Faculty of Resource Science and Technology

BIODEGRADATION, EFFICACY, LEACHABILITY AND STRENGTH PROPERTIES OF ORGANOTIN(IV)-TREATED *HEVEA BRASILIENSIS, ALSTONIA SCHOLARIS* AND *MACARANGA TRILOBA* WOOD

Md. Masudur Rahman

Doctor of Philosophy

(Plant Science)

2014

Faculty of Resource Science and Technology

BIODEGRADATION, EFFICACY, LEACHABILITY AND STRENGTH PROPERTIES OF ORGANOTIN(IV)-TREATED *HEVEA BRASILIENSIS, ALSTONIA SCHOLARIS* AND *MACARANGA TRILOBA* WOOD

Md. Masudur Rahman

Doctor of Philosophy

(Plant Science)

2014

BIODEGRADATION, EFFICACY, LEACHABILITY AND STRENGTH PROPERTIES OF ORGANOTIN(IV)-TREATED *HEVEA BRASILIENSIS, ALSTONIA SCHOLARIS* AND *MACARANGA TRILOBA* WOOD

MD. MASUDUR RAHMAN

A thesis submitted
in fulfillment of the requirement for the degree of

Doctor of Philosophy

(Plant Science)

Department of Plant Science and Environmental Ecology
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2014

AUTHOR'S DECLARATION

This is to certify that the dissertation work entitled "Biodegradation, Efficacy, Leachability and Strength Properties of Organotin(IV)-Treated *Hevea brasiliensis, Alstonia scholaris* and *Macaranga triloba* Wood" has been done by the candidate himself and does not contain any material extracted from elsewhere or from a work published by anybody else. The work for this dissertation has not been submitted elsewhere by the author for any degree or diploma.

……..

Md. Masudur Rahman
Matric Number: 10011486
Dept. of Plant Science and Environmental Ecology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ACKNOWLEDGEMENTS

Alhamdulillah, all praises for the most omnipotent, omnipresent and omniscient Almighty Allah S.W.T, Who blessed me to accomplish my doctoral research work. All respect for his last Holy Prophet (Peace be upon him), whose teaching inspired me to widen my thoughts and deliberate the things deeply.

I would like express my ardent spirit of gratitude, profound regard and indebtedness to my respected supervisor Assoc. Prof. Dr. Ismail Jusoh for his constant guidance, dedication, untiring efforts, active encouragement and inspiration at all stages during the entire period of this study. I wish to greatly appreciate to my co-supervisors, Assoc. Prof. Dr. Awang Ahmad Sallehin Awang Husaini of Department of Molecular Biology, Faculty of Resource Science and Technology, UNIMAS and Dr. Idris Abu Seman of the Malaysian Palm Oil Board (MPOB) for their thoughtful criticism, suggestions, financial support and encouragement throughout this work.

I also want to express my special thank to Prof. Dr. Sinin Hamdan, Faculty of Engineering, UNIMAS for his laboratory support. I am extremely thankful to Assoc. Prof. Dr. Abu Affan for his immense help, cooperation, guidance and assistance in experiments.

A special thanks to my laboratory fellows for their assistance and nice company. I am greatly honoured to mention the nice cooperation of all employees and laboratory assistant of the Department of Plant Science and Environmental Ecology. I would like to thank the Universiti Malaysia Sarawak for financial support under the Principal Investigator Fund (DPI) E14052 F07 49 791/2011(01). I am also thankful to the MPOB for their support which made this research possible.

Last but not least, my heartfelt gratitude and appreciation goes to my parents, my wife Israt Jahan Sharmeen and my pretty daughter Nazrat Maisharah, without their encouragement and prayers, I would have not been able to complete this tough task. They suffered and missed me a lot through my studies.

Md. Masudur Rahman

2014

ABSTRACT

Organotin compounds have been known to possess biological activities and had been used as wood preservatives. Safety and environmental issues limit the use of tributyltin oxide (TBTO) and tributyltin naphthenate (TBTN) wood preservatives to aboveground and industrial applications only. Interest in monosubstituted and disubstituted organotin(IV) compounds is increasing due to their interesting structural features and biocidal properties. The specific objectives of this study were to determine the efficacy of newly synthesized monosubstituted and disubstituted organotin(IV) compounds against wood decay fungi, microdistribution of tin in treated wood cells, enzymes involved in wood biodegradation, and mechanical properties of organotin(IV)-treated woods. Three non-durable tropical wood species namely *Alstonia scholaris* (pulai), *Macaranga triloba* (mahang) and *Hevea brasiliensis* (rubberwood) were chemically treated with two monosubstituted and three disubstituted newly synthesized organotin(IV) complexes using full-cell treatment method. Ten 19 mm x 19 mm x 19 mm sized wood cubes of each species were treated with three levels of concentration (0.1, 0.5 and 1%) of monomethyltin(IV) (MMT) and monophenyltin(IV) (MPT) of the monosubstituted organotin(IV), and dimethyltin(IV) (DMT), diphenyltin(IV) (DPT) and dibutyltin(IV) (DBT) of the disubstituted organotin(IV). Chemical retentions were determined following treatment and the threshold value was determined based on the soil block test results. Chemical bonding in wood cells and microdistribution of organotin(IV) were determined using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscope-energy dispersive X-ray (SEM-EDX) analyses, respectively. Enzyme bioassays were carried out to determine the lignolytic enzyme activities of fungi involved in wood biodegradation. Results showed that all the selected woods are treatable with the newly synthesized organotin(IV) complexes with retention at

10.59 kg m^{-3} was achieved with *A. scholaris* which was treated with 1% DMT. FTIR spectra and SEM-EDX analyses revealed that organotin(IV) complexes bind with wood and tin was present in fibre cell wall, respectively suggesting that the organotin(IV) complexes are capable of penetrating the cell wall. Leaching test results showed no tin was release after 270 hours. The strength properties were not affected following organotin(IV) complexes except for samples treated with DMT. Enzyme bioassays indicated that MnP was most likely to be the predominating enzymes causing lignin degradation in *A. scholaris, M. triloba* and *H. brasiliensis* wood cubes and enzymes activities were reduced in treated wood. Results showed that the best protection against decay fungi was provided by dibutyltin(IV) complex followed by diphenyltin(IV), dimethyltin(IV), monophenyltin(IV) and monomethyltin(IV) complexes. This study showed that the newly synthesized organotin(IV) complexes are effective against *T. versicolor* and *G. trabeum* and disubstituted organotin(IV) provide better protection than monosubstituted organotin(IV) to *A. scholaris, M. triloba* and *H. brasiliensis*. However further studies such as treating a wide range of wood species, exposing to other wood decay fungi and field tests are necessary to evaluate the true potential of these newly synthesized organotin(IV) complexes.

