer to the pith being less durable than outer heartwood, which implies that the extractive concentration in the inner heartwood is lesser than that present in the outer heartwood.

Similarly, the lateral absorption showed some inconsistency with increasing incubation period, with an initial decreasing trend observed, before an increase was noticed at the latter incubation period for both fungi. Maximum axial and lateral absorptions were

observed in wood bioincised for 9 weeks, except for wood samples bioincised with *I. dryophilus*, where maximum axial dye absorption was observed at 5 weeks.

Axial uptake of dye in the samples bioincised with *I. dryophilus* was about twice that of the control samples while those bioincised with *G. adspersum* was almost thrice that of the control samples. On the contrary, improvement of lateral uptake of the dye in the bioincised wood samples was lower when compared to that observed for the axial absorption, even though the average lateral dye absorption exceeded the axial dye absorption. Samples bioincised with the *I. dryophilus* and *G. adspersum* fungi yielded a 22% and 53% increase in the lateral dye absorption, respectively compared to the 53% and 68% increase in axial dye absorption for *I. dryophilus* and *G. adspersum* fungi, respectively. This implies that the dominant pathway for the movement of the fungi species during the wood colonization was through the vessels, thereby creating new openings for more axial flow of the dye in the wood. *G. adspersum* significantly enhanced the dye absorption in the wood when compared to that of *I. dryophilus*, indicating that it is more effective in improving the permeability of *G. arborea* heartwood than *I. dryophilus*. The average dye absorption observed after 9 weeks of incubation was almost twice that observed for all the other incubation periods, which implies that higher exposure period of the wood to the fungi species led to a more improved permeability of the wood. Overall, higher flow of the dye rather occurred transversely as opposed to axially. According to Comstock (1970) and Lihra *et al.* (2000), flow in the axial plane is considerably more than lateral flow due to the high conductivity of vessels in hardwoods. Vessels, when they are large in size, number and free from occlusions are considered to be primarily responsible

for fluid flow in hardwoods (Rudman, 1965; Sujatha and Venmalar, 2019). However, contrary to this assertion, bioincised *Gmelina arborea* heartwood in this study absorbed more dye laterally than axially due to the inability of the fungi species to substantially unclog the vessels of the tyloses present within the cell lumina, coupled with the ineffectiveness of the wood fibres and axial parenchyma in the uptake of the dye solution. A contrary observation to the result noted in this study was reported by Keith and Chauret (1988) where longitudinal flow of CCA preservative in Spruce wood (*P. glauca*) was observed to be about 15 to 20 times more than the transverse flow.

5.1.6 Axial penetration depth and penetration area

To evaluate the effectiveness of a preservative, the absorption or net retention of the injected preservative is inadequate as parameters for preservative effectiveness evaluation and should be considered alongside with the penetration depth and area of penetration of the preservative (Kollmann and Côté 1968; Lebow 2010; Reinprecht, 2016). Bioincision of the wood samples with *I. dryophilus* increased the penetration depth of the dye in the axial direction at the sub-surface section from 5.39 mm to 20.76 mm at 7 weeks of incubation, indicating an improvement of 74%, while that of the middle section increased from 8.55 mm to 22.96 mm at 9 weeks, enhancing axial penetration in this section by almost 63%. Though the axial penetration depth was enhanced by the bioincising fungi, the axial penetration of the dye is still appreciably low, considering that the wood dimension in the study is small compared to the dimension of wood in service, where a much deeper penetration depth of the treating substance would be required. Penetration area, on the other hand, was enhanced in the samples bioincised with *I. dryophilus* by approximately 97% and 98% at both the sub-surface and middle sections, respectively. For the samples bioincised with *G. adspersum*, a more appreciable improvement was observed, with axial penetration depth enhanced by about 81% and 75% at the sub-surface and middle sections, respectively. This shows that *G. adspersum* was more effective in degrading the tyloses blocking the vessels than *I. dryophilus*, thereby facilitating more axial flow of

the dye. However, *G. adspersum* only enhanced the penetration area by approximately 93% and 94% at both the sub-surface and middle sections, respectively. The higher penetration area observed in samples bioincised with *I. dryophilus* could be attributable to the inherent high variability among the wood samples bioincised with the fungus, which perhaps, led to the high variation in the penetration area of the wood samples bioincised with the fungus when compared to those bioincised with *G. adspersum*.

The penetration depth of the dye increased consistently with prolonged exposure periods while the dye penetrated deeper and was better distributed at the middle section of the wood than the sub-surface area. The improvement in penetration depth should be more pronounced at the sub-surface section due to its closeness to the growing fungi mycelia and higher accessibility to moisture as a result of its close proximity to the culture medium. The proximity of the sub-surface section is therefore, expected to experience higher fungal activities and consequently have a higher permeability than the middle section. However, the reverse was the case as the wood specimens experienced higher axial dye penetration depth and area at the middle section than the sub-surface section (Plate 4.4 and Table 4.16). This implies that the fungi species, due to the toxicity of the extractive in the wood, rather colonized the wood by moving through the areas of least resistance within the wood (i.e. cells with less extractive concentration and occlusions) (Blanchette, 1991) or may be due to the mobilization of the degradation products towards the sub-surface section of the wood as the moisture content of the wood increased during bioincision, consequently constituting an obstruction to the ingress of the dye into the wood. This assumption could also be substantiated based on the colour changes experienced on the wood surface directly exposed to the fungi mycelia during bioincision, which are evidences of the presence of degradation products' deposits in this region. Nonetheless, the insignificant difference in the axial penetration depth and area between both section depths indicates a relatively uniform activity in fungal activities within both sections and hence, an identical axial dye penetration depth and distribution within both wood sections.

5.1.7 Lateral penetration depth and penetration area

Transverse penetration depth of the dye in the wood was greatly enhanced by the bioincision of the wood as maximum improvements of 97% and 98% was observed in the radial penetration depth of the dye after bioincision with *I. dryophilus* and *G. adspersum*, respectively, while improvements of 40% and 88% was observed in the penetration depth of the dye tangentially after bioincision with *I. dryophilus* and *G. adspersum*, respectively. This implies that fluid flow in the lateral axis of the wood extended beyond both the surface and sub-surface layer of the wood down to the border zone as a result of the bioincision based on the classification adopted by Lehringer *et al.* (2010). The range of radial penetration depth recorded in this study is slightly comparable with that reported by Emaminasab *et al.* (2015) for normal and tension wood of poplar bioincised with *P. vitreus* and *X. longipes*, though their study revealed that bioincision rather caused a reduction in the radial permeability of the wood. Similar to the axial penetration area, the improvement in lateral penetration area was about 100% for both *I. dryophilus* and *G. adspersum*. However, a maximum lateral penetration area of about 17% can be considered too low as this is appreciably lesser than a quarter of the entire area of the wood, indicating a poor transverse distribution of the dye within the impregnated *G. arborea* wood.

The penetration depth and penetration area of the bioincised samples along both anatomical planes were discovered to vary greatly after incubation for 9 weeks, particularly those bioincised with *G. adspersum*. This suggests that heterogeneity of fungal colonization on the wood increased with prolonged exposure period, but was more prominent in wood bioincised with *G. adspersum*. Lehringer *et al.* (2009a) also noted a heterogeneous colonization of Norway spruce after bioincising with *P. vitreus*, which led to a high variability in substance uptake and penetration of the treating substance. Heterogeneity in the colonization of bioincised spruce and pine wood by the fungus *Dichomitus squalens* was also reported by Dale *et al.* (2019).

The range of percentage improvement in lateral penetration of impregnated substance in the wood observed in this study (80 – 93%) is comparably higher than that reported by Lehringer (2011), where a 68% improvement in transverse penetration depth of Norway spruce heartwood treated with hydrophobation substances stained with Rhodamin dye after bioincising with *P. vitreus* for 9 weeks. Radial penetration of dye was twice as much as that observed along the tangential plane, which implies that the activities of the bioincising fungi was more predominant within the ray cells, which are

major path for radial flow (Cote, 1963; Nicholas and Siau, 1973; Hansmann *et al*., 2002), thereby facilitating higher flow of the dye radially than in the tangential direction. The higher radial dye penetration observed in this study is in agreement with that reported by Keith and Chauret (1988), where they observed a higher radial penetration of CCA preservative in Spruce wood (*P. glauca*) than that along the tangential plane.

5.2 Effect of bioincision on anatomical structures of *G. arborea* heartwood

A comparison of the bioincised wood for both fungi and that of the control in the light microscopy analysis shows an obvious reduction in the occlusions present in the vessel lumina of the bioincised wood. This indicates that both fungi acted on the tyloses present within the vessels and degraded it. The presence of the blue dye in the vessels and ray parenchyma also depicts that these structures were predominantly the pathways for the colonization of the wood by the fungi, and this observation was explicitly revealed in the SEM analysis accordingly. This is in consonance with reports by Schwarze and Baum (2000) for the beech wood exposed to *G. adspersum*, where they observed major activities of the fungus in the axial parenchyma and vessel lumina, thereby degrading the occlusions present within them. Similarly, Otjen and Blanchette (1982) reported that large earlywood vessels of Oaks exposed to *I. dryophilus* were rapidly colonized by the fungus. There were also some activities of *I. dryophilus* observed within the axial parenchyma of *G. arborea* heartwood in this study, which is consistent with the reports of Otjen and Blanchette (1982) for the fungus on Oak heartwood. Other activities of *G. adspersum* noticeable in the heartwood of *G. arborea* consist of delamellation and cell wall degradation of two coalesced axial parenchyma cells, together with the delignification of the vessel from the lumen outwards. These patterns of degradation are generally attributes of white rot fungi (Zabel and Morell, 1992), and the attack induced on virtually all the major chemical constituents of the wood indicates that the fungus is a simultaneous white rot fungus. This is in contrast to reports by Blanchette (1984c) for *Ganoderma spp*. and Schwarze and Baum (2000) for *G. adspersum* as they were described as selective delignifying basidiomycetes. *I. dryophilus*, on the other hand, exhibited similar decay attributes to that of *G. adspersum*, though at a slightly lower intensity. Otjen and Blanchette (1982) observed that most occluded cells of the heartwood of Oak species were not affected by *I. dryophilus*. This may explain why the intensity of degradation

of the *G. arborea* wood cells was minimal for *I. dryophilus* when compared to *G. adspersum*. However, stained pits on the radial wall of vessels were observed which may indicate penetration of the pits by the fungal hyphae into adjoining cells or perhaps, a free passage of the blue dye within an unoccluded vessel cell through the pits into adjoining cells. No evidence of bleached appearance of the wood was noticed for both fungi, which implies that there was no extensive degradation of the lignin in the wood.

Scanning electron micrograph, however, further buttresses the general observations for the light microscopy but with additional degradation features seen at higher resolutions. In the case of *G. adspersum*, there were formation of erosion troughs and enlargement of pit aperture on the radial wall of the vessel, with evidences of degradation of possibly encrusted or aspirated vessel pits by the fungal hyphae. As reported by Schwarze (2007), colonisation of hardwood species via the pits, leading to their enlargement and subsequent coalescence with an adjoining pit is a general tendency of white rot fungi.

5.3 Mechanical properties

5.3.1 Impact resistance

According to Panek *et al.* (2013), alterations in the impact bending strength of wood is an important index of detecting early changes in the anatomical and molecular structure of wood. Impact resistance appeared to slightly increase after bioincision with both fungi. However, the insignificant change in the impact resistance of the bioincised samples in comparison with the control indicates that there was little or no effect of the bioincision on the wood samples. This agrees with reports from similar studies on bioincision on the insignificant effect of bioincision on the impact bending strength of wood (Efransjah *et al.*, 1989; Panek and Reinprecht, 2011; Panek *et al.*, 2013). However, the results of these studies are in dissonance with the observation from this study in that while slight increment in impact resistance was noticed after bioincision in this study, they reported slight decreases in the impact bending strength of the bioincised samples. Inconsistency in impact resistance with increasing incubation may be generally due to the heterogeneity of the wood material. Significantly lower impact resistance value was observed for wood samples bioincised with *G. adspersum*, which indicates higher activities of the fungus on the wood

polymeric units than *I. dryophilus*. This is also in line with results of the weight loss and moisture content of the bioincised samples previously discussed.

5.3.2 MOE and MOR

Though the MOE and MOR appeared to vary inconsistently with increasing incubation period for both fungi, the general trend appears to be a decreasing trend in the MOE and MOR with prolonged exposure period. This observation corresponds well with the microscopy of the bioincised wood which revealed some activities of the fungi species on the lignin and polysaccharide fractions of the wood cell wall, thereby reducing the bending strength and stiffness of the wood with prolonged exposure time to both fungi species. This is in agreement with reports of Lehringer *et al.* (2011a), Panek and Reinprecht, (2011), Thaler *et al.* (2012), Panek *et al.* (2013) and Emaminasab *et al.* (2015) where they all observed a decreasing trend in the bending strength and stiffness of the bioincised wood with increasing incubation period. As expected, *G. adspersum* caused a significant reduction in the MOR of the bioincised samples compared to *I. dryophilus*. This may be attributed to the higher activities of the fungus on the structural components of the wood; this is also reflected in the higher weight loss and moisture content of the wood bioincised with the fungus. The significantly lower MOR at 7 weeks, however, may be due to the heterogeneity of the wood substrate. The MOE and MOR followed a similar trend across the incubation period.