Biodegradasi, efikasi, kebolehlarutresapan dan ciri-ciri kekuatan kayu *Hevea brasiliensis, Alstonia scholaris* dan *Macaranga triloba* yang telah dirawata oleh organotimah(IV)

ABSTRAK

Sebatian organotimah diketahui mempunyai aktiviti biologi dan telah pun digunakan sebagai bahan pengawet kayu. Walau bagaimanapun isu-isu keselamatan dan persekitaran telah menghadkan penggunaan pengawet kayu tributiltin oksida (TBTO) dan tributiltin naftenat (TBTN) untuk kegunaan atas tanah dan industri. Minat terhadap sebatian-sebatian ekatukar ganti dan dwitukar ganti organotimah(IV) meningkat kerana ciri-ciri strukturnya yang menarik, sifat-sifat biosidnya dan ianya mesra alam. Objektif-objektif kajian ini adalah untuk menentukan efikasi sebatian-sebatian ekatukar ganti dan dwitukar ganti organotimah(IV) yang telah disintesis terhadap kulat pereput kayu, agihan mikro timah dalam sel-sel kayu terawat, enzim-enzim yang terlibat dalam biodegradasi kayu, dan sifat-sifat fisikal dan mekanikal kayu yang telah dirawat dengan organotimah(IV). Tiga spesies tropika tidak tahan reput iaitu *Alstonia scholaris* (pulai), *Macaranga triloba* (mahang) dan *Hevea brasiliensis* (getah) telah dirawat secara kimia dengan dua sebatian ekatukar ganti dan tiga sebatian dwitukar ganti organotimah(IV) menggunakan kaedah rawatan penuh-sel. Sepuluh kiub kayu bersaiz 19 mm x 19 mm x19 mm dari setiap spesies telah dirawat dengan tiga tahap kepekatan (0.1, 0.5 dan 1%) monometiltin(IV) (MMT) dan monofeniltin(IV) (MPT) daripada ekatukar ganti sementara sebatian-sebatian dwimetiltin(IV) (DMT), difeniltin(IV) (DPT) dan dwibutiltin(IV) (DBT) daripada sebatian dwitukar ganti organotimah(IV). Retensi kimia ditentukan selepas rawatan dan nilai ambang kimia ditentukan berdasarkan keputusan ujian blok tanah. Ikatan kimia yang terbentuk ditentukan oleh spektroskopi inframerah transformasi Fourier (FTIR) dan analisis mikroskop elektron imbasan-

penyebaran tenaga sinar-X (SEM-EDX). Bioasai enzim dijalankan untuk menentukan aktiviti-aktiviti enzim lignolitik kulat yang terlibat dengan biodegradasi kayu. Keputusan menunjukkan semua kayu yang diuji boleh dirawat dengan sebatian organotimah(IV) di mana retensi 10.59 kg m^{-3} dicapai oleh *A. scholaris* setelah dirawat dengan 1% DMT. Spektra FTIR dan analisis SEM-EDX masing-masingnya mengambarkan sebatian organotimah(IV) membentuk ikatan dengan kayu dan timah terdapat dalam dinding sel gentian kayu, menunjukkan sebatian tersebut telah menembusi dinding sel. Keputusan ujian melarut lesap menunjukkan tiada timah larut lesap setelah 270 jam. Sifat-sifat kekuatan tidak terjejas kecuali sampel kayu yang dirawat oleh DMT. Bioasei enzim menunjukkan enzim MnP berkemungkinan menguasai degradasi lignin dalam kayu *A. scholaris*, *M. triloba* and *H. brasiliensis* dan aktiviti enzim adalah rendah dalam kayu yang dirawat. Keputusan menunjukkan perlindungan terhadap kulat pereput kayu yang terbaik adalah rawatan dari sebatian dwibutiltin(IV) diikuti oleh dwifeniltin(IV), dwimetiltin(IV), monofeniltin(IV) dan monometiltin(IV). Kajian ini telah menunjukkan sebatian organotimah(IV) yang disintesis adalah berkesan terhadap kulat pereput kayu *T. versicolor* and *G. trabeum* dan dwitukar ganti organotimah(IV) menghasilkan perlindungan lebih baik dari ekatukar ganti organotimah(IV). Walau bagaimanapun kajian lanjut perlu dijalankan seperti merawat pelbagai spesies kayu, mendedahkan kayu yang telah dirawat kepada kulat pereput kayu yang lain dan menjalankan ujian lapangan supaya potensi sebenar sebatian organotimah(IV) yang disintesis ini untuk bahan pengawet kayu dapat dinilai.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

%	:	Percentage
% g/g	:	Percentage gramme per gramme
°C	:	Degree Celcius
µl	:	Microlitre
APCT	:	2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone
Bu	:	Butyl
C	:	Concentration
CCA	:	Chromated copper arsenate
Cl	:	Chlorine
cm^{-1}	:	Per centimetre (wavenumber)
D	:	Density
DBT	:	Disubstituted butyltin(IV) complex
DMSO	:	Dimethylsulphoxide
DMT	:	Disubstituted methyltin(IV) complex
DPT	:	Disubstituted phenyltin(IV) complex
EDXA	:	Energy dispersive X-ray analysis
FRIM	:	Forest Research Institute of Malaysia
FTIR	:	Fourier Transform Infrared
g	:	Gramme
Hg	:	Mercury
hrs	:	Hours
KBR	:	Potassium Bromide
kg	:	Kilogramme
L	:	Litre

Lac	:	Laccase
LiP	:	Lignin peroxidase
LOSP	:	Light Organic Solvent Preservatives
MC	:	Moisture content
Me	:	Methyl
MEA	:	Malt extract agar
MEB	:	Malt extract broth
mg	:	Milligramme
min	:	Minute
mm	:	Millimetre
mM	:	Millimolar
MMT	:	Monosubstituted methyltin(IV) complex
MnP	:	Manganese peroxidase
MOE	:	Modulus of elasticity
MOR	:	Modulus of rupture
MPT	:	Monosubstituted phenyltin(IV) complex
nm	:	nanometre
PCP	:	Pentachlorophenol
Ph	:	Phenyl
psi	:	Pounds per square inch
rpm	:	Revolutions per minute
SEM	:	Scanning electron microscopy
Sn	:	Tin
TBTN	:	Tributyltin nephthenate
TBTO	:	Tributyltin oxide
U	:	Unit

V	:	Volume
VA	:	Veratryl alcohol
W	:	Weight
WL	:	Weight loss
ν	:	Nu

CHAPTER ONE

INTRODUCTION

1.1 General background

Wood is one of the most attractive materials because of its complex structure and wide range of applications in the world. It is a complex cellular material of biological origin made up mainly of cellulose, hemicelluloses and lignin (Bowyer *et al.*, 2003). These three polymeric cell wall components are the main factors influencing wood properties. The physical, mechanical and chemical properties of wood can be changed by changing these three cell wall component (Rowell, 2005). Some wood species are naturally more durable which are preferred building and construction materials due to their physical, mechanical and aesthetically pleasing performance. But most of tropical wood species are non-durable or less durable which limit their use to indoor and outdoor applications (Chao and Lee, 2003; Brelid *et al.*, 2000).

The wood in living trees and also in used products would start to decay and decompose with the attack of organisms, hence termed as wood biodegradation. Biodegradation is one of the major economic losses to any biological products. Wood products are subjected to various bio-hazard attacks if preventive measures are not taken. Several types of biodegradation have been recognized in wood, mainly due to fungal decay, wood destroying termites, bacterial degradation and insect attack (Stirling, 2009; Salmiah *et al.*, 2007; Wong *et al.*, 2005). Among all types of degradation fungal decay is one of the most important and widespread type of degradation (Schwarze *et al.*, 2000; Zabel and Morrell, 1992). Fungi use various processes to degrade woody tissues resulting in three forms of decay namely brown-rot, white-rot and soft-rot (Eriksson *et al.*, 1990). Decay fungi caused

significant softening and weakening of wood which ultimately results changes in physical, chemical and mechanical properties of wood (Bowyer *et al.*, 2003).

Wood especially the less durable tropical species such as *Alstonia scholaris* (pulai), *Macaranga triloba* (mahang) and *Hevea brasiliensis* (rubberwood) must be treated with preservatives in order to protect wood from fungi and insects attack. In addition, to provide a long economic service life of the wood for many end uses, preservative treatment indirectly contributes to conservation of forest resource (Eaton and Hale, 1993).

Most of the conventional preservatives causing environmental pollution and a few of them are hazardous to animals and human beings (Onuorah, 2000). The toxicity of the conventional water based wood preservative chromated copper arsenate (CCA) is higher prior to impregnation. Thus CCA presents a high risk for workers exposed to liquid solutions (Eaton and Hale, 1993). Environmental and health concerns with the use of CCA, including possible arsenic exposure to humans have resulted in its use being significantly restricted or limited (Pohleven *et al*., 2002). Creosote and creosote solution are also among the oldest and most used tar oil preservatives (Richardson, 1993). Though it is toxic to wood destroying organisms and makes treated wood more resistant, but it cannot be used indoors because their vapours are sensitive to plants, animals, humans and treated wood cannot be painted (Bowyer *et al.*, 2003). Creosote raise a bad smell and black oily liquid which can cause pollution of environment such as water pollution, air pollution and take many years to break down once it is in contact to the ground water (Eaton and Hale, 1993). Its bad smell can cause bad effect to human respiration system resulted badly effect human health. When creosote solution flow into the water such as river or sea, water surface is covered with black creosote layer, also

preventing respiration of fish and algae make its use almost eliminated (Eaton and Hale, 1993).

Organotin(IV) compounds are chemical compounds based on tin with hydrocarbon substituent. Trialkyltin compounds like TBTO (tri-n-butyltin oxide) and TBTN (tri-n-butyltin naphthanate) are both trialkyltin compounds and are used as fungicides worldwide (Schweinfurth *et al*., 1991). Both of these compounds are liquid form at room temperature. These compounds are most effective against wood decay fungi. Schweinfurth *et al*. (1991) observed that the undiluted active ingredient of TBTO was found to be severely irritating to the skin of rabbit and human. The application of the compounds onto the skin of human showed severe reddening and slight swelling. This shows the danger in using these trialkyltin compounds as fungicides. The precautions and safety of usage cannot be guaranteed and strict supervision is needed if both these compounds were to be used. Although it is very effective organotin to treat wood but there have also several disadvantages. Therefore, TBTO is recommended only for aboveground use, such as mill work. It has been used as a marine antifoulant, but this use has been almost eliminated because of the environmental impact of tin on shellfish. However, organotin(IV) continues to be of interest due to their bioactivities potentials.

This project was carried out in three parts. The first part of this study was to look into the efficacy of selected organotin(IV) compounds in protecting *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood. Specifically this study investigated the efficacy of newly synthesized organotin(IV) compounds against decay fungi. The second part of this study examined the microdistribution of organotin in treated-wood cells. This part of the study examined whether the organotin(IV) compounds have penetrated into the wood cells

following treatment. The third part of this study was to investigate the activities of lignolytic enzymes involved in wood biodegradation.

1.2 Problem statement

The consumption of wood has been rapidly increasing year by year due to population increase. In contrast, however, the production of wood has been drastically decreasing. Due to this reason there exists an imbalance between demand and supply of forest product (Tolunay *et al.*, 2008). This has driven researchers to look for alternative low-quality resources, such as non-durable tropical wood, for value-added applications (Cai *et al.*, 2007; Deka and Saikia, 2000). In addition, the declining supplies and rising costs of the durable woods or heavy hardwoods, has created interest in the utilization of lower grade woods such as non-durable or less durable tropical woods, whose usage can be extended by applying proper wood preservatives (Oluwafemi and Adegbenga, 2007; Kazemi, 2007; Ayer *et al.*, 2003; Chao and Lee, 2003). One of the most effective ways is to apply suitable wood preservatives needed to improve low-quality resources in order to meet end-use requirements (Wang *et al.*, 2007; Zhang *et al.*, 2006). In addition, preservatives treatment can improve low-quality wood properties, sometimes making them ever better than hardwoods (Schneider, 1994).

Huge non-durable tropical wood species are abundantly available in Southeast Asia (Deka *et al.*, 2002; Yalinkilic *et al.*, 1999). In Malaysia, especially Sarawak has the third biggest rainforest in the world. Several tropical wood species are abundantly available in Sarawak but they have not been sufficiently developed and utilized. Therefore, satisfactory use of these types of woods depends on proper treatment using proper preservatives. Besides there is now an increased awareness of the hazards associated with the production and application of wood treatment chemicals and the disposal of treated wood and unused

solutions (Eaton and Hale, 1993). For this reason, it is necessary to search for new preservatives which are environmentally friendly and safe to use.

Currently there is no alternative except preservative treatment to increase the service life of many non-durable or less durable and highly susceptible woods. On the basis of this ground, non-durable or less durable wood species must be treated with proper preservatives to extend their service life. The chemistry of organotin(IV) compounds continues to be of interest due to their interesting structural features and also because of their potentials as agricultural biocides, antitumor agents and other biological activities which are currently being investigated by many researchers (Singh and Kaushik, 2008; Benetollo *et al.*, 2005). In recent years, organotin(IV) compounds have been used extensively as agrochemical fungicides, biocides and antifouling agents (Hanif *et al.*, 2010). Therefore, the current study includes preservatives treatment using selected newly synthesized organotin(IV) compounds of selected non-durable tropical wood species using full-cell treatment method.

1.3 Objectives of study

The general aim of this study was to investigate the efficacy of mono- and disubstituted organotin(IV) compounds against wood decay fungi to protect non-durable tropical wood.

The specific objectives of this study were -

i) To determine the efficacy of newly synthesized mono- and disubstituted organotin(IV) compounds against wood decay fungi.

ii) To determine the microdistribution of organotin(IV) compound in the wood cells of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* following treatment.

iii) To determine the leaching rate of 1% organotin(IV)-treated woods.

iv) To determine the enzymes involved in wood biodegradation, and

v) To determine the mechanical properties of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood species following treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biodegradation of wood

Biodegradation can be defined as any undesirable changes in the properties of a nonliving material caused by the activities of living organisms (Zabel and Morrell, 1992). The major processes involved are assimilation, mechanical damage, corrosion of metal and function impairments. It is also the ability of the heartwood of a timber to resist decay or the inherent resistance of timber against wood destroying fungi or insects (EN 350-1, 1993). Decay resistance of wood is commonly attributed to the fungi toxic extraneous materials or extractives deposited during heartwood formation (Eaton and Hale, 1993; Fengel and Wegener, 1983).

Natural durability or natural decay resistance is an important property of wood. It is the ability of wood to resist decay by wood destroying fungi. The presence of the toxic extractives in the wood is reported to be responsible for the natural durability of wood (Desch and Diwoodie, 1996; Schultz and Nicholas, 2000; Taylor *et al*., 2002). Other factors such as lignin type and quality (Zabel and Morrell, 1992), wood permeability, nitrogen content, presence and observe of mineral deposits and rate of growth of wood (Singh *et al*., 1992). Wood extractives are deposited in the heartwood. Sapwood of all the species contains little or no toxic extractives and is considered to be highly susceptible to decay (Vrenon, 2000).

Several types of biodegradation have been recognized in wood, namely fungal decay, bacterial degradation and insect attack. Fungi are the main principle cause of wood decay and there are three types of wood decay fungi such as white rot, brown rot and soft rot fungi.