5.3.3 Maximum Compressive Strength

The maximum compressive strength parallel to grain generally decreased after bioincision. The overall trend was more of a decreasing trend in compressive strength with increasing incubation period for both fungi. This decreasing trend in compressive strength with incubation period is in agreement with reports of Thaler *et al.* (2012), Emaminasab *et al.* (2015) and Emaminasab *et al.* (2016) for other bioincised wood. Reduction in compressive strength of the wood by both fungi across all incubation periods was generally infinitesimal. This implies that the delignification induced by both fungi species in the bioincised wood was rather insignificant such as to cause an obvious reduction in the compressive strength of the wood. Contrary to this, Thaler *et al.* (2012) reported a significant decrease in the compressive strength of Norway

spruce after exposure to *Antrodia vaillanttii* and *Hypoxylon fragiforme* for 30 and 45 days.

5.3.4 Tensile strength

The bioincision generally induced an insignificant effect on the wood tensile strength. This is in contrast to reports by Lehringer *et al.* (2011b) where a significant reduction in tensile strength of Norway spruce exposed to *Physisporineus vitreus* after 7 and 9 weeks was observed. However, Lehringer *et al.* (2011b) also observed slight increases in the tensile strength of the heartwood of Norway spruce after exposure to *P. vitreus* for 3 and 5 weeks and attributed this to the possibility of cross-linking or polymerization of the cellulose or lignin fraction of the wood during incipient decay of the wood. Tensile strength across the incubation period varied inconsistently, which may be associated with the material heterogeneity.

5.3.5 Janka Hardness

Delignification of wood leads to a reduction in hardness due to the crosslinking function of lignin, which bears the compression forces acting on the cell wall of the wood (Adsumalli *et al.*, 2010). Hence, the changes in Janka hardness of the bioincised samples can be attributed to the removal of lignin from the wood by the bioincising fungi. Marginal changes in the hardness (estimated as the pooled mean of the radial and tangential hardness) of the bioincised wood samples were observed for both fungi for all the incubation periods, which indicates that a negligible amount of delignification of the wood occurred during the wood colonization by both fungi. Reports by Lehringer *et al.* (2011a), however is in dissonance with this observation, as considerable reductions in hardness were observed after 7 and 9 weeks of incubation of the sapwood and heartwood of Norway spruce to *Physisporineus vitreus*. As observed from this study, there was a marked difference in the hardness values between the radial and tangential planes, with significantly lower hardness observed on the radial face of the bioincised wood. This is unexpected, considering that the tangential face was in direct contact with the fungal mycelia, which implies that higher fungal activity, and consequently, lower hardness is expected to occur along this plane in comparison with the radial plane. Therefore, this difference in hardness between the two planes may be associated with density variation between the two wood axes, as density is reported to be positively correlated with the hardness of wood (Kollmann

and Côté 1968; Wiemann and Green, 2007; Lehringer *et al.*, 2011a) This, however, is in contrast to reports by Lehringer *et al.* (2011a) where an insignificant difference in hardness was observed between the radial and tangential planes of Norway spruce bioincised with the fungus *Physisporineus vitreus*.

5.4 FT-IR

The intensity of specific functional groups and chemical bonds which exists in polymers can be determined with the aid of FT-IR (Windeisen *et al.*, 2007), through the interactions of these chemical bonds with infrared radiation. As a result of the interaction of these chemical bonds, a bending or stretching vibration of the molecules occurs. Changes in intensity of a given peak represent the quantity of that bond type present. Consequently, in this study, any reduction of the peaks implies an increased percentage transmittance or reduced percentage absorbance in the spectra due to fungal degradation.

The spectrum obtained for the control samples is similar to that reported by Ashaduzzaman (2014) for untreated *G. arborea* wood. Slight differences were however noted between both spectra which may be due to the ethanol extraction of the wood prior to the conduction of the FTIR. Comparison of the control spectrum with that of *G. adspersum* inner and *I. dryophilus* inner samples showed no obvious difference. However, the near perfect similarity among the three spectra indicate little or no change in the wood chemical structure due to bioincision in the inner parts of the wood, or perhaps, it is a reflection of the mild degradation of cell wall components induced by both fungi on the wood as evidenced by the marginal loss in the weight of the wood after bioincision.

Due to the colour change in the outer parts of the bioincised wood samples, it became necessary to obtain separate spectra for this region for both fungi. As reported earlier, this colour change is possibly due to the deposition of fungal metabolites on the wood surface, which is anticipated to bring about an obvious deviation from the initial spectra obtained for both the control samples and the inner samples of the bioincised wood for both fungi. As predicted, there were noticeable changes in the spectra obtained from this region for both fungi, ranging from considerable increase to decline in the observed peaks, as well as some shifts in peak positions. For those obtained for the *G. adspersum* outer samples, four categories of spectra were identified based on

the strength of the peaks at 1595 cm^{-1} and 1322 cm^{-1}. The gradual reduction in the absorption intensities of the peaks at 1595 cm^{-1} and 1322 cm^{-1} from Type 1 to Type 4 suggests differing levels of degradation by the fungus.

When compared with the control, decline in peaks within the lignin region (1230 cm^{-1} and 1734 cm^{-1}), loss of the 1504 cm^{-1} and 1648 cm^{-1} peak, increase in the peaks within the lignin region (1322 cm^{-1}, 1595 cm^{-1} and 2846 cm^{-1}), together with a corresponding increase in the peaks within the sugar region (793 cm^{-1} and 2915 cm^{-1}) all indicate the degradation of all the three major wood cell wall components. Type 1 spectra showed a significant deviation from the control spectra, with a noticeable flatter peak and lower absorption intensity at 3333 cm^{-1}, a very weak absorption at 1734 cm^{-1}, 1504 cm^{-1}, 1455 cm^{-1}, 1418 cm^{-1} and 1159 cm^{-1}, a stronger absorption at 1595 cm^{-1}, 1322 cm^{-1} and 793 cm^{-1}, coupled with the loss of the 1230 cm^{-1} and 1648 cm^{-1} peak. Type 2 looks a bit more similar to the Type 1, except for stronger absorption intensities at 3333 cm^{-1}, 2915 cm^{-1}, 2846 cm^{-1}, 1734 cm^{-1}, 1504 cm^{-1}, 1455 cm^{-1}, 1418 cm^{-1}, 1230 cm^{-1}, 1159 cm^{-1}, and weaker absorption intensities at 1595 cm^{-1}, 1322 cm^{-1}, and 793 cm^{-1}. Type 3 and 4 looks very similar to the control spectrum, except for the weaker absorptions at 1734 cm^{-1}, 1648 cm^{-1}, broader peak at 1595 cm^{-1} (Type 4), which is possibly due to a combination of peaks of similar absorption intensities for both 1595 cm^{-1} and 1648 cm^{-1}. Based on the similarity between the Type 4 and Type 3 spectra to that of the control, it is suggested that degradation seemed to proceed from Type 4 to Type 1, with Type 3 being the least degraded, while the Type 1 is the most degraded. Thus, the sequence of degradation can be summarized as: Type 3 ➤ Type 4 ➤ Type 2 ➤ Type 1.

The Type 1 spectrum, which is typified by partial or total degradation of cellulose (3333 cm^{-1} and 1159 cm^{-1}), hemicellulose (1734 cm^{-1}, 1230 cm^{-1} and 793 cm^{-1}) and lignin (1504 cm^{-1}, 1455 cm^{-1}, 1418 cm^{-1} and 1230 cm^{-1}) shows degradation of all the wood structural constituents. The pronounced peak at 793 cm^{-1} may indicate the formation of carboxylic acid salts arising from the cleavage of the alpha linkage bonds of the hemicelluloses (Lv *et al.*, 2015), while the increase in intensity of the 1595 cm^{-1} and 1322 cm^{-1} lignin bands and decrease in the 1734 cm^{-1} and 1230 cm^{-1} carbohydrate bands were also reported by Pandey and Pitman (2003) for beech wood exposed to the brown-rot fungus *Coniophora puteana* for 12 weeks. The increase in the 1322 cm^{-1} band could be as a result of selective carbohydrate removal, thereby making the C–O vibration in syringyl unit of the lignin fraction more prominent (Pandey and Pitman,

2003). The degradation of all the wood structural components further confirms the results of the anatomical investigation that the fungus is a simultaneous white-rot fungus (Eaton and Hale, 1993; Kubicek, 2013). Similar observations were reported by Karim *et al.* (2017) for hornbeam wood exposed to degradation by the white rot fungus *Trametes versicolor*. Shang *et al.* (2013) also reported a simultaneous degradation of *Betula platyphylla* wood cell wall components by three white rot fungi through the FTIR analysis of the decayed wood.

The *I. dryophilus*, on the other hand, produced rather more complex spectra (particularly the Type 1), probably due to the highly pigmented nature of the wood bioincised with the fungus, which was eventually grouped into two categories. For the Type 1 spectra, the shape of the sugar regions (1734 cm^{-1}, 1030 cm^{-1}, 1055 cm^{-1}) and lignin region (near 1100 cm^{-1} and 1250 cm^{-1}) appeared to differ slightly, indicating that there may be varying levels of degradation of both the carbohydrate and lignin fractions of the wood, even though the pigmentation effect is similar. Also, the varying absorbance intensities at the 1618 cm^{-1} and 1313 cm^{-1} among the Type 1 spectra also suggests differing levels of lignin and cellulose degradation by the fungus. The major difference between the Type 2 and control was a gradual transition from having a strong peak at 1593 cm^{-1}, with the absence of the 1629 cm^{-1} peak (control) to having a strong peak at 1629 cm^{-1}, with a shoulder nearby at 1593 cm^{-1} (Type 2). Thus, a comparison of all these spectra show a gradual loss in the 1593 cm^{-1} peak due to fungal degradation, transitioning from the strong 1593 cm^{-1} peak (control), to a gradual loss of the peak from Type 2 and then to Type 1. Furthermore, another transition was observed among the spectra, which is the gradual loss of the 1648 cm^{-1} peak of the control specimens, transitioning to the shift of this peak to 1629 cm^{-1} in Type 2 (though with a slightly weaker absorption), to a shift in the peak to in Type 1, which had a stronger absorption. Based on these observations, the sequence of degradation can be said to proceed from the control to Type 2 (transition stage) and then to Type 1. Appearance of the 778 cm^{-1} peak in the Type 1 and a gradual loss of the peak from Type 2 to the control spectrum may also indicate the destructive activities of the fungus on the hemicellulose molecule of the wood. Also, there is higher absorption intensity at the 1313 cm^{-1} band and weaker absorbance at the 1030 cm^{-1} in Type 1 when compared to the Type 2 and control spectra.

The loss of the 1648 cm^{-1} peak in the control specimens to the emergence of the 1618 cm^{-1} (Type 1) may indicate the steady degradation of the cellulose molecule and structural modification of the lignin after exposure to the fungus, resulting in the formation of lignin molecules with more aromatic units which caused the complete shift to 1618 cm^{-1} band experienced in the Type 1 spectra. Faix *et al.* (1991) also observed a similar trend for beech wood degraded by some white-rot fungi, where there was an increased intensity in the 1646 cm^{-1} peak of the degraded wood, occasioned by the structural change in the lignin molecule due to the decreasing intensities of the 1506 cm^{-1} and 1596 cm^{-1} bands. The exhibition of stronger intensity of the 778 cm^{-1} peak may indicate the production of carboxylic acids produced from the degradation of the hemicellulose of the wood (Lv *et al.*, 2015). The loss of the 1593 cm^{-1} peak of the control samples after degradation indicates loss of aromatic units of lignin, which was also reported by Faix *et al.* (1991) for degraded beech wood after exposure to three white-rot fungi and Popescu *et al.* (2007) for pulp samples of *Eucalyptus globulus*. The reduction in the 1030 cm^{-1} peak with increasing degradation (i.e. from control to Type 1) indicates cellulose degradation in the wood (Pandey and Pitman, 2003). However, the increase in the 1313 cm^{-1} peak could be as a result of the decreasing intensity of the 1250 cm^{-1} peak due to xylans degradation rather than the lignin, thereby making the lignin band of the 1313 cm^{-1} more prominent in the degraded wood. The gradual reduction in the hemicellulose band of the 1734 cm^{-1} peak with increasing degradation was equally reported by Pandey and Pitman (2003) for beech wood degraded by *Phanerochaete chrysosporium* but this peak remained unchanged in undegraded and degraded beech wood exposed to *Lentinus edodes*, *Coriolus versicolor* and *Pleurotus ostreatus* (Faix *et al.*, 1991).