Some decay fungi utilize only cellulose, and change the structure of the lignin slightly and turn it brown. These fungi are therefore referred to as brown rot fungi (Myneni *et al*., 2001; Zabel and Morrell, 1992; Eaton and Hale, 1993). Another group of fungi use both cellulose and lignin as food and as these components are removed from the wood and becomes bleached. Fungi affecting wood in this manner are referred to as white rot fungi (Schwarze *et al*., 2000). Soft rot fungi are a special group of wood destroying organisms that usually attack S_2 portion of cell wall which is at too high moisture content for the ones of wood decay as described previously. Hardwoods are most prone to soft rot attack, but its occurrence is not uncommon in softwoods under the wood at high moisture content (Zabel and Morrell, 1992). Schwarze *et al*. (2000) reported that in the wood decay, wood structures as well as the enzymatic potential of the fungi are very important. Structural differences of anatomical and individual cell wall layers pose differing attractiveness for fungal enzymes to break them down. Biodegradation caused by fungi, bacteria, insects, termites degrade wood by oxidation, hydrolysis, reduction and also by chewing the wood (Rowell *et al*., 1988). Wood is degraded biologically because these organisms have very specific enzyme systems capable of hydrolyzing carbohydrate polymers into digestible unit.

Fungi can produce hyphae and different enzymes that deteriorate the cell wall and cause deterioration whereas the insects, termites chew and feed on the sapwood and heartwood and causes great losses (Behbood, 2003). Timbers are susceptible to rapid deterioration by variety of organisms. Deterioration of wood by decay fungi and insects are the threat to longevity of wood used. Kirk and Cullen (2005) showed that white rot fungi are able to fragment the major structural polymers of wood and other lignocellulosics - lignin, cellulose, and hemi cellulose and further metabolize the fragments. The hyphae of fungi rapidly invade wood cells and lie along the lumen walls where they secrete the enzyme to

depolymerize the hemi cellulose, cellulose and fragmentation of lignin**.** The white rot fungi degrade wood by removing cellulose, hemi cellulose and lignin more or less simultaneously.

Twigs and branches of the crown of trees are sapwood, and heartwood is located at the center of tree trunks which in many species are darker in colour and less degradable compared to sapwood (Ross, 2002). Zabel and Morrell (1992) reported that some wood is more resistance to fungal deterioration because of the characteristics constituents in wood cell wall. Eaton and Hale (1993) observed that the heartwood of some species like teak (*Tectona grandis*) and greenheart (*Ocotea rodiaei*) will last for decades even in areas of high decay risk.

2.2 Wood protection

It has been established that drastic losses of timber in the forest and in harvested woods or in wood products caused by wood rot fungi if proper steps to control of wood decay are not taken. Hence they require effective and appropriate approach under certain situation to avoid decay and to extend their service life to fulfill wood industry demand. The most practical way is to prevent wood decay by applying wood preservatives (Brischke and Rapp, 2008). Wood preservation can be defined as chemical application to the wood for protecting the wood from biodegradation.

There are many methods for applying wood preservatives as there are various types of preservatives. There are two types of wood preservation treatment: short-term and long-term wood protection.

2.2.1 Short-term wood protection

Short-term treatments involve brushing, dipping or spraying of fungicides onto lumber or log surfaces. According to Zabel and Morrell (1992), thin surface coating is the prophylactic barrier that prevents fungal spores from germinating on the wood surface and provides only short-term protection, around five to six months or as low as four weeks. Brushing home furniture such as living sets, dining sets, chairs and desk with fungicides such as copper naphthene is one of the examples of short-term treatment. The preservative is not absorbed into the wood, but only in-contact with the wood surface. Therefore, when the chemical layer is removed due to weathering or high temperature, the wood is exposed to fungi infection and insect attack.

2.2.2 Long-term wood protection

Long-term treatments are necessary for wood used when conditions are conducive to decay prevail (Zabel and Morrell, 1992). These treatments often divided into non-pressure and pressure treatment by the American Wood Preservers Association (AWPA, 1990).

The non-pressure treatment method does not require deep penetration, high retention levels and precise treatment as in pressure treatment. The effectiveness of non-pressure treatment depends on the kind of wood, its moisture content, method and duration of treatment and types of preservatives used. This treatment provides only a thin shell of treated wood and provides useful protection to wood exposed above ground and exposed to intermittent wetting. Non-pressure treatment can improve wood service life, but the high variation in preservatives penetration and distribution, along with the limited protection

periods these treatments provide, makes them less attractive where wood in costly structures must be exposed under adverse conditions.

For a long, reliable service life for wood used in decay-hazard conditions, pressure treatment with effective preservatives is mandatory. When performed properly, pressure-treatment results in deeper, more uniform treatment of the wood. There are three basic processes that we can consider pressure treatments namely thermal, full-cell and empty-cell process.

Thermal process uses the natural development of small pressure differences inside the wood to force solution into the wood. In this process, dry wood is placed into an open tank, and preservative solution is added to the tank and heated to 150 to 230ºF (65.5 to 110 ºC) for periods ranging from 26 to 48 hours. Then the preservative solution is withdrawn, and a cooler preservative solution is pumped in. The cool solution results in a pressure differential in the wood that draws preservatives into the wood, increasing uptake. Thermal process is generally used with oil borne chemicals, which are less likely to evaporate due to high temperature of the baths.

The full-cell or Bethell process, developed in 1839. In this process, wood is placed in a treating cylinder (retort), a vacuum is drawn to remove as much air as possible from the wood, and the preservative is added to cylinder. The pressure is gradually raised to maximum of 150 to 200 psi (1050 to 1408 kPa) and held until gauges on the outside of the cylinder indicate that a sufficient amount of solution has been forced into the wood (Zabel and Morrell, 1992). This level, called gauge retention, depends on the volume of wood being treated, as well as on the specified retention and penetration values required by specifications for a given commodity. The pressure is released after specified time. At this time, the

pressure release causes air in the wood to expand and force outward a certain amount of preservative. The amount of preservatives released from the wood is called kickback. Additional periods of heating in solution, steaming, and vacuum may also be used to remove surface deposits and reduce subsequent bleeding of preservative once the wood is placed in service. The full-cell process requires more elaborate equipment and result in deeper, more uniform penetration than other processes. This process results in a maximal uptake, or retention of preservative for a given depth of penetration. The concentration of chemicals used in this process can be changed to achieve the desired retention.

In empty-cell or Rueping process, no vacuum is used. The solution is added and the pressure is raised and held until the desired amount of chemical is forced into the wood. The pressure is then released and air that was trapped in the wood expands outward, forcing excess preservative from the wood. The empty-cell process results in larger kickback of preservative at the end of the treatment cycle than the full-cell process, producing a lower retention for a given depth of penetration (Zabel and Morrell, 1992).

2.3 Types of wood preservatives

Numerous chemical preservatives have been developed and there are three major categories of wood preservatives: oil based preservatives, water based preservatives and organic solvent based preservatives (Rayner and Boddy, 1988; Eaton and Hale, 1993; Richardson, 1993). These preservatives types are classified based on the solvent in which the preservative is dissolved. Three broad categories of wood preservatives are used in industrial and commercial applications: oil based preservatives, water based preservative and organic solvent based preservatives.

2.3.1 Oil based preservatives

Creosotes (coal tar oil) patented in 1836 by German chemist Franz Moll and first used for wood impregnation by John Bethel in 1838. In the 19th and 20th centuries, creosotes were the most commonly used wood preservatives throughout the world (Eaton and Hale, 1993). Creosote and creosote solution are among the oldest and most used tar oil preservatives. Creosote is a complex mixture consist from 200 to 800 polynuclear aromatic hydrocarbons (PAH) and it made from distilling coal tar. According to Richardson (1993), the tar was originally derived principally as a by-product of the manufacture of town gas using horizontal, vertical or inclined retorts within continuous operation at high or low temperature. As creosote was derived from town gas production, it is no longer readily available. However, coal-tar is still available in enormous quantities from the coke ovens which are associated with the metal smelting industries, but it is difficult to prepare creosote (Richardson, 1993). Creosote is toxic to wood destroying organisms, low volatility (no leaching) and makes treated wood more resistant to weathering. However, they cannot be used indoors because their vapours are sensitive to plants, animals, humans and treated wood cannot be painted (Bowyer *et al.*, 2003). Creosote raise a bad smelly and black oily liquid which can cause pollution of environment such as water pollution and take many years to break down once it is in contact to the groundwater (Eaton and Hale, 1993).

Copper naphthenate, a copper complex of naphthenic acids derived from the oil-refining process, has been promoted. This chemical, which is less toxic to humans than penta, has been successfully tested over a 40-year of period and appears to be a promising new chemical, as its cost becomes more competitive with other preservatives. It has been used extensively for many years in the retail industry as a brush-on preservative for home and

marine use. In cases where the green color of the preservative is objectionable, the colorless but less effective zinc salt is used.

Many broad-spectrum chemicals have been subjected to public criticism due to their broad toxicity. For example, creosote which these products are mixtures of many chemicals created by high-temperature treatment of beech and other woods, coal or from the resin of the creosote bush. This is released to water and soil mainly as a result of its use in the wood preservation industry when it enters the environment. These also may dissolve in water and may move through the soil to the ground water which it is raise bad smelly and very dirty can cause pollution of environment. Once it is in groundwater, it may take many years for it to break down and can adversely affect humans, plants and animals.

2.3.2 Water based preservatives

Water based preservatives have been used in the United States for over 100 years, beginning with mercuric chloride in the Kyanizing process (Richardson, 1993). Water used as solvent reduce solution costs and leave wood surface clean and paintable. There are abundant of different water-borne preservatives on the market throughout the world such as Borate, copper-crome-arsenic (CCA), ammoniacal copper arsenate (ACA) and ammoniacal copper quat (ACQ).

Borates are widely used as wood preservatives. This compound is based on boric acid or sodium borate. Borate is very effective as insecticides for borer and termites and fungicides for decay fungi (Richardson, 1993). Luo *et al.* (2005) pointed out that boron compounds belong to traditional preservatives for wood protection against wood-destroying fungi and insects in interior exposure. It is usually applied in water solutions to wood.

Advantage of this compound is low toxicity to human and the environment. In order to reach a better efficacy, it has to be applied in higher amounts, approximately from 3 to 20 kg m^{-3} of wood (Lyon *et al*., 2009; Reinprecht, 2007; Pallaske, 2004). Borate diffuse with moisture and completely penetrate into wood, which normally applied by dipping followed by a four to eight weeks wet-storage period. Borate is safe, but their application is limited by high susceptibility of leaching (Zabel and Morrell, 1992).

Copper-chrome-arsenic (CCA) is one of the most widely used water borne preservative which is the most advanced in the wood preservation. CCA is known as Chromated-Copper-Arsenate. CCA is a wood preservative containing copper, chromium and arsenic (Jusoh and Kamdem, 2000). CCA preservative is a very good and effective preservative throughout the world. Pankras *et al*. (2009) pointed that copper is effective against fungi in various inorganic compounds like CCA. Copper in the form of Cu^{2+} cation is especially effective against soft-rot fungi (Ray *et al*., 2010). According to Wong (1989), CCA is the dominant wood preservative for Malaysian timber exposed to a decay hazard. In Malaysia, CCA-treated wood is still commonly used to protect roof trusses under hazard class H2 situation, i.e, protection from termites and beetle borers (Wong *et al*., 2005). However, European countries have banned CCA because of highly toxic particularly based on their arsenic content. It also pointed out that CCA has high toxicity against mammals and environment hazard (Salmiah *et al*., 2005). Preston (2000) and Evans (2003) highlighted that usage of arsenic in the copper-chromated-arsenate (CCA) and in other preservatives already have been restricted in most of the countries of the world.

Ammoniacal copper arsenate (ACA) is used in the treatment of western wood species. It has a recently developed replacement which is known as ammoniacal copper zinc arsenate

(ACZA). In contrast to CCA, ACC does not have chromium to hasten fixation (Zabel and Morrell, 1992), but they have ammonia component which helps improve preservative penetration and reacts strongly with wood component to provide excellent long-term protection.

Ammoniacal copper quaternary (ACQ) is another wood preservative in the market. Since ACQ contains no arsenic or chromium, it is considered environmentally safe. The low toxicity of this compound makes achievement of durability levels equivalent to copper or arsenic pressure with no impact on the mechanical properties of wood. From studies made by Pankras *et al*. (2009), it is evident that copper is effective against fungi in the form of ACQ. However, copper preservatives are not effective against all types of wood-inhibiting fungi. It is well known that most brown-rot fungi which use Fenton reaction at depolymerization of cellulose, are tolerant towards copper based wood preservatives as a consequence of creation the non-active copper oxalate crystals, as well as other gene predictions (Hastrup *et al*., 2005; Tang *et al*., 2010; Schilling and Inda, 2010; Woo and Morris, 2010). Copper oxalate is insoluble in water and copper in this form has a greatly reduced inhibitory effect on fungal growth (Humar *et al*., 2001). Thereby, broad-spectral preservatives require the addition of suitable co-fungicide (e.g. boron-compound), to protect wood against copper tolerant fungi.

Methylene bisthiocyanate (MBT) has also been used in the forestry sector to protect unseasoned sapwood timber from fungal degradation (Goldstein, 1988). A key attribute of MBT is its ability to diffuse below the wood surface of unseasoned wood (Williams *et al*., 1985; Kennedy and Woods, 1996), which is important for effective protection of unseasoned timbers from microbial invasion. Recent research in New Zealand has further investigated the mobility of MBT and has led to the development of an antisapstain product that has proved

highly successful in arresting fungal pre-infections in *radiata* pine export logs (Eden *et al*., 1999; Kreber *et al*., 2001). Singh *et al*. (2001, 2004) also found that established mycelium of the test fungi is more tolerant to MBT in liquid culture and on wood than their respective propagules. Recent studies showed that the vapours released from MBT also had an inhibitory effect on fungi. But it is highly toxic for human beings as well as environment (Singh *et al*., 2004).

Although the water based preservatives are effective but it has some disadvantages like treated wood swells, rising the grain, treated wood requires redrying, some salt treatments are not chemically fixed in wood and will leach, and some treatments impart slight losses in strength (Tillott and Coggins, 1981).

2.3.3 Organic solvent based preservatives

Organic solvent preservatives were first introduced in the 1920s and 1930s. They are composed of biocidal compounds–insecticides and / or fungicides – dissolved in a volatile or non-volatile, non-polar organic solvent. The volatile solvent types are the most common and leave the wood in a paintable condition (Hilditch, 1991). In general, the organic solvent preservatives have traditionally been described as either light organic solvent preservatives (LOSPs) or those made up in heavy oil such as pentachlorophenol (PCP) which are non-paintable. Light organic solvent preservatives applied by dipping, brushing, spraying and double vacuum treatment are very suitable for the pretreatment of joinery timber. Because of their absorptive capacities, they are widely used in the remedial treatment of wood in buildings following fungal and insect infestations (Eaton and Hale, 1993). They have certain advantages over other preservatives like treated wood dries quickly, active ingredient is more

or less insoluble in water and therefore not readily leached or minimum leach, treated wood can be painted and glued if a suitable solvent is used, etc.