The activities of this fungus on both the sugar and lignin component of the wood also show non-selectivity by the fungus on the wood polymers, which also corroborates the results of the anatomical investigation. Formation of new peaks in degraded wood and their subsequent disappearance during fungal degradation was also reported by Bari *et al.* (2018) for beech wood degraded by *Trametes versicolor* and *Pleurotus ostreatus* fungi. According to them, formation of a new peak and its subsequent disappearance is as a result of liberation of degraded cell wall components in the form of small molecules by the fungi and a subsequent assimilation of these liberated molecules in carbon form. Karim *et al.* (2017) noted that several kinds of hydrolysing enzymes are

possibly released by white rot fungi to modify and break the linkage bonds of wood cell wall components, thereby releasing them as small molecules of absorbable forms, which could be subsequently assimilated by the fungus.

A juxtaposition of the outer spectra of the wood bioincised with both fungi indicates some differences and similarities; the similarities, in addition to the fact that both fungi exhibited a simultaneous mode of wood decay by white rot fungi, include increase in the intensity of the 1322/1313 cm^{-1}, and 793/778 cm^{-1} peaks, and a reduction in the intensity of the 1734 cm^{-1}, 1030/1038 cm^{-1}, and 1230/1250 cm^{-1} peaks. However, the major contrasts between both fungal activities on the wood can be splitted into two. The first can be noticed in the 1595/1593 cm^{-1} peak, where a reduction in intensity after degradation occurred in samples bioincised with *I. dryophilus*, while an increase in intensity with degradation occurred in *G. adspersum*. Second, the loss of the 1648 cm^{-1} peak in the samples bioincised with *G. adspersum*, but a shift was rather observed in this band for samples bioincised with *I. dryophilus* from the 1648 cm^{-1} to 1618 cm^{-1}. By implication, while *G. adspersum* appeared to completely assimilate the conjugated carbonyl groups of the lignin molecule, *I. dryophilus* caused a restructuring of the lignin molecule to produce that comprising of more aromatic units.

5.5 Decay resistance of the treated bioincised wood

One of the most commonly employed methods used in evaluating the efficacy of a chemical treatment in wood against fungal decay is weight loss. Thus, the efficacy of bioincision in considerably enhancing chemical uptake in *G. arborea* heartwood and whether the process ultimately improved decay resistance of the wood against deterioration by *Trametes vesicolor* (white rot fungus) and *Coniophora puteana* (brown rot fungus) was assessed. Mean weight loss observed for the control samples (treated unincised samples) was below 5%, which is within the range of weight loss values reported by Adegeye *et al.* (2009) for treated *G. arborea* wood when exposed to attack by *Pleurotus squarrosullus* and *Lentinus subnudus*. This implies that the Tanalith treatment of the wood, together with the wood extractive was highly toxic against the two fungal strains. The treated bioincised samples, however, both had a lower weight loss for both the incising fungi than that of the control samples, which implies that enhanced uptake of the preservative as a result of the bioincision contributed to a better resistance of the treated wood to the decay fungi attack.

However, significant improvement in the resistance of the treated bioincised wood could not be achieved as the weight loss observed between the treated control samples and treated bioincised samples were statistically similar. This is in contrast to reports by Schubert *et al.* (2011) where a marked improvement in resistance of treated heartwood of Norway spruce bioincised with *Physisporineus vitreus* against some decay fungi was observed. This may be due to the better distribution of the preservatives within the Norway spruce wood, which was facilitated by the bioincising fungus, as opposed to the poor preservative distribution of the Tanalith preservative in the *G. arborea* heartwood. Wood samples bioincised with *I. dryophilus* gave a lower weight loss when compared to those bioincised with *G. adspersum*, though a statistical difference could not be established between their weight loss. This could be as a result of inherent variation within the wood specimen.

As for preservative effectiveness related to incubation period after bioincision, there was a progressive decrease in weight loss with increasing exposure time, except for the slight increment at 7 weeks. Samples bioincised for 9 weeks recorded a significantly lower weight loss when compared to most of the other incubation periods. This could possibly be as a result of the higher preservative uptake, coupled with a better distribution and deeper penetration of the treating chemical in the wood observed for the wood specimens bioincised for this duration. When the treatment method was considered, steeping yielded a higher but insignificant weight loss in comparison to the pressure-treated specimens. Pressure treatment of wood with preservatives is commonly employed for most refractory species of wood due to the energy input involved during the process which enhances the ingress of the chemical into the wood (Lehringer *et al.*, 2009a; Thaler *et al.*, 2012). This could be attributed to a significantly higher uptake of the preservative in the wood due to the pressure treatment, consequently improving the resistance of the wood against fungal decay than the steep-treated wood.

Based on the decay fungi, higher weight loss was induced on the wood samples by *T. versicolor* (white rot) than *C. puteana* (brown rot). This is in agreement with reports by Venmalar and Nagaveni (2005) and Ajala *et al.* (2014) where higher weight losses and hence, higher virulence were reported for white rots than brown rots. However, this observation is in dissonance with reports of Adetogun *et al.* (2006), Ogunsanwo and Adedeji (2010) and Adenaiya *et al.* (2016). Reasons advanced for this is that brown rot

fungus primarily attacks the cellulose and hemicelluloses component of the wood cell wall, whose effect on the wood mechanical properties is highly significant (Curling *et al*., 2001). Lignin, on the other hand, is the main target of white-rot fungi and plays less structural role when compared to cellulose and as such, its degradation may not result in a considerable decline in the wood's mechanical properties. However, it must be stated here that this reason may only hold true for selective delignifying white rot fungi species, as some white rot fungi are simultaneous rotters, attacking all the polymeric units of the wood simultaneously at a relatively uniform rate (Blanchette, 1991). *T. versicolor* belongs to the group of simultaneous rotters (Kubicek, 2013) and as such, the higher weight loss encountered in the wood samples is justified owing to the simultaneous attack on all the wood cell wall components. On the basis of exposure period to the fungi, there was a progressive but insignificant increase in weight loss with increasing exposure period. This is due to the removal of wood cell wall materials with increasing exposure due to the fungal activities on the wood. Similar observation was also documented by Adebawo *et al.* (2020).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

This study investigated the potentials of bioincision using *Inonotus dryophilus* and *Ganoderma adspersum* in improving the permeability of refractory *Gmelina arborea* heartwood. While investigating the efficacy of the procedure in the permeability improvement of the wood, the impact of the process on the mechanical, chemical and

cellular structure of the wood, as well as the improvement in the decay resistance of the treated bioincised wood was also assessed.

The two fungi species utilized generally showed promising outcomes, most particularly *Ganoderma adspersum*. The colonization of the wood by both fungi was heterogeneous as evident from the results of some of the physical properties such as the discoloration observed in the wood bioincised with both fungi which appeared localized. Wood samples bioincised with *G. adspersum* for 9 weeks had the highest weight loss (0.8%), while the least was observed in samples bioincised with *G. adspersum* for 3 weeks (0.02%). Overall range of weight loss for both fungi was marginal (i.e., <1%) which is one of the goals of a successful bioincision. Colour changes were observed in the bioincised species, but wood samples bioincised with *Inonotus dryophilus* did exhibit an obvious change in colour, capable of compromising the aesthetic value of the wood and therefore, making it unsuitable for applications where aesthetic quality is of paramount importance. There was equally an observed increase in moisture content with increasing incubation time owing to the increased metabolism of the wood cell wall material by the fungi species. Highest moisture content was observed after bioincising the wood for 7 weeks with both fungi species while the least was observed after 3 weeks of bioincision with both fungi. A decline in the moisture content of the bioincised wood was noticed after bioincising the wood samples for 9 weeks with both fungi species, indicating a drop in the overall metabolic activities of both fungi at this period of incubation.

Generally, absorption and retention of preservative in the bioincised wood was not significantly enhanced, even though both fungi opened up the wood cellular structure due to their activities on the occluding structures within the wood. However, prolonged exposure of the wood appeared to inhibit further uptake of the treating chemical possibly due to the blocking of accessible flow paths created by the fungi by the deposition of their metabolic products. Pressure treatment was discovered to be more effective in treating bioincised *G. arborea* heartwood than steeping as it produced a considerably higher ($p<0.05$) preservative absorption and retention in the bioincised wood than the steep-treated bioincised samples.

Axial and lateral uptake of chemical substance were considerably improved after 9 weeks of incubation, particularly in wood bioincised with *G. adspersum*, while the

least axial (27.28 Kg/m^3) and lateral chemical uptake (28.10 Kg/m^3) were observed in samples bioincised with *I. dryophilus* for 3 and 5 weeks, respectively. It was discovered that the dominant pathway for the ingress of the treating chemical into the wood was via the axial axis by virtue of the percentage improvement of axial chemical uptake of 53% and 68% observed in the *I. dryophilus* and *G. adspersum* bioincised wood samples, respectively when compared to that of the lateral chemical uptake of 22% and 53% for the *I. dryophilus* and *G. adspersum* bioincised wood samples, respectively. This implies that the activities of the fungi species were predominantly on the vessel occlusions (tyloses). The penetration depth of the chemical substance in the wood axially was substantially enhanced after 9 weeks by *G. adspersum* but the chemical distribution along this plane was abysmally poor. Activities of the fungi along the axial plane were rather pronounced at the middle section of the wood rather than at the surface in close proximity to the fungus, owing to the higher but insignificant ($p>0.05$) axial penetration depth of the chemical substance at the middle section than at the surface. This was possibly due to the fungi colonization proceeding via the zones of least resistance within the wood. Furthermore, depth of the treating substance in the lateral plane, which occurred principally through the radial axis, extended beyond the half-way point of the wood length in samples bioincised with *G. adspersum*, and a significantly higher penetration depth ($p<0.05$) was recorded after 9 weeks of incubation. Similar to the chemical distribution in the axial axis, the distribution of the treating substance in the lateral plane was extremely poor.

Anatomical investigations revealed that both fungi species were simultaneous rot fungi, having the ability to metabolize all the wood cell wall constituents. Majority of the fungal hyphae were found within the vessels and ray parenchyma. Expectedly, the impregnated dye was mostly found within the two cells in the light microscopy analysis, indicating that the two cells were predominantly the pathways for chemical flow within the wood. Both fungi degraded the tyloses within the vessel lumina. *G. adspersum* induced delamellation and cell wall degradation and subsequent coalescence of two axial parenchyma cells of the wood, coupled with the formation of erosion troughs and enlargement of pits on the radial wall of the vessel. Activities of *I. dryophilus* on the wood cellular structures was however minimal when compared to that *G. adspersum*, possibly due to its lower tolerance to the toxicity of the wood extractives.

The adverse effect of the bioincision on the wood mechanical properties was generally insignificant, which shows the suitability of both fungi in the bioincision of the wood species. Only the MOR was significantly reduced by *G. adspersum* at 7 weeks of incubation, while there was slight but insignificant ($p>0.05$) increase in the impact resistance, tensile strength and hardness of some of the bioincised wood. Chemical analysis using FTIR indicated that virtually all the chemical components were metabolized by both fungi and also showed that a great deal of variation in the decay pattern and intensity of decay was induced by both fungi on the wood, most especially by the samples bioincised with *I. dryophilus*. For the decay test, the decay resistance of the treated bioincised wood against *Trametes versicolor* (white rot fungus) and *Coniophora puteana* (brown rot fungus) was generally enhanced, but was not appreciable enough to be considered effective in preventing the decay of the wood by both fungi. The bioincising fungi, treatment type, decay fungi and exposure period had no considerable effect ($p>0.05$) on the weight loss of the treated *G. arborea* heartwood after exposure to fungi attack, while only incubation period of the bioincising fungi had a marked effect ($p<0.05$) on the wood weight loss. It was discovered that the incubation period of 9 weeks for the bioincision had a significantly lower weight loss ($p>0.05$) than the other incubation periods. Interaction effects of all the factors considered in the decay test also had no pronounced effect ($p>0.05$) on the weight loss of the wood. Overall, *G. adspersum* appeared to be a more promising candidate fungus species for the bioincision of *G. arborea* heartwood.

6.2 Recommendations

Based on the findings of this study, the following recommendation is suggested:

1. The complexity of the cellular structure of hardwoods, coupled with the heterogeneous distribution of moisture during the bioincision process are some of the factors responsible for the heterogenous colonization of the wood by both fungi. Thus, homogeneity of the wood colonization and property improvement could be improved on by increasing the water activity of the wood (amount of free water available in the wood for fungal biochemical activities) before bioincising.
2. A more detailed study on the optimum incubation requirements of both fungi species for their optimal growth is essential for an improved outcome of the bioincising process.

3. The extractives within the wood was apparently an inhibiting factor to the optimal growth of the fungi in the wood. Hence, preparation of an extractive-free wood before bioincising might help improve the outcome of the bioincising process for subsequent impregnation of chemical into the wood.
4. Most of the permeability indices of the wood increased with prolonged exposure period as observed in the study. Thus, further research on the effect of higher incubation times with both fungi should be conducted to determine the optimal incubation period required for an optimal improvement in the permeability of the wood.
5. Also, a further study on the extraction of the enzymes of both fungi and subsequent impregnation of *G. arborea* heartwood with the enzymes is needed as this could make the bioincision process more effective. This is because of noticeable inhibition of optimal fungal development in the wood due to the toxicity of the wood extractives. This could therefore, be avoided by the direct impregnation of the wood with the fungi enzymes, as this could help improve the activities of the enzymes on the target wood occluding structures.

6.3 Contributions to knowledge

1. The suitability of bioincision using *Inonotus dryophilus* and *Ganoderma adspersum* in improving the permeability of refractory *Gmelina arborea* heartwood was demonstrated.
2. Information on the decay pattern and the impact of the activities of *Inonotus dryophilus* and *Ganoderma adspersum* on the cellular structure of *Gmelina arborea* heartwood was provided.
3. The effect of *Inonotus dryophilus* and *Ganoderma adspersum* on the mechanical properties of *Gmelina arborea* heartwood was revealed.
4. The effect of *Inonotus dryophilus* and *Ganoderma adspersum* on the chemical constituents of *Gmelina arborea* heartwood using FT-IR was provided.

5. Information on the decay resistance of treated bioincised *Gmelina arborea* heartwood against a white and brown rot fungus was provided.

REFERENCES

Adam, K. A. and Krampah, E. 2005. *Gmelina arborea* Roxb. ex Sm. In: Louppe, D., Oteng-Amoako, A. A. and Brink, M. (Eds). PROTA (Plant Resources of Tropical Africa/ Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://www.prota4u.org/search.asp>. Accessed 3rd August 2019.

Adebawo, F. G., Naithani, V., Sadeghifar, H., Tilotta, D., Lucia, L. A., Jameel, H. and Ogunsanwo, O. Y. 2016. Morphological and interfacial properties of chemically-modified tropical hardwood. *RSC Advances* 6: 6571-6576.

Adebawo, F. G., Ogunsanwo, O.Y. and Olajuyigbe, S. O. 2020. Decay Resistance of the Acetylated Tropical Hardwood Species. *Journal of Forest and Environmental Science* 36.3: 225-232.

Adegeye, A. O., Ogunsanwo, O. Y. and Olajuyigbe, S. O. 2009. Antifungal activities of heartwood extract (HWE) of Teak *Tectona grandis* against two white rots in woods of *Gmelina arborea* and *Triplochiton scleroxylon*. *Academic journal of plant sciences* 2.4: 279-285.

Adenaiya, A. O., Ogunsanwo, O. Y. and Onakpoma, I. 2016. Weight loss and compressive strength of Castor oil-treated *Pinus caribaea* (Morelet) wood exposed to fungi. *PRO LIGNO* 12.4: 41-52.

Adeniyi, I. M., Adejoba, O. R., Akinlabi, F. M., Alao, O. J., Fakorede, C. O. and Adubi, O. A. 2017. Anatomical structure of plantation-grown *Gmelina arborea* ROXB. In: Adekunle, V.A.J., Ogunsanwo, O.Y. and Akinwole, A.O. (Eds.) Harnessing the Uniqueness of Forests for Sustainable Development in a Diversifying Economy. *Proceedings of the 39th Annual Conference of the Forestry Association of Nigeria held in Ibadan, Oyo State, Nigeria, 20th - 24th February, 2017*, pp67-73.

Adetogun, A. C. and Adegeye, A. O. 2002. Aqueous leaching of cashew nut shell liquid (CNSL) treated wood of *Triplochiton scleroxylon* K. Schum exposed to basidiomycetes decay fungi. *Journal of Tropical Forest Resources* 18: 69-74.

Adetogun, A. C., Aina, O. M., Ogunsanwo, O. Y. and Omole, A. O. 2006. Resistance of three Nigerian hardwoods to decay caused by brown and white rot basidiomycetes. *ASSET an International Journal* 6.2: 205-208.

Adewuyi, O. I. and Oladapo, A. G. 2011. Use of geophysical methods in landfill site investigation: A case study of Ibadan, South-western Nigeria. *New York Science Journal* 4.10: 1-10

Adusumalli, R. B., Mook, W. M., Passas, R., Schwaller, P. and Michler, J. 2010. Nanoindentation of single pulp fibre cell walls. *Journal of Materials Science* 45.10: 2558–2563

Ahmed, S. A. and Chun, S. U. 2007. Descriptions of the wood anatomy and safranine impregnation in *Gmelina arborea* Roxb. from Bangladesh. *Journal of the Korea Furniture Society* 18.2: 100-105.

Ajake, A. O. and Enang, E. E. 2012. Demographic and Socio-economic attributes affecting forest ecosystem exploitation and management in the rural communities of Cross River State, Nigeria. *American International Journal of Contemporary Res*earch 2.1: 174-184.

Ajala, O. O., Adebawo, F. G., Yekeen, O. M. and Owoade, O. D. 2014. Potentials of seed oil extract of *Azadirachta indica* (A. JUSS) as preservative against wood-decaying fungi of *Aningeria robusta* (A. CHEV.) In: Sudano-sahelian landscape and renewable natural resources development in Nigeria. Ogunsanwo, O. Y., Akinwole, A. O., Azeez, I. O., Adekunle, V. A. J. and Adewole, N. A. (Eds).

Proceedings of the 37th Annual conference of the Forestry Association of Nigeria held in Minna, Niger State, 9th - 14th November, 2014, 588-595.

Archer, K. and Lebow, S. 2006. *Wood preservation*. In: Primary wood processing: principles and practice. Walker, J. C. F. (ed.), 2nd Edition, Springer, Netherlands. pp.297-338.

Arsenault, R. D. 1973. Factors influencing the effectiveness of preservative systems. In: Wood deterioration and its prevention by preservative treatments, Vol II: Preservatives and Preservative Systems, D. D. Nicholas (ed). Syracuse University Press, Syracuse, New York. Pp 121-278.

Asafu-Adjaye, O. A. 2012: Characterization of the physico-mechanical properties of the different zones of *Borassus aethiopum* (*Mmaa Kube*). Thesis submitted to the Department of wood science and technology *Kwame Nkrumah University of Science and Technology, FRNR-KNUST, Kumasi-Ghana* in partial fulfilment of the requirements for the degree of Master of philosophy in wood technology faculty of renewable natural resources college of agriculture and natural resources management. 244pp

Ashaduzzaman, M., Hale, M.D., Tverezovskiy, V. and Ormondroyd, G.A. 2013. *Effect of bio-resin from cashew nut shell liquid (CNSL) on decay resistance properties of wood*. Proceedings of the 44th Annual Conference of International Research Group on Wood Protection, IRG/WP 13-40649, Stockholm, Sweden, June 16-20, 2013.

Ashaduzzaman, Md. 2014. Physico-mechanical and decay resistance properties of bio-resin modified wood. A Ph.D Thesis submitted to the School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, United Kingdom. 210pp.

Awoyemi, L. 2006. Heat treatment of less-valuable Nigerian-grown *Ceiba pentandra* wood for improved properties. Paper prepared for the 37th Annual Meeting Tromso, Norway, 18 - 22 June, 2006. IRG/WP 06-40332.

AWPA Standards. 1971. American Wood-Preservers’ Association, Washington, D.C.

Baines, E.F. and Saur, J. M. 1985. Preservative treatment of spruce and other refractory wood. Proceedings of the 81st Annual Meeting of the American Wood-Preserver’s Association, MGM Grand Hotel, Reno, Nevada, May 5-8, 1985, 81: 136-147.

Banks, W. B. 1970. Some factors affecting the permeability of Scots pine and Norway spruce. *Journal of the Institute of Wood Science* 5.1: 10-17.

Bari, E., Mohebby, B., Naji, H. R., Oladi, R., Yilgor, N., Nazarnezhad, N., Ohno, K. M. and Nicholas, D. D. 2018. Monitoring the cell wall characteristics of

degraded beech wood by white-rot fungi: anatomical, chemical, and photochemical study. *Maderas. Ciencia y tecnología* 20.1: 35 – 56.

Bari, E., Taghiyari, H. R., Mohebby, B., Clausen, C. A., Schmidt, O., Ghanbary, M. A. T. and Vaseghi, M. J. 2015. Mechanical properties and chemical composition of beech wood exposed for 30 and 120 days to white-rot fungi. *Holzforschung* 69.5: 587-593.

Barnes, H. M. and Murphy, R. J. 1995. Wood Preservation. The Classics and New Age. *Forest Products Journal* 45.9: 16-23.

Behr, E. A., Sachs, I. B., Kukachka, B. F. and Blew, J. O. 1969. Microscopic examination of pressure treated wood. *Forest Products Journal* 19.8: 31-40.

Bell, A. A. 1980. The time sequence of defense. In: Plant disease: An advanced treatise. Horsfall, J. G. And Cowling, E. B. (eds). How plants defend themselves. Academic press, New York, 534pp.

Bergman, R., Cai, Z., Carll, C. G., Clausen, C. A., Dietenberger, M. A., Falk, R. H., Frihart, C. R., Glass, S. V., Hunt, C. G., Ibach, R. E., Kretschmann, D. E., Rammer, D. R., Ross, R. J., Stark, N. M., Wacker, J. P., Wang, X., White, R. H., Wiedenhoeft, A. C., Wiemann, M. C. and Zelinka, S. L. 2010. Wood Handbook, Wood as an Engineering Material. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI: 508pp.

Blanchette R. A. 1991. Delignification by wood-decay fungi. *Annual Review of Phytopathology* 29: 381-398.

Blanchette, R. A. 1980a. Wood decomposition by *Phellinus* (Fomes) *pini*: a scanning electron microscopy study. *Canadian Journal of Botany* 58.13: 1496-1503

Blanchette, R. A. 1984a. Selective delignification of eastern hemlock by *Ganoderma tsugae*. *Phytopathology* 74: 153-160.

Blanchette, R. A. 1984b. Manganese accumulation in wood decayed by white rot fungi. *Phytopathology* 74: 725-730.

Blanchette, R. A. 1984c. Screening wood decayed by white-rot fungi for preferential lignin degradation. *Applied Environmental Microbiology* 48: 647–653

Blanchette, R. A. 2005. Microbes in Trees and Wood. Research projects, University of Minnesota. Retrieved from http://www.forestpathology.coafes.umn.edu on the 13th April, 2013.

Blanchette, R. A., Farrell, R. L., Burnes, T. A., Wendler, P. A., Zimmerman, W., Brush, T. S. and Snyder, R. A. 1992a. Biological control of pitch in pulp and paper production by *Ophiostoma piliferum*. *TAPPI Journal* 75: 102–106.

Bolden, S. and Greaves, H. 2008. Guide to the specification, installation and use of preservative treated engineered wood products. Forest and wood products Australia project publication PR08.1068 report, 41pp.

Bon, E. P. S. and Ferrara, M. A. 2007. Bioethanol production via enzymatic hydrolysis of cellulosic biomass on "The role of agricultural biotechnologies for production of bioenergy in developing countries". FAO seminar, Rome.

Bowyer, J. L., Shmulsky, R. and Haygreen, J.G. 2007. *Forest products and wood science: an Introduction*, 5th ed. Iowa State University Press, USA. 576pp.

Brischke, C., Soetbeer, A. and Meyer-Veltrup, L. 2017. The minimum moisture threshold for wood decay by basidiomycetes revisited. A review and modified pile experiments with Norway spruce and European beech decayed by *Coniophora puteana* and *Trametes versicolor*. *Holzforschung* 71.11: 893–903.

British Standards 373 1957. *Method of Testing Small Clear Specimens of Timber*. British Standard Institute, London. 32pp.

BS EN 350-2, 1994. *Durability of wood and wood-based products- Natural durability of solid wood*. Part 2: Guide to natural durability and treatability of selected wood species of importance in Europe. 36pp.

BSI 1961. British Standard Institute: *Methods of Test for Toxicity of Wood Preservatives to Fungi*. BS838 London, England.

Butler, R. 2012. How to save tropical rainforest. Available at http://rainforests.mongabay.com/1001.htm. Accessed on 2/27/2015.

Butterfield, B. 2006. *The structure of wood: form and function*. In: Primary wood processing: principles and practice. Walker, J. C. F. (ed.), 2nd Edition, Springer, Netherlands. pp. 1-22.

Cech, M. Y. 1971. Dynamic transverse compression treatment to improve drying behavior of yellow birch. *Forest Products Journal* 21.2: 41-50.