Pentachlorophenol (PCP) in heavy oil is one of widely used organic solvent based wood preservatives. The value of PCP as a wood preservative increased in the late 1940s when creosote was in short supply. It is easily synthesized by successive chlorinations of phenol. It functions as a biocide by uncoupling oxidative phosphorylation. Pentachlorophenol, and its water soluble sodium salt, exhibited broad spectrum toxicity to fungi and insects, were relatively inexpensive, and could be supplied large quantities. As a result, PCP rapidly replaced creosote in many applications. Despite its efficacy and range of uses, PCP is no longer regarded as universally acceptable for wood treatment. Pentachlorophenol (PCP) is not allowed in Malaysia because it is very harmful to humans and environment. Previous studies reported that PCP is linked to long term health problems such as persistent fever, cancer, weight loss, nausea, etc. The Malaysian government has banned the water borne NaPCP from the market. The presence of minute amounts of highly toxic dioxins during manufacture and handling, and concerns about its impact on the environment has resulted in the banning of PCP in most of the countries (Eaton and Hale, 1993).

2.4 TBTO and TBTN as organotin wood preservatives

TBTO (tri-n-butyltin oxide) and TBTN (tri-n-butyltin naphthanate) are both trialkyltin compounds and are used as fungicides worldwide during the 1990's (Schweinfurth *et al*., 1991). Both of these compounds are liquid at room temperature. These compounds are most effective against wood decaying brown rot fungi which destroy the wood cellulose, but are

less effective against white rot fungi and soft rot fungi which destroy both cellulose and lignin.

According to Schweinfurth *et al*. (1991), the undiluted active ingredient TBTO was found to be severely irritating to the skin of rabbit and human. In an experiment, the application of the compounds onto the skin of human volunteers was carried out. After two to four hours, all application areas showed severe reddening and slight swelling. This shows the danger in using these trialkyltin compounds as fungicides. The precautions and safety of usage cannot be guaranteed and strict supervision is needed if both these compounds were to be used. The wood can dry quickly after treatment using organotin compound. The active ingredient is insoluble in water hence the preservatives applied do not readily leached. There is no dimensional change in the wood after treatment.

The compounds were commercially used as biocides until today are trialkyltin compounds. The biocidal activity triorganotin compounds have been known since the early 1950s. Starting from the 1970s trialkyltin compounds were commercially used on a large scale as biocides in the fields of plant protection, antifouling, material protection and wood preservation (Schweinfurth *et al*., 1991). There are two trialkyltin compounds namely tri-n-butyltin oxide (TBTO) and tri-n-butyltin naphthenate (TBTN). TBTO [$\{Bu_3Sn\}_2O$], TBTN and the water-soluble tributylmesylimide used as wood preservatives (Hoch, 2001; Evans, 1998; Schweinfurth *et al*., 1991)). Although it is very effective but can be harmful to human and environment (Schweinfurth *et al*., 1991). For this reason most countries have already banned or restrict the use of TBTO and TBTN as wood preservatives.

2.5 Organotin(IV) complexes

2.5.1 Ligand

Ligand is an ion or molecule that binds to a central metal atom/ion to form a coordination compound. The bonding between metal/metal ion and ligand generally involves formal donation of one or more of the ligand's electron pairs. The nature of metal-ligand bonding can range from covalent to ionic character. Furthermore, the metal-ligand bond order can range from one to three. Ligand selection is a critical consideration in many practical areas, including bioinorganic and medicinal chemistry, homogeneous catalysis and environmental chemistry. There are various types of ligands such as semicarbazone, thiosemicarbazone, hydrazone, carbohydrazone, thiocarohydrazone etc. (Lever *et al*., 2004). Substituted thiosemicarbazone ligand was chosen in this research as this can be synthesized without a complicated synthesis route and easily optimizes to get high yield (Salam, 2012).

2.5.2 Thiosemicarbazone

A thiosemicarbazone is an analog of a semicarbazone which contains a sulfur atom in place of the oxygen atom (Figure 2.1).

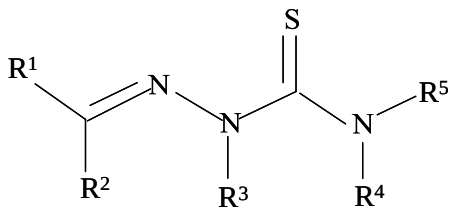
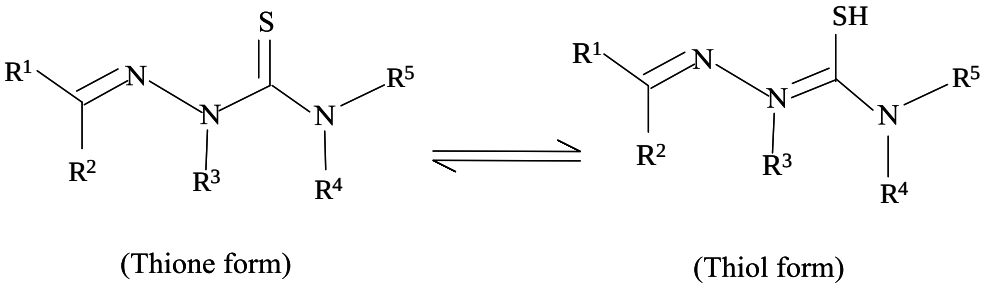


Figure 2.1. General chemical structure of a thiosemicarbazone

Thiosemicarbazones have a very special tautomerization feature within the structure. In solid state, the thiosemicarbazone exists as thione form but in solution it can converted into thiol form (Figure 2.2) (Salam, 2012).



(Thione form) (Thiol form)

Figure 2.2. Tautomerization of thiosemicarbazone

2.5.3 Tin

In Periodic Table, the atomic number of tin is 50 which is a member of Group 14. The symbol for tin is 'Sn'. Tin has been used for thousands of years in the form of alloys. In common with the other group 4 elements, Carbon (C), Silicon (Si), Germanium (Ge) and Lead (Pb), Sn has four electrons in the valence shell and thus forms compounds in which it has oxidation states +II and +IV (Heiserman, 1992). Many compounds that have both oxidation states are known. The +IV oxidation state is the more stable state and tin(II) compounds are moderately strong reducing agents. Tin and its compounds have many important applications starting from small preparative to large industrial scales. Tin forms a large variety of different compounds in combination with other elements. Tin and organotin compounds have been extensively studied due to interesting catalytic or biological applications (Heiserman, 1992).

2.5.4 Organotin compounds

Organotin(IV) compounds are chemical compounds based on tin with hydrocarbon substituent which can be included in the group of organic solvent preservatives. Organotin(IV) compounds have at least one tin-carbon bond exists, which have the +4 oxidation state. Organotin compounds have general formula:

$R_nSnX_{4-n,}$

Where, n=1, 2, 3 and 4

R= Me, Bu, Ph, Et, Vinyl etc.

X= Halides (Cl, Br, I), oxides etc.

A large number of organotin compounds exist but there are four major classes of these compounds, depending on the number of organic groups: tetra-organotins (R_4Sn), tri-organotins (R_3SnX), di-organotins (R_2SnX_2) and mono-organotins ($RSnX_3$) where R is an alkyl or aryl group (organic group), Sn is a tin atom and X is an anionic group (halide, oxide, hydroxide, carboxilate or thiolate) or a group attached to tin through oxygen, sulphur, nitrogen, halogen and so on (Salam, 2012). The toxicity of these compounds depends on the nature and the number of the alkyl groups (Tian *et al*., 2005). Generally the alkyltin toxicity increases with the number of the organic groups and the greatest fungicidal activity develops with 9-12 carbon atoms (Richardson, 1993). Triorganotin compounds are generally more toxic than other organotin classes and trialkyltins with linear organic groups cannot be used as agriculture biocides due to their high toxicity.

The synthesis of the first organotin compound diethyltin diiodide by Edward Frankland in 1849 was the beginning of a new area in the field of tin chemistry (Frankland, 1849).

$$2EtI + Sn \longrightarrow Et_2SnI_2$$

Lowich (1852) described a reaction of alkyl halides with a tin-sodium alloy yielding alkyl tin compounds. Kuivila *et al.*, (1962) showed that the reaction of trialkyltin hydrides with alkyl halides (hydrostannolysis) was a radical chain reaction involving short-living trialkyl tin radicals, $R_3Sn^{+}\cdot$.

$$R_3SnH + R'X \longrightarrow R_3SnX + R'H$$

Depending on the organic groups attached, they can be powerful bactericides and fungicides. Tributyltins are used as industrial biocides, antifungal agents in textiles and paper, wood pulp and paper mill system. Cardwell and Sheldon (1986) pointed out that tributyltin at low concentration is highly toxic to various non-target organisms. Though tributyltin is highly toxic to some sensitive aquatic organisms, its degradation products (dibutyl-, monobutyl- and inorganic tin) are at least one or two orders of magnitude less toxic (Wong *et al.*, 1982). Triphenyltins are used as active compounds of antifungal paints and agricultural fungicides. In addition, the nature of the alkyl (R_3) group is important and a total carbon atom content of 12 (3 x C_4) produced maximum fungal toxicity (Miller, 1972). Anderson (1979) pointed out that organotin compounds have been impregnated directly into the cell lumen of wood through direct impregnation and the resultant modified woods have been resistant to attack by fungi (Ellis and Rowell, 1984).

Saxena and Tandon (1984) studied on some organotin(IV) complexes structure. They synthesized and characterized of some five- and six-coordinated di and tri-n-butyl tin(IV) semi- and thio-semi carbazones. Screening of organotin compounds revealed that the structure of organotin compounds reflects their biocidal activity and those tri-alkyl (R3) tin derivatives in particular, showed good anti-fungal activity (Schweinfurth *et al*., 1991). The chemistry of organotin(IV) has been the area of interest for many years because of their industrial and biomedical applications (Singh *et al*., 2001). Several organotin(IV) complexes have been found as effective as antifouling, anti-microbial (Nath *et al.,* 1995; Nath *et al.,* 1999) and antiviral agents. Organotin compounds have a range of applications; including their use as boat paint additives to prevent attack by microorganisms (Rouhi, 1998) and as insecticides and fungicides (Piver, 1973 and van der Kerk, 1976).

The increasing interest in organotin(IV) compounds is attributed to their important biological properties and their considerable structural diversity. In general, organotin(IV) compounds show significant in vitro antifungal, antibacterial, antiviral, wood preservatives, pesticides and antitumor activities (Khan *et al*., 2004; Girasolo *et al*., 2006). The effectiveness of organotin(IV) compounds are essentially related to the number and nature of the organic groups attached to the central tin (Sn) atoms, however, the role of attached ligand cannot be ignored. Usually, triorganotin(IV) compounds display a higher biological activity than their di- and monoorganotin(IV) compounds (Davies and Smith, 1980). Pellerito *et al.,* (2006) reported the biological activity of organotin(IV) complexes and also gave a review of the different experimental procedures used in biological studies. Research dealing with metal complexes of thiosemicarbazones have expanded enormously with increasing attention

devoted to thiosemicarbazones complexes of organotin(IV) in view of their chemical properties, biological significance, industrial importance, and structural variety.

The use of organotin for agriculture and industrial purposes has been increasing steadily in these last 20 years. Industrial use of organotin compounds has risen dramatically as a result of their wide range of technical applications and their environmental and toxicological properties (Kidwai *et al*., 2000). Organotin(IV) compounds showed a wide range of industrial (Yin and Chen, 2006), agricultural and biocidal (Carraher *et al.*, 2008) activities. Organotin compounds have a range of applications, including their use as boat paint additives, as insecticides and fungicides ((Rouhi, 1998; Piver, 1973). A number of triorganotin compounds have been developed as agrochemicals and they are successfully used in specialized applications. The main advantage of organotin(IV) compounds as agrochemicals includes low phyotoxicity, they are less harmful to non-targeted organism and they can easily degrade in the environment eventually forming harmless tin residues (Blunden *et al.*, 1985).

Tian *et al.* (2005) reported that the biological activity is greatly dependant on the structure of the organotin(IV) compounds. Besides, the understanding of mono-, di- and triorganotin(IV) derivatives is equally important as they may show antitumor and biocidal properties which make some of these species suitable for pharmaceutical, industrial and agricultural applications.

Hadi *et al*. (2008) observed that the compound synthesized in general exhibit greater fungi toxicity than the organotin chloride, the intermediate products and the carboxylic acids. The fact that the organotin moiety plays an important role in deciding the antifungal activity of organotin compounds. Recent studies have shown that thiosemicarbazones and their

organotin(IV) derivatives present a wide range of bioactivities (Sharma *et al.*, 2011; Momeni *et al.,* 2009).

2.6 Enzymatic studies specifically on lignin degradation

2.6.1 Lignin and lignin degradation

Lignin is a heterogeneous polymer that provides strength and rigidity to wood, protects cellulose and hemicellulose from microbial attack, and is the major precursor of coal (Robinson, 1990). Lignin is the most abundant organic material on earth after cellulose. It is found in the secondary wall and middle lamella of higher plants (Darah and Ibrahim, 1996). Lignin is a naturally occurring substance produced by plants to strengthen their tissues. The cellulose walls of the wood become impregnated with lignin, a process called lignifications, which greatly increases the strength and hardness of the cell and gives the necessary rigidity to the tree. The lignin content of tropical hardwoods is quite high (Kim *et al*., 2006; Nilsson *et al*., 1988). Kim *et al*. (2006) illustrated a relatively high amount of condensed lignin in the cengal heartwood. Softwood lignin is composed of guaiacyl unit, while hardwood lignins contain guaiacyl and syringyl units. Lignin is generally considered to be the polymer most resistant to biological degradation (Rowell, 1984).