Cech, M. Y. and Huffman, D. R. 1970. Dynamic transverse compression treatment of spruce to improve intake of preservatives. *Forest Products Journal* 20.3: 47-52.

Chaubey, B. B., Dobriyal, P. B. and Kumar. S. 1986. Structural factors affecting penetration of fluids in Indian hardwoods: A microscopic study. *Journal of the Timber Development Association of India* 33.3: 25-35.

Chen, H. 2014. *Biotechnology of lignocellulose: theory and practice*. Beijing: Chemical Industry Press and Springer Science. pp. 510.

Chirra, F. 1995. Ohio pesticide applicator training: In Kick-Raack, J. (ed.) Wood preservation student workbook bulletin 821-13. The Ohio State University Extension.

Chudnoff, M. 1984. *Tropical Timbers of the World*. Agricultural Handbook 607. USDA Forest Service, Washington DC. 466 p.

Clausen, C. 2010. *Biodeterioration of wood*. Pp. 14.1-14.16. *In*: Wood Handbook: Wood as an Engineering Material. General Technical Report FPL-GTR-190. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 508pp.

Cochran, V. W. 1958. "*Physiology of Fungi*." John Wiley New York.

Collins, B. and Parke, J., 2008. Spatial and temporal aspects of tylosis formation in tanoak inoculated with *Phytophthora ramorum*. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M. (Eds.), Proceedings of the Sudden Oak Death Third Science Symposium, U.S. Department of Agriculture General Technology Report PSW-GTR-214, p. 335.

Comstock, G. L. 1968. Relationship between permeability of green and dry eastern hemlock. *Forest Products Journal* 18.8: 20-23.

Comstock, G. L. 1970. Directional permeability of softwoods. *Wood and Fiber* 1.4: 283-289.

Cooney, D. G., and Emerson, R. 1964. "*Thermophilic Fungi*." W. H. Freeman, San Francisco.

Cote, W. A. and Krahmer, R. L. 1962. The permeability of coniferous pits demonstrated by electron microscopy. *Tappi* 45.2: 119-122.

Côté, W. A. 1963. Structural factors affecting the permeability of wood. *J. polymer Sci.* Part C, 2: 231-242.

Côté, W. A. 1990. Colley lecture: In search of pathways through wood. Proceedings of the 86th Annual Meeting of the American Wood Preservers' Association, Vol. 86, Opryland Hotel, Nashville, Tennesse, April 30 -May 2, 1990, AWPA, P.O. Box 849, Stevensville, MD 21666.

Curling, C. F., C.A. Clausen, and J.E. Winandy. 2001. The effect of hemicellulose degradation on the mechanical properties of wood during brown rot decay. Doc. No. IRG/WP 01-20219. International Res. Group on Wood Preservation, Stockholm, Sweden.

Curling, S. F., Clausen, C. A. and Winandy, J. E. 2002. Relationships between mechanical properties, weight loss and chemical composition of wood during incipient brown-rot decay. *Forest Products Journal* 52.7-8: 34-39.

Dale, A., Morris, P. I., Uzunovic, A., Symons, P. and Stirling, R. 2019. Biological incising of lodgepole pine and white spruce lumber with *Dichomitus squalens*. *European Journal of Wood and Wood Products* 77.6:1161–1176.

Dashtban, M., Schraft, H., Syed, T. A. and Wensheng, Q. 2010. Fungal biodegradation and enzymatic modification of lignin. *International Journal of Biochemistry and Molecular Biology* 1.1: 36-50.

Desch, H. E. and Dinwoodie, J. M. 1981. *Timber: its structure, properties and utilisation*. 6th Edition. London: Macmillian Education Ltd. pp. 410.

Desch, H. E. and Dinwoodie, J. M. 1996. *Timber structure, properties, conversion and use.* 7th Edition, Basingstoke, Hampshire, UK: Macmillian Press. pp. 306.

Dickinson, D. J. and Sorkhoh, N. A. A. H. 1976. The micro-distribution of wood preservatives. Scanning Electron Microscopy I.I.T.R.I., Chicago. Pp. 549-554.

Dinwoodie, J. M. 1989. Wood: *Nature's cellular, polymeric fibre composite*. Pub. The Institute of Metal London.

Dinwoodie, J. M. 1989. Timber: Its nature and behaviour. 2nd Edition. London and New York: E & FN Spon. pp. 257.

Duncan, C. G. 1967. Effect of light on the rate of decay of three wood-destroying fungi. *Phytopathology* 57.10: 1121-1125.

Dungani, R., Bhat, I.U.H., Khalil, A.H.P.S., Naif, H.P.S. and Hermawan, D. 2012. Evaluation of antitermitic activity of different extract obtained from Indonesian teak wood (*Tectona grandis*). *Journal of Bioresources* 7.2: 1452-1461.

Durmaz, S., Yildiz, U. C. and Yildiz, S. 2015. Alkaline enzyme treatment of spruce wood to increase permeability. *Bioresources* 10.3: 4403-4410.

Eaton, R. A. and Hale, M. D. C. 1993. Wood: Decay, pests and protection. Chapman & Hall, New York, NY. 546pp.

Efransjah, F., Kilbertus, G. and Bucur, V. 1989. Impact of water storage on mechanical properties of spruce as detected by ultrasonics. *Wood and Science Technology* 23.1: 35-42

Emaminasab, M., Tarmian, A. and Pourtahmasi, K. 2015. Permeability of poplar normal wood and tension wood bioincised by *Physisporinus vitreus* and *Xylaria longipes*. *International Biodeterioration & Biodegradation* 105: 178-184.

Emaminasab, M., Tarmian, A. and Pourtahmasi, K. and Avramidis, S. 2016. Improving the permeability of Douglas-fir (*Pseudotsuga menziesii*) containing compression wood by *Physisporinus vitreus* and *Xylaria longipes*. *International Wood Products Journal* 7.3: 110-115.

Encyclopaedia Britannica 2000. Wood: cellular composition of wood. Accessed on 14th Sept., 2018. Retrieved from https://kids.britannica.com/students/assembly/view/55253.

Erickson, H. D. and Balatinecz, J. J. 1964. Liquid flow paths into wood using polymerization techniques- Douglas-fir and styrene. *Forest Products Journal* 14.7: 293-299.

Erickson, H. D. and Crawford, R. J. 1959. The effect of several seasoning methods on the permeability of wood to liquids. Proc. Am. Wood-Preservers Association., 55: 210-219.

Eriksson, K. L., Blanchette, R. A. and Ander, P. 1990. Morphological aspects of wood degradation by fungi and bacteria. In: Microbial and enzymatic degradation of wood and wood components. Timell, T. E. Ed. Springer Series in Wood Science. Vol 12, Heidelberg, Springer-Verlag Berlin. pp1-87.

European Standard EN 113 1996. *Wood preservatives – Method of test for determining the protective effectiveness against wood destroying basidiomycetes* - Determination of the toxic values. European Committee for Standardization (CEN), Brussels, Belgium.

Evans, P. D. 2003. Emerging technologies in wood protection. *Forest Products Journal*, 53.1: 14-22.

Faix, O., Bremer, J., Schmidt, O. and Stevanovic, J. 1991. Monitoring of chemical changes in white-rot degraded beech wood by pyrolysis-gas chromatography and Fourier-transform infrared spectroscopy. Journal of Analytical and Applied Pyrolysis 21: 147-162.

Farinde, M. A and Oni, S. O. 2015. Geophysical and geotechnical characterization of newly constructed Abadina-Ajibode road, University of Ibadan, Ibadan. *Journal of Multidisciplinary Engineering Science and Technology* 2.1: 363-378.

Farmer R.H, 1972: Handbook of Hardwood. Second Edition. Her Majesty's Stationary Office London 2, 49-50pp

Feist, W. C. and Hon, D. N. S. 1984. Chemistry of weathering and protection. In: Rowell, R. M., (Ed). The Chemistry of Solid Wood, Advances in Chemistry Series No. 207. American Chemical Society, Washington, DC. Eds.: 401-451.

Fengel, D. and Wegener, G. 1989. *Wood: Chemistry, Ultrastructure, Reactions*. Walter De Gruyter: Berlin, Germany. 611pp.

Findlay, W. P. K. (ed.) 1985. Preservation of timber in the tropics. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, Netherlands, 273pp.

Flynn, K. A. 1995. A review of the permeability, fluid flow, and anatomy of spruce (*Picea spp.*). *Wood and Fiber Science* 27.3: 278-284

Food and Agricultural Organisation [FAO] 1986. Wood Preservation Manual. FAO Forestry Paper 76, Mechanical Wood Products Branch, Forest Industries Division, FAO Forestry Department, pp 152.

Forest Products Laboratory 1999. Wood handbook - Wood as an engineering material. Gen. Tech. Rep. FPL–GTR–113. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 463 p.

Forest Products Laboratory, 2010: Wood Handbook—Wood as an Engineering Material. General Technical Report FPL-GTR-190. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. pp508.

Fowlie, I. M. and Sheard, L. 1983. Developments in the use of home grown spruce poles for use as overhead line supports. Rec. Brit. Wood Pres. Assoc. Ann. Conv., 47-60.

Freeman, M. H. 2003. Wood Preservative Formulation Development and Systems: Organic and Inorganic Based Systems: In Schultz, T. P., Militz, H., Freeman, M. H., Goodell, B. and Nicholas, D. D. (Eds) Development of commercial wood preservatives: Efficacy, environmental and health issues, America Chemical Society, Washington, DC, USA, ACS Symposium series 982, Pp 408-426

Freeman, M. H., Shupe, T. F., Vlosky, R. P. and Barnes, H. M. 2003. Past, present and future of the wood preservation industry. *Forest Products Journal* 53.10: 8-15.

Fujii, T., Suzuki, Y. and Kuroda, N. 1997. Bordered pit aspiration in the wood of *Cryptomeria japonica* in relation to air permeability. *IAWA Journal* 18.1: 16-76. DOI: 10.1163/22941932-90001462.

Gerwing, J. J., Jones, J. S. and Vidal, E. 1996. Reducing waste during logging and log processing. Forest conservation and utilization. FAO, Unasylva, No. 187.

Gilbertson, R. L. 1980. Wood-rotting fungi of North America. *Mycologia* 72: 1-49.

Goodell B., Nicholas, D.D. and Schultz, T.P. 2003. Introduction to wood deterioration and preservation. In: Goodell B, Nicholas DD and Schultz TP (ed.), Wood deterioration and preservation: advances in our changing world. ACS Symposium Series 845. American Chemical Society, Washington DC, pp2-7.

Goodell, B., Kamke, F. A. and Liu, J. 1991. Laser incising of spruce lumber for improved preservative penetration. *Forest Products Journal* 4.9: 48-52.

Goodell, B., Yuhui, Q. and Jellison, J. (2008). Fungal decay of wood: soft rot-brown rot-whiterot. In: T. P. Schult, H. Militz, M. H. Freeman, B. Goodell, D. D. Nicholas (Eds.), Development of Commercial Wood Preservatives, ACS symposium series, American Chemical Society. pp. 9–31.

Greaves, H. 1970. The effect of some wood inhabiting bacteria on the permeability characteristics and microscopic features of Eucalyptus regnans sapwood and Pinus radiata sapwood and heartwood. *Holzforschung* 24: 6–17

Greaves, H. 1977. An illustrated comment on the softrot problem in Australia and Papua New Guinea. *Holzforschung* 28.5: 193-200.

Greaves, H. and Levy, J. F. 1978. Penetration and distribution of copper-chrome-arsenic preservative in selected wood species. 1. Influence of gross anatomy on penetration, as determined by X-ray microanalysis. *Holzforschung* 32.6: 200-208.

Green III, F. and Highley, T. 1997. Mechanism of brown-rot decay: paradigm or paradox. *International Biodeterioration and Biodegradation* 39: 113-124.

Green, D. W., Evans, J. W. and Craig, B. A. 2003. Durability of structural lumber products at high temperatures I: 66°C at 75% RH and 82°C at 30% RH. *Wood and Fiber Science* 35.4: 499–523.

Gunzerodt, H., Walker, J. C. F. and Whybrew, K. 1986. Compression rolling and hot-water soaking: effects on the drying and treatability of Nothofagus fusca heartwood. *New Zealand Journal of Forestry Science* 16.2: 223-236.

Gupta S, Prakash J. 2009 Studies on Indian green leafy vegetables for their antioxidant activity. *Plant Foods and Human Nutrition* 64: 39–45.

Hansmann, C., Gindl, W., Wimmer, R., Teischinger, A. 2002. Permeability of wood - A review. *Wood Research* 47.4: 1-16.

Harper, C. J., Bomfleur, B., Decombeix, A., Taylor, E. L., Taylor, T. N. And Krings, M. 2012. Tylosis formation and fungal interactions in an Early Jurassic conifer from northern Victoria Land, Antarctica. *Review of Palaeobotany and Palynology* 175: 25-31

Harris, J. M. 1954. Heartwood formation in *Pinus radiata* (D Don.). *The New Phytologist* 53.3: 517-524.

Harris, W. V. 1971. *Termites. Their recognition and control*. 2nd Edition, Longman Group Ltd., London

Hartford, W. H. 1973. Chemical and physical properties of wood preservatives and wood-preservative systems. In: Wood deterioration and its prevention by preservative treatments, Vol II: Preservatives and Preservative Systems, D. D. Nicholas (ed). Syracuse University Press, Syracuse, New York. Pp 1-120.

Hatt, W. K. 1906. Experiments on the strength of treated timber. USDA Forest Serv. Circular No. 39.

Haygreen J. G. and Bowyer, J. L. 1996. Forest Product and Wood Science. An Introduction. Third Edition. IOWA State University Press/ AMES 232pp.

Hernandez, R. E. 2007. Swelling properties of hardwoods as affected by their extraneous substances, wood density and interlocked grain. *Wood and Fiber Science* 39: 146–158.

Highley, T. L. 1999. Biodeterioration of Wood. In: Wood Handbook – Wood as an Engineering Material. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI. General Technical Report FPL-GTR-113. 463pp.

Hihara, L. H., Adler, R. P. I. and Latanision, R. M. (eds.) 2013. Environmental Degradation of Advanced and Traditional Engineering Materials. Taylor and Francis/CRC Press.

Hill, C.A.S. 2006. Wood modification: chemical, thermal and other processes. John Wiley and Sons Ltd, The Atrium, Southern Gate Chichester, UK. 260pp.

Hillis, W. E. (1987). Heartwood and tree exudates. Springer-Verlag, Berlin, New York.

Hingston, J. A., Collins, C. D., Murphy, R. J. and Lester, J. N. 2001. Leaching of chromated copper arsenate wood preservatives: a review. *Environmental Pollution* 111: 53–66.

Homan, W. J. and Jorissen, J. M. 2004. Wood modification developments. *HERON* 49.4: 361-386.

Hon, D. N.S 1996. *Chemical Modification of Lignocellulosic Materials.* Marcel Dekker: New York. 370 pp.

Ibach, R. E. 1999. Wood preservation. In: Wood Handbook – Wood as an Engineering Material. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI. General Technical Report FPL-GTR-113. 463pp.

Illston, J. M., Dinwoodie, J. M. and Smith, A. A. 1987: Concrete, Timber and Metals. T.J. Press, Padstow Limited. Padstow. 63p.

Islam, M. S., Hamdan, S., Rusop, M., Rahman, M. R., Ahmed, A. S., and Idrus, M. A. M. (2012). Dimensional stability and water repellent efficiency measurement of chemically modified tropical light hardwood. *BioResources* 7.1: 1221-1231.

Jensen, M. 1995. Trees commonly cultivated in Southeast Asia; Illustrated field guide. RAP Publication: 1995/38, FAO, Bangkok, Thailand. p.93.

Johnson, B. R. and Giovik, L. R. 1970. Effect of *Trichoderma viride* and a contaminating bacterium on microstructure and permeability of loblolly pine and Douglas fir. *Am. Wood Preserv Assoc*. 66: 234– 242.

Kang, S., Paik, K. and Kim, G. 1997. Studies on improving preservative treatability of Japanese larch heartwood by presteaming. *Mokchae Konghak* 25.1: 15-22.

Karim, M., Daryaei, M. G., Torkaman, J., Oladi, R., Ghanbary, M.A.T., Bari, E. and Nural Y. 2017. Natural decomposition of hornbeam wood decayed by the white rot fungus *Trametes versicolor. Annals of the Brazilian Academy of Sciences* 89.4: 2647-2655.

Keith, C. T. and Chauret, G. 1988. Anatomical studies of CCA penetration associated with conventional (tooth) and with micro (needle) incising. *Wood and Fiber Science* 20.2: 197-208.

Khramer, R. L. and Cote, W. A. 1963. Changes in coniferous wood cells associated with heartwood formation. *Tappi* 46.1: 42-49.

Kim, H., Song, B. and Koo, J. 2008. Spatial Distributions of Chromium, Copper, and Arsenic Concentrations in Soils Near Three Log Structures and a Sound Barrier, All Constructed with CCA-treated Wood. *Journal of KoSSGE* 13.2: 12-20

Kirk, T. K. and Cullen, D. 1998. *Enzymology and molecular genetics of wood degradation by wood-degrading fungi*. In: Young, R. A. and Akhtar, M. (eds), Environmentally Friendly Technologies for the Pulp and Paper Industry. New York: John Wiley & Sons. Pp 273-307.

Kirk, T. K. And Highley, T. L. 1973. Quantitative changes in structural components of conifer woods during decay by white- and brown-rot fungi. *Phytopathology* 55: 739-745.

Kirk, T. K., Shultz, E., Connors, W. J., Lorentz, L. F. and Zeikus, J. G. 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Archives of Microbiology* 117: 277-285.

Kleist, G. and Seehann, G. 1997. Colonization patterns and topochemical aspects of sap streak caused by *Stereum sanguinolentum* in Norway spruce. *Eurasian Journal of Forest Path*ology 27: 351-361

Klemm, D., Heublein, B., Fink, H. and Bohn, A. 2005. Cellulose: fascinating biopolymer/sustainable raw material. *Ang Chem Intl Edn Engl.* 44: 3358–3393.

Kollmann, F. F. P. and Côté, W. A. 1968. *Principles of wood science and technology I: Solid wood*. Springer-Verlag: Berlin Heidelberg. 592pp.

Koyani, R. D. and Rajput, K. S. 2015. Anatomical characterisation of wood decay pattern in *Azadirachta indica* A. Juss. by the white-rot fungi *Irpex lacteus* Fr. (Fr.) and *Phanerochaete chrysosporium* Burds. *Anales de Biología* 37: 97-106.

Kretschmann, D. E. 2008. The influence of juvenile wood content on shear parallel, compression, and tension perpendicular to grain strength and mode I fracture toughness of loblolly pine at various ring orientation. *Forest Products Journal* 58.7–8: 89–96.

Kubicek, C. P. 2013. *Fungi and lignocellulosic biomass*. Wiley-Blackwell, New Delhi, India. 290pp.

Kumar, S. and Dobriyal, P. B. 1993. Penetration indices of hardwoods: a quantitative approach to define treatability. *Wood and Fiber Science*, 25.2: 192-197.

Kumar, S. and Morrell, J. J. 1993. Effect of fatty acid removal on treatability of Douglas-fir. International Research Group on Wood Preservation. (IRGWP 92-1531), Stockholm.

Kumar, S. and Sharma, R. P. 1982. Pressure impregnation of hardwoods. In: Treatment schedules for easy-to-treat wood species. *Journal of the Timber Development Association of India* 28.4:24-29.

Kumar, S., Sharma, R.P., Dobriyal, P.B. and Chaubey, B.B. 1990. Pressure impregnation of hardwoods: Treatment schedules for easy-to-treat Indian Hardwoods. *Wood and Fiber Science* 22.1: 3-9.

Lande, S., Høibø, O. A, Larnøy, E. 2009. Variation in Treatability of Scots Pine (Pinus sylvestris) by the Chemical Modification Agent Furfuryl Alcohol Dissolved in Water. *Wood Science and Technology* 44.1: 105-118.

Lantican, D. M., Cote, W. A. and Skaar, C. 1965. Effect of ozone treatment on the hygroscopicity, permeability, and ultrastructure of the heartwood of western redcedar. *Industrial and Engineering Chemistry Product Research and Development* 4.2: 66-70.

Larnøy, E., Lande, S. & Vestøl, G. 2008. Variations of Furfuryl alcohol and Wolmanit CX-8 treatability of pine sapwood within and between trees. The International Research Group on Wood Protection Document NO IRG/WP/08-40421.

Lebow, S. T. 2010. Wood preservation: In Centennial (ed.) wood as an engineering material Wood handbook General technical report FPL, GTR-190. Madison, WI, U.S. Dept. of Agriculture, Forest Service, Forest Products Laboratory, 28pp.

Lee, H. V., Hamid, S. B. A. and Zain, S. K. (2014). Conversion of lignocellulosic biomass to nanocellulose: Structure and chemical process. *The Scientific World Journal*, Volume 2014, Article I.D 631013. 20pp.

Lehringer, C. 2011. Permeability improvement of Norway spruce wood with the white rot fungus *Physisporinus vitreus*. A Ph.D Thesis submitted to the Department of Wood Biology and Wood Products, Faculty of Forest Sciences and Forest Ecology, Georg-August University, Göttingen. 32pp.

Lehringer, C., Arnold, M., Richter, K., Schubert, M., Schwarze, F. W. M. R. and Militz, H. 2009a. Bioincised wood as substrate for surface modifications. European Conference on Wood Modification, 2009.

Lehringer, C., Hillebrand, K., Richter, K., Arnold, M., Schwarze, F. W. M. R. and Militz, H. 2010. Anatomy of bioincised Norway spruce wood. *International Biodeterioration and Biodegradation* 64: 346-355.

Lehringer, C., Koch, G., Adusumalli, R., Mook, W. M., Richter, K. and Militz, H. 2011a. Effect of *Physisporinus vitreus* on wood properties of Norway spruce. Part 1: Aspects of delignification and surface hardness. *Holzforschung* 65: 711–719.

Lehringer, C., Richter, K., Schwarze, F. W. M. R. and Militz, H. 2009b. A review on promising approaches for liquid permeability improvement in softwoods. *Wood and Fibre Science* 41.4: 373-385.

Lehringer, C., Koch, G., Adusumalli, R., Mook, W. M., Richter, K. and Militz, H. (2011a). Effect of *Physisporinus vitreus* on wood properties of Norway spruce. Part 1: Aspects of delignification and surface hardness. *Holzforschung*, 65: 711–719.

Lehringer, C., Saake, B., Zivkovic, V., Richter, K. and Militz, H. 2011b. Effect of *Physisporinus vitreus* on wood properties of Norway spruce. Part 2: Aspects of microtensile strength and chemical changes. *Holzforschung* 65: 721–727.

Liese, W. and Bauch, J. 1967. On anatomical causes of the refractory behavior of spruce and Douglas-fir. J. Inst. *Wood Sci*. 19: 3-14.

Liese, W. and Pechmann, H. 1959. Untersuchungen über den Einfluß von Moderfäulepilzen auf die Holzfestigkeit. *Forstwissenschaftliches Centralblatt*, 78(9-10): 271-279.

Lihra, T., Cloutier, A. and Zhang, S.Y. 2000. Longitudinal and transverse permeability of Balsam fir wetwood and normal heartwood. *Wood and Fiber Science* 32: 164–178.

Lindgren, R. A. and Harvey, G. M. 1952. Decay control and increased permeability in southern pine sprayed with fluoride solutions. *Forest Products Journal* 2.5: 250-256.

Lv, P., Almeida, G. and Perré, P. (2015). TGA-FTIR analysis of torrefaction of lignocellulosic components (cellulose, xylan, lignin) in isothermal conditions over a wide range of time durations. *BioResources* 10.3: 4239-4251.

Machado, G., Leon, S., Santos, F., Lourega, R., Dullius, J., Mollmann, M. E. and Eichler, P. 2016. Literature review on furfural production from lignocellulosic biomass. *Natural Resources* 7: 115-129.

Mai, C., Kües, U. and Militz, H. 2004. Biotechnology in the wood industry. *Applied Microbiology and Biotechnology* 63.5: 477 – 494.

Maity, S. K. 2015. Opportunities, recent trends and challenges of integrated biorefinery: Part I. *Renewable and Sustainable Energy Reviews* 43: 1427-1445.

Malaka, S. L. O. 1996: Termites in West Africa, University of Lagos Press, Lagos, Nigeria. Pp 165

Malakani, M., Khademieslam, H., Hosseinihashemi, S. K. and Zeinaly, F. 2014. Influence of fungal decay on chemi-mechanical properties of beech wood (*Fagus orientalis*). *Cellulose Chem. Technol.*, 48.1-2: 97-103.

Măluţan, T. H., Nicu, R., Popa, V. I., 2008. Contribution to the study of hydroxymethylation reaction of alkali lignin. *BioResources* 3: 13-20.

Mangindaan, B., Matsushita, Y., Aoki, D., Yagami, S., Kawamura, F. and Fukushima, K. 2017. Analysis of distribution of wood extractives in *Gmelina arborea* by gas chromatography and time-of-flight secondary ion mass spectrometry. *Holzforschung* 71.4: 299–305.

Mantanis, G.I., Young, R.A. Rowell, R.M. 1994. Swelling of wood. Part I: swelling in water. *Wood Science and Technology* 28: 119–134.

Martínez-Iñigo, M. J., Immerzeel, P., Gutierrez, A., Carlos del Río, J. and Sierra-Alvarez, R. 1999. Biodegradability of extractives in sapwood and heartwood from Scots Pine by sapstain and white rot fungi. *Holzforschung* 53: 247–252.

Mattheck, C. and Weber, K. 2003. Manual of wood decays in trees. Aboricultural Association, 127pp.

Militz, H. (1993b) Der Einfluß enzymatischer Behandlungen auf die Tränkbarkeit kleiner Fichtenproben. *Holz Roh Werkst* 51:135–142.

Militz, H. and Homan, W. J. 1993. Vorbehandlung von Fichtenholz mit Chemikalien mit dem Ziel der Verbesserung der Imprägnierbarkeit. *Holz Roh Werkst* 51:14–20.

Mohebby, B. and Sanaei, I 2005. Influences of the hydro-thermal treatment on physical properties of beech wood (*Fagus orientalis*). Paper prepared for 36th Annual Meeting Bangalore, India 24 – 28 April, 2005. IRG/WP 05-40303.

Morrell, J. J. 2011. Resistance of selected wood-based materials to fungal and termite attack in non-soil contact exposures. *Forest Products Journal* 61: 685-687.

Morrell, J. J. and Morris, P. I. 2002. Methods for improving preservative penetration into wood: a review. International Research Group on Wood Preservation (IRG/WP 02-40227), Stockholm.

Morrell, J. J. and Winandy, J. E. 1987. Incising practices used to improve preservative treatment in Western Species: A preliminary survey. *Proc. Am. Wood Preserv. Assoc.*, 83: 400-404.

Morrell, J. J., Gupta, R., Winandy, J. E. and Riyanto, D. S. 1998. Effect of incising and preservative treatment on shear strength of nominal 2-inch lumber. *Wood and Fibre Science* 30.4: 374-381.

Moya, R. and Berrocal, A. 2015. Evaluation of biodeterioration and the dynamic modulus of elasticity of wood in ten fast-growing tropical species in Costa Rica exposed to field testing. *Wood Research* 60.3: 359-374.

Nicholas, D. D. 1977. Chemical methods of improving the permeability of wood. In: Wood Technology: Chemical Aspects; Goldstein, I. (ed.); ACS Symposium Series 43; American Chemical Society, Washington, D.C.; pp. 33-46.

Nicholas, D. D. and Siau, J. F. 1973. Factors influencing treatability. In: Wood deterioration and its prevention by preservative treatments, Vol II: Preservatives and Preservative Systems, D. D. Nicholas (ed). Syracuse University Press, Syracuse, New York. Pp 299-343.

Nicholas, D. D. and Thomas, R. J. 1968a. Influence of steaming on the ultrastructure of bordered pit membrane in loblolly pine. *Forest Products Journal* 18.1: 57-59.

Nicholas, D. D. and Thomas, R. J. 1968b. The influence of enzymes on the structure and permeability of loblolly pine. Proc. Am. Wood-Preservers' Assoc., 64: 1-7.

Nicholas, D.D. 1971. Unpublished. Experimental determination of the effect of dynamic transverse compression on Douglas-fir heartwood.

Nilsson, T. 2009. Biological wood degradation. Pp. 121-144. *In*: M. Ek, G. Gellerstedt, and G. Henriksson (eds.), Pulp and Paper Chemistry and Technology: Wood Chemistry and Wood Biotechnology. Vol. 1. Walter de Gruyter and Co.

Noguchi, M., Nishimoto, K., Imanura, Y., Fujii, Y., Okamura, S. and Miyauchi, T. 1986. Detection of very early stages of decay in western hemlock wood using acoustic emissions. *Forest Products Journal* 36.4: 35-36.

Ofong, A. U. and Pearce, R. B. 1994. Suberin degradation by *Rosellinia desmazieresii*. *European Journal of Forest Pathology* 24: 316–322.

Ogunsanwo, O.Y and Adedeji, G.A. 2010. Effect of bark extract of *Erythrophleum suaveolens* (Guillemin & Perrottet) Brenan on fungal activities in wood of *Triplochiton scleroxylon* K. Schum. *Journal of Environmental Extension* 9: 56-62.

Okon, K. E. 2014. Variations in specific gravity and shrinkage in wood of a 25-year-old *Gmelina arborea* in Oluwa forest reserve, south west Nigeria. *Archives of Applied Science Research* 6.4: 271-276

Olajuyigbe, S. O., Ogunsanwo, O. Y. and Adegeye, A. O. 2010. Compressive Strength in Heartwood Extract of Teak (HWE) Treated Hardwoods After Exposure to White Rot Attack. *International Journal of Biological and Chemical Sciences* 4.3: 571-578.

Oluyege, A. O. 2007. Wood: A Versatile Material for National Development. 45th Inaugural Lecture Series. Delivered at The Federal University of Technology, Akure on 26th June, 2007, pp 19-21.

Otjen, L. and Blanchette, R. A. 1982. Patterns of decay caused by *Inonotus dryophilus* (Aphyllophorales; Hymenochaetaceae), a white-pocket rot fungus of oaks. *Canadian Journal of Botany* 60.12: 2770-2779.

Owoyemi, J. M. 2010. The influence of preservative viscosity on fluid absorption by *Gmelina arborea* wood. *Forests and Forest Products Journal* 3: 32-39.

Owoyemi, J. M. and Kayode, J. O. 2008. Effect of incision on preservative absorption capacity of *Gmelina arborea* wood. *Biotechnology* 7.2: 351-353.

Pandey, K. K. 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR Spectroscopy. *Journal of Applied Polymer Science* 71: 1969-1975.

Pandey, K. K. and Pitman, A. J. 2003. FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *International Biodeterioration and Biodegradation* 52: 151-160.

Panek, M. and Reinprecht, L. 2011. *Bacillus subtilis* for improving spruce wood impregnability. *Bioresources* 6.3: 2912-2931.

Pánek, M., Reinprecht, L. and Mamoňová, M. 2013. *Trichoderma viride* for improving Spruce wood impregnability. *Bioresources* 8.2: 1731-1746.

Panshin, A.J. and deZeeuw C. 1980. Textbook of wood Technology, 4th Edition, McGraw Hill, New York, 722pp.

Parham, R. A. and Gray, R. L. 1984. *Formation and structure of wood*. In: R. M. Rowell and M. Roger, (eds), The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: American Chemical Society. pp 3-56.

Peterson, C. A. and Cowling, E. B. 1973. Influence of various initial moisture contents on decay of Sitka spruce and sweetgum sapwood by *Polyporus versicolor* in the soil-block test. *Phytopathology* 63: 235-237.

Pettersen, R. C. 1984. *The chemical composition of wood*. In: R. M. Rowell and M. Roger, (eds), The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: American Chemical Society. pp 57-126.

Petty, J. A. and Preston, R. D. 1969. The dimensions and number of pit membrane pores in conifer wood. Proc. Ro. Soc. Lond. B172: 137-151.

Popescu, C. M., Popescu, M. C., Singurel, G., Vasile, C., Argyropoulos, D. S. and Willfor, S. (2007). Spectral Characterization of Eucalyptus Wood. *Applied Spectroscopy* 61.11: 1168-1177.

Purushotham, A., Das, N. R., Singh, S., Subrahmanayam, I. V., Shivaramakrishna, V. R., Pillai, S. R. M., Badola, K. C. and Gahlot, H. S. 1967. Natural durability of commercially important timber species and efficacy of wood preservatives on land (Part I). *Journal of the Timber Development Association of India* 13.1: 3-88.

Rahman, K.S., Islam, Md. N., Musa, S.M. and Alam, D.M.N. 2011. Incising as an aid for the preservative treatment of wood - a review. *Recent Patents on Materials Science* 4: 201-208.

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P. F, Marita, J. M, Hatfield, R. D, Ralph, S. A, Christensen, J. H. and Boerjan, W. 2004. Lignins: natural polymers from oxidative coupling of 4-hydrophenylpropanoids. *Phytochemistry Reviews* 3: 29-60.

Rasineni, G. K., Chinnaboina, M. and Reddy, A. R. 2010. Proteomic approach to study leaf proteins in a fast-growing tree species, *Gmelina arborea* Linn. Roxb. *Trees* 24: 129–138.

Raven, P. H., Evert, R. F. And Eichhorn, S. E. 2013. *Biology of Plants*, 8th edn., NY New york: W. H. Freeman/ Palgrave Macmillian. 900pp.

Rayner, A.D.M and Boddy, L. 1988. *Fungal decomposition of wood*. Its biology and ecology. Wiley, Chichester

Reinprecht, L. 2016. *Wood Deterioration, Protection and Maintenance*, First Edition, Chichester, United Kingdom: John Wiley & Sons, Ltd. 357pp.

Richards, D. B. 1954. Physical changes in decaying wood. *Journal of Forestry* 52: 260-265.

Richardson, B. A. 1993. *Wood preservation*. 2nd Edition, London, UK; E & FN Spon. 226pp.

Robinson, S. C., Richter, D. L. and Laks, P. E. 2007. Colonization of sugar maple by spalting fungi. *Forest Products Journal* 57.4: 24–32.

Rosner, B., Messner, K., Tucker, E. and Bruce, A. 1998. Improved preservative penetration of spruce after pre-treatment with selected fungi. In: Fungal pre-

treatment of pole sections. IRG Document No.: IRG/WP/98-40117. The International Research Group on Wood Preservation, Stockholm, Sweden. pp1 – 14

Rowell, R. M. 2005. Handbook of Wood Chemistry and Wood Composites. CRC Press, Florida, USA: 487pp.

Rowell, R. M. (Ed.) 1984. *Penetration and reactivity of cell wall components*. In: The chemistry of solid wood; Advances in chemistry, vol. 207, Washington, DC: American Chemical Society. Pp 173-210.

Rudman, P. 1965. Studies in wood preservation. Part 1. The penetration of liquids into eucalypt sapwoods. *Holzforschung* 19.1: 5-13.

Samuel, J. R. 2004. The Mechanical Properties of Wood. Project Gutenberg Ebook

Scheffer T. C. 1973. Microbiological degradation and its casual organisms. In: Nicholas, D. D. (Ed.). Wood deterioration and its prevention treatments: Degradation and protection of wood. Syracuse: Syracuse University, Volume 2, pp 31-106.

Scheffer, T. C. 1936. Progressive effects *of Polyporus versicolor* on the physical and chemical properties of Sweet-gum sapwood. U.S.D.A. Technical Bulletin No. 527, Washington, D.C.

Scheffer, T. C. 1986. O_2 requirements for growth and survival of wood-decaying and sapwood-staining fungi. *Canadian Journal of Botany* 64: 1957-1963.

Scheffer, T. C. and Cowling, E. B. 1966. Natural resistance of wood to microbial deterioration. *Annual Review of Phytopathology* 4: 147-170.

Scheffer, T. C. and Morrell, J. J. (1998). Natural durability of wood: A worldwide checklist of species. Forest Research Laboratory, Oregon State University. Research Contribution 22. 58p.

Schmidt, O. and Liese, W. 1994. Occurrence and significance of bacteria in wood. *Holzforschung* 48: 271–277.

Schubert, M., Dengler, V., Mourad, S. and Schwarze, F. W. M. R. 2009. Determination of optimal growth parameters for the bioincising fungus *Physisporinus vitreus* by means of response surface methodology. *Journal of Applied Microbiology* 106: 1734-1742.

Schubert, M., Volkmer, T., Lehringer, C. and Schwarze, F.W.M.R. 2011. Resistance of bioincised wood treated with wood preservatives to blue-stain and wood-decay fungi. *International Biodeterioration & Biodegradation* 65.1:108-115.

Schultz, T.P., Nicholas, D.D. 2000. Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. *Phytochemistry* 54: 47-52.

Schwarze, F., Engels, J. 1998. Cavity formation and the exposure of peculiar structures in the secondary wall (S_2) of tracheids and fibres by wood degrading basidiomycetes. *Holzforschung* 52: 117-123.

Schwarze F., Lonsdale D., Mattheck C. 1995. Detectability of wood decay caused by *Ustulina deusta* in comparison with other tree-decay fungi. *European Journal of Forest Pathology* 25: 327-341.

Schwarze, F.W.M.R and Baum, S. 2000. Mechanisms of reaction zone penetration by decay fungi in wood of beech (*Fagus sylvatica*). *New phytologist* 146: 129-140.

Schwarze, F.W.M.R., Baum, S. and Fink, S. 2000a. Dual modes of degradation by *Fistulina hepatica* in xylem cell walls of *Quercus robur*. *Mycological Research* 104: 846-852

Schwarze FWMR, Engels J. and Mattheck, C. 2004. Fungal strategies of wood decay in trees. Springer, Heidelberg, 185pp.

Schwarze, F.M.W.R. and Fink, S. 1998. Host and cell type affect the mode of degradation by *Meripilus giganteus*. *New Phytologists* 139: 721-731.

Schwarze, F.W.M. R. 2007. Wood decay under the microscope. *Fungal Biology Reviews* 21: 133-170.

Schwarze, F.W.M.R. 2008. Procedure and composition for the improvement of the uptake and distribution of soaking compositions in woods. EP1681145, Empa - Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, 8600 Dübendorf, CH.

Schwarze, F. W. M. R. and Landmesser, H. 2000. Preferential Degradation of Pit Membranes within tracheids by the basidiomycete *Physisporinus vitreus*. *Holzforschung* 54.4: 461-462.

Schwarze, F.W.M.R., Landmesser, H., Zgraggen, B. and Heeb, M. 2006. Permeability changes in heartwood of *Picea abies* and *Abies alba* induced by incubation with *Physisporinus vitreus*. *Holzforschung* 60: 450-454.

Schwarze, F.W.M.R., Spycher, M. and Fink, S. 2008. Superior wood for violins - Wood decay fungi as a substitute for cold climate. *New Phytologist* 179.4: 1095-104.

Schwarze, F.W.M.R. and Schubert, M. 2009. Enhanced uptake of wood modification agents in ‘bioincised’ wood. The International Research Group on Wood Protection, Paper prepared for the 40th Annual Meeting Beijing, China 24-28 May 2009. IRG/WP 09-40445.

Shahzadi, T., Mehmood, S., Irshad, M., Anwar, Z., Afroz, A., Zeeshan, N., Rashid, U. and Sughra, K. 2014. Advances in lignocellulosic biotechnology: A brief review

on lignocellulosic biomass and cellulases. *Advances in Bioscience and Biotechnology* 5: 246-251.

Shang, J., Yan, S. and Wang, Q. 2013. Degradation mechanism and chemical component changes in *Betula platyphylla* wood by wood-rot fungi. *Bioresources* 8.4: 6066-6077

Shigo, A. L. 1984. Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* 22: 189–214

Shmulsky, R. and Jones, P. 2011. Durability and Protection. *In*: Forest Products and Wood Science: An Introduction, 6th ed. Pp.229-252.

Shrivastava, M. B. 1997: Wood Technology. Vikas Publishing House PVT LTD New Delhi 181pp.

Shupe, T. S., Lebow, S. T. and Ring, D. 2008. Causes and control of wood decay, degradation and stain. Res. & Ext. Pub, no. 2703, Zachary, LA, Louisiana State University Agricultural Center. 27pp.

Siau, J. F. 1984. *Transport processes in wood*. Springer Verlag, Heidelberg, Germany, 245p.

Siau, J. F. 1995. *Wood: Influence of moisture on physical properties*. Dept. of Wood Science and Forest Products, Virgnia Polytechnic Institute and State University, USA: 227pp.

Silvester, F. D. 1967. Timber: Its mechanical properties and factors affecting its use. 1st Edition, Oxford: Pergamon Press. 152pp.

Sint, K. M., Militz, H., Hapla, F. and Adamopoulos, S. 2011. Treatability and penetration indices of four lesser-used Myanmar hardwoods. *Wood Research* 561: 13-22.

Sjöström, E. 1993. *Wood Chemistry: Fundamentals and Applications*, 2nd edition, Academic Press, San Diego, CA, USA, 293 p.

Sjöström, E. and Westermark, U. 1999. *Chemical composition of wood and pulps: Basic constituents and their distribution*. In: E. Sjöström *et al.* (eds.), Analytical Methods in Wood Chemistry, Pulping, and Papermaking. Springer-Verlag Berlin Heidelberg. Pp 1-19.

Smith, K.T. and Shortle, W.C. 1991. Decay Fungi Increase the Moisture Content of Dried Wood. In: Biodeterioration and Biodegradation, H.W. Rossmoore, ed.). Elsevier Science Publishers, Ltd. 611 pp.

Solo-Gabriele, H. M., Townsend, T. G., and Cai, Y. 2004. Environmental impacts of CCA-treated wood within Florida, USA. Proceedings of the Environmental

Impacts of Preservative-Treated Wood Conference February 8–10, 2004, Orlando, Florida.

Stamm, A. J. (1964). Wood and Cellulose Science. Ronald Press, NewYork.

Sujatha, M. and Venmalar, D. 2019. Anatomical approach to evaluating the treatability class of five species of plantation timbers. *J Indian Acad Wood Sci.*, 16.1: 15–21

Sulaiman, A. and Lim, S. C. 1989. Some timber characteristics of *Gmelina arborea* grown in a plantation in peninsular Malaysia. *Journal of Tropical Forest Science* 2.2: 135-141.

Talboys, P. W. 1964. A concept of the host-parasite relationship in Verticillium wilt diseases. *Nature* 361: 361–364.

Tarmian, A. and Perre, P. 2009. Air permeability in longitudinal and radial directions of compression wood of Picea abies L. and tension wood of Fagus sylvatica L. *Holzforschung* 63: 352-356.

Teesdale, C. H. and Maclean, J. D. 1918. Relative resistance of various hardwoods to injection with creosote. USDA Bull. No. 606. 36pp.

Telmo, C. and Lousada, J. 2011. The explained variation by lignin and extractive contents on higher heating value of wood. *Biomass and Bioenergy* 35: 1663-1667.

Thaler, N., Lesar, B., Kariz, M. and Humar, M. 2012. Bioincising of Norway spruce wood using wood inhabiting fungi. *International Biodeterioration & Biodegradation* 68: 51-55.

Theden, G. 1941. Investigation on the moisture requirements ofmost important wood-destroying fungi occurring in buildings (In German). Dissertation. Friedrich Wilhelms-University, Berlin. *Ang. Bot.* 23: 189–253.

Thomas, R. J. 1976. Anatomical features affecting liquid penetrability in three hardwood species. *Wood and Fibre* 74: 256-263.

Thomas, R. J. 1981. Wood anatomy and ultrastructure. In: Wood – its structure and properties, Wangaard, F. F. (ed.), USDA Forest Product Laboratory and The University of Wisconsin-Extension, Pennysylvania State University, University Park, PA 16802.

Thomas, R. J. and Kringstad, K. P. 1971. The role of hydrogen bonding in pit aspiration. *Holzforschung* 25.5: 143-149.

Thomas, R. J. and Nicholas, D. D. 1966. Pit membrane structure in loblolly pine influenced by solvent exchange drying. *Forest products Journal* 16.3: 57-59.

Thomasson, G., Capizzi, J., Morrell, J. and Miller, D. 2006. Wood preservation and wood products treatment – Training manual. Extension service, Oregon State University. Retrieved from https://ir.library.oregonstate.edu/downloads/d791sg46f. Accessed on 24th Dec, 2018. 22pp.

Tijani, M. 2007. Society and sustainable management of natural resources. Proceedings of International Conference on Forest, Society and Sustainable Development, Cassablanca, Morocco, 19th - 21st March, 2007.

Timell, T. E. 1982. Recent progress in the chemistry and topochemistry of compression wood. *Wood Science and Technology* 16.2: 83-122.

Timings, R.L. 1991: Engineering Materials, Vol. l, Longmann Scientific and Technical Limited, UK.

Trade Investment Nigeria 2013: Forestry and Paper. Available at www.tradeinvestmentnigeria.com/pls/coms/Ti-SECOUT-dev?p/. Accessed 4/20/2013.

Tripathi, S. 2012. Treatability evaluation of meranti with ZiBOC and CCA. *International Wood Products Journal* 3.2: 70-76.

Tschernitz, J. L. 1973. Enzyme mixture improves creosote treatment of kiln-dried Rocky mountain Douglas-fir. *Forest Products Journal* 23.3: 30-38.

Tsoumis, G. 1991: Science and Technology of Wood: Structure, Properties and Utilization. Van Nostrand Reinhold; New York. 494pp.

Unanaonwi, E. 2015. Sustainable utilization of forest resources: A step to sustainable agriculture. *Journal of Agriculture and Ecology Research International* 2.3: 196-200.

Unligil, H. H. 1971. Permeability of white spruce after water storage. J. Inst. Wood Sci., 5.6: 30-35.

UN-REDD 2013: The State of Nigeria's Forest. Available at www.unredd.org/about UNREDD programme/National Programme/Nigeria/tabid/199/default.aspx). Accessed 4/01/2013.

Usta, I. and Hale, M. D. 2006. Comparison of the bordered pits of two species of spruce (Pinaceae) in a green and kiln-dried condition and their effects on fluid flow in the stem wood in relation to wood preservation. *Forestry* 794: 467-475.

Venmalar, D. and Nagaveni, H.C. 2005. Evaluation of copperized cashew nut shell liquid and neem oil as wood preservatives. Paper prepared for the 36th Annual Meeting Bangalore, India 24 - 28 April, 2005. IRG/WP 05-30368.

Wakefield K.E. 1957. Determination of the strength properties and physical

characteristics of Canadian woods, Forest Branch Bulletin No. 119. Canada.

Wang, J. Z. and DeGroot, R. 1996. Treatability and Durability of Heartwood. In: Ritter, M.A.; Duwadi, S.R.; Lee, P.D.H., ed(s). National conference on wood transportation structures; 1996 October 23-25; Madison, WI. Gen. Tech. Rep. FPL- GTR-94. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. Pp 252 – 260.

Watanabe. T., Ohnishi, Y., Kaizu, Y. S. and Koshijima, T. 1989. Binding site analysis of the ether linkages between lignin and hemicelluloses in lignin-carbohydrate complexes by DQD-oxidation. *Agric. Biol. Chem.* 52: 2233-2252.

Whetten, R. and Seredoff, R. 1995. Lignin biosynthesis. *The Plant Cell* 7: 1001-1013.

Wiemann, M. C. and Green, D. W. 2007. Estimation of Janka hardness from specific gravity for tropical and temperate species. United States Department of Agriculture, Forest Service, Forest Products Laboratory, research paper FPL-RP-643. 21pp.

Wilcox, W.W. 1978. Review of literature on the effects of early stages of decay on wood strength. *Wood and Fibre* 9.4: 252–257.

Winandy, J. E. and Morrell, J. J. 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. *Wood and Fibre Science*, 25.3: 278-288.

Winandy, J. E. and Rowell, R. M. 1984. *The chemistry of wood strength*. In: The chemistry of solid wood. Rowell, R. M. (Ed). Advances in chemistry, vol. 207, Washington, DC: American Chemical Society. Pp 211-255.

Windeisen, E., Strobel, C. and Wegener, G. 2007. Chemical changes during the production of thermo-treated beech wood. *Wood Science and Technology* 41: 523-536.

Witec, 2007. Confocal Raman Microscopy - Characterization of Wood Cells and Cellulose Fibers. Witec Application note retrieved on 26th December, 2014 from *http://www.witec.de/assets/Literature/Files/WITec-AppNote-Wood-Raman.pdf*

Wong, A. H. H. and Ling, W. C. 2009. Natural durability variations of Malaysian timber from Sarawak after 26 years exposure by stake test. International Research Group on Wood Protection Document No: IRG/WP 09-10704, 26pp.

Wong, A. H. H., Kim, Y. S, Singh, A. P and Ling, W. C. 2005. Natural Durability of Tropical Species with Emphasis on Malaysian Hardwoods – Variations and Prospects. *The International Research Group on Wood Preservation. Doc No. IRG/WP/05-10568.* Paper Prepared for the 36th Annual Meeting Bangalore, India, 24-28 April 2005.

Wong, A. H. H., Wilkes, J. and Heather, W. A. 1983. Influence of wood density and extractives content on the decay resistance of the heartwood of *Eucalyptus delegatensis* R. T Baker. *Journal of the Institute of Wood Science* 9: 261- 263.

Xu, G. and Goodell, B. 2001. Mechanisms of wood degradation by brown-rot fungi: chelator-mediated cellulose degradation and binding of iron by cellulose. *Journal of Biotechnology* 87: 43-57.

Yamada, T. 2001. Defense mechanisms in the sapwood of living trees against microbial infection. *Journal of Forest Research* 6: 127–137.

Yamamoto, K. (1982). Yearly and seasonal process of maturation of parenchyma cells in Pinus species. Res Bull Exp. *For Hokkaido Univ*. 39: 245-296.

Yildiz, S. Canakci, S., Yildiz, U. C., Ozgenc, O. and Tomak, E. D. 2012. Improving of the impregnability of refractory spruce wood by *Bacillus licheniformis* pretreatment. *Bioresources* 7.1: 565-577.

Zabel, R. A. and Morrell, J. J. 1992. *Wood microbiology: Decay and its prevention*. United Kingdom: Academic Press Ltd. 476pp.

Zimmermann, T., Pöhler, E. and Geiger, T. 2004. Cellulose fibrils for polymer reinforcement. *Advanced Engineering Materials* 6: 754-761.