Cells in wood are composed of two parts: cell wall and lumen. The lumen is the void space inside the cell that allows for water conduction in the living tree. The structure enclosing the lumen, or cell wall, contains three layers: the middle lamella, primary wall, and secondary wall. The middle lamella and primary wall are formed during initial growth of the cell, while the secondary wall is formed during the thickening stage. The secondary cell wall is divided into three sub layers, S1, S2 and S3, based on orientation of the cellulose

microfibrils, S3 layer being closest to the cell lumen. The microfibrils in S1 and S3 layers are nearly perpendicular to the length of the cell; while those in the S2 layer run almost parallel to the longitudinal axis. The S1 and S3 layers are thin. S1 layer contains only 40-70% polysaccharides, while the S3 layer has the highest cellulose and hemicelluloses content, around 85% (Panshin and Zeeuw, 1980). The S2 layer is the thickest layer and is, thus, responsible for most of the physical and mechanical properties of the cell wall. The middle lamella is the layer between the adjacent cells, and this area is often called compound middle lamella due to the difficulty of differentiating the middle lamella from the two primary walls of the adjacent cells (Saka, 2000; Wiedenhoeft and Miller, 2005). Although the proportion of lignin in the S2 layer is low compared to S1 and S3 layers, the S2 is the thickest layer and therefore contains most of the lignin in the entire cell wall. Lignin content in the S1 and S3 layers remains relatively constant, at around 15% (Panshin and Zeeuw, 1980; Sjostrom, 1981).

Lignin degradation is considered the rate-limiting steps in carbon cycling on the earth since there are only a few organisms capable of degrading this structurally complex aromatic biopolymer (Orth *et al.*, 1993). The enzymes, those are extracellular, oxidative and unspecific, with the ability to liberate the highly unstable products which further undergo many different oxidative reactions, able to catalyze the initial steps of lignin depolymerization.

Microorganisms that able to degrade lignin include the wood-rotting fungi and, to a lesser extent, certain actinomycetes and bacteria (Coll *et al.*, 1993). These microorganisms produce ligninolytic enzymes, which consisted of two major families of enzymes: peroxidases and laccases. They have the ability to degrade the lignin. Lignin is an insoluble

polymer. Therefore, the initial steps in its biodegradation must be extracellular. Due to its hydrophobicity and complex random structure that lacked the regular hydrolysable bonds, lignin is poorly biodegraded by most microorganisms. The organism known to extensively degrade lignin is fungi and, to a lesser extent, certain actinomycetes and bacteria (Coll *et al*., 1993; Mester and Field, 1998).

2.6.2 Ligninolytic fungi

Lignin degrading fungi or also known as ligninolytic fungi are classified into three major categories based on the type of wood decay caused by these organisms: white-rot fungi, brown-rot fungi and soft-rot fungi (Haglund, 1999; Dhouib *et al*., 2005).

2.6.2.1 *Trametes versicolor* (white rot fungus)

Trametes versicolor previously known as *Polyporus versicolor*, *Coriolus versicolor* and *Polystictus versicolor*, is normally found on wounded or dead standing trees, hardwood logs and stumps, softwoods, also observed on pulpwood chips, in mine timbers, in railway sleepers, and from in-service timbers out of ground contact. For the growth and development of *Trametes versicolor*, optimum temperature is 30°C and moisture content is 40-45%, but can withstand a wide range of temperature and moisture (Eaton and Hale, 1993). The white-rot fungus *Trametes versicolor* belongs to Basidiomycetes (Hatakka, 2001).

In the past few years remarkable progress has been made in determining the enzymatic nature and probable pathways of lignin digestion since the initial identification of a ligninase (Tien and Kirk, 1983, 1984; Gold *et al*., 1984). The principal enzymes now

believed to play a vital role in this process are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) which are responsible for the initial fragmentation of the lignin polymer and production of low molecular mass breakdown products in white-rot fungi (Kirk and Shimada, 1985; Kirk, 1988). It has been detected that LiP appears to be the key lignin degrading enzyme in *Trametes versicolor*, the most studied white rot fungus to date. However not all white-rot fungi produce all the three enzymes (Rieble *et al*., 1994).

White rot fungi are able to fragment the major structural polymers of wood and other lignocellulosics- lignin, cellulose, and hemi cellulose and further metabolize the fragments. The hyphae of fungi rapidly invade wood cells and lie along the lumen walls where they secret the enzyme to depolymerize the hemi cellulose, cellulose and fragmentation of lignin (Kirk and Cullen, 2005). The white rot fungi degrade wood by removing cellulose, hemi cellulose and lignin more or less simultaneously. This is more dangerous and harmful than brown rot since it affects all the contents of cell wall thus causing accidental collapse and damages. White rot fungi have the capacity to degrade all cell wall components, including lignin. The extent of lignin degradation can vary considerably among species of white-rot fungi (Blanchette, 1991). Some species, such as *Trametes versicolor*, are nonselective in how they degrade the wood, i.e., they simultaneously degrade lignin, cellulose and hemicelluloses. In nonselective degradation approximately equal amounts of all fractions of lignocelluloses are degraded (Blanchette, 1995; Hatakka, 2001). *Trametes versicolor* can destroy polysaccharides (cellulose and hemicelluloses) present in the cell walls and also can degrade the lignin (Arantes *et al*., 2010; Schmidt, 2006). Other species, such as *Phellinus pini, Ceriporiopsis subvermispora,* and *Phlebia tremellosa,* cause preferential degradation of

lignin (Otjen *et al*., 1987). White rot fungi are involved in the extensive degradation of lignin by means of their extracellular ligninolytic system (Kirk and Farrell, 1987). *Trametes versicolor* and *Phellinus sp*. white rot strains are of more interest in lignin degradation. In general, lignin degradation is only significant from 20 days onwards. The overall percentage of lignin weight loss is within the range of 1.02–26.90% over the biodegradation periods (Liew *et al*., 2011).

The white rot fungi are by far the most efficient ligninolytic organisms described to date. The capability to degrade lignin is due to their extracellular nonspecific and nonstereoselective enzyme systems (Tekere *et al*., 2001). Because the key components of the white rot degrading systems are extracellular, these fungi have the potential to be used in various biotechnological applications including the degradation of very insoluble chemicals such as lignin, hazardous waste remediation (Hammel, 1997; Asgher *et al*., 2006), industrial processing of paper and textiles and bioconversion of feeds (Eriksson *et al*., 1990).

2.6.2.2 *Gloeophyllum trabeum* (brown rot fungus)

Gloeophyllum trabeum previously known as *Lenzites trabea*. Generally it occurs in standing trees, external timber including felled logs, stumps, domestic timbers in contact with soil and mortar, roofing timbers, window joinery, mine timbers, telegraph poles, railway sleepers, bridge timbers, cooling tower timbers, wooden boats, timber in storage, etc. Duncan and Lombard (1965) pointed that *G. trabeum* can cause rapid decay of both soft and hardwoods including naturally durable softwoods. Eslyn (1986) recorded 59% weight loss of southern pine exposed by the soil block method for 12 weeks. It can survive a wide range of growth conditions. Humphrey and Siggers (1933) reported that for the growth and development of this fungus optimum temperature 35°C and moisture content 30-50% but will withstand a wide fluctuation in temperature and moisture content.

Brown rot fungi cause rapid and extensive depolymerization of cellulose early in the decay process (Kirk and Cowling, 1984; Eriksson *et al*., 1990). Wood polysaccharides are degraded, lignin modification occurs, and relatively small amounts of lignin are lost as decay progresses (Blanchette *et al*., 1990; Goni *et al*., 1993). Brown rot fungi are common decomposes in conifer forests and also are responsible for most decay found in buildings and wood in service.

It is well known that brown rot takes place through oxidative reactions involving metals either in free form like iron in Fenton reaction for brown-rot fungi or as key components of enzymes like copper, iron or manganese in laccase and peroxidases (Hammel, 1997; Kerem *et al*., 1999). Brown rot fungi cause rapid and extensive depolymerization of cellulose early in the decay process (Eriksson *et al*., 1990). Wood polysaccharides are degraded, lignin modification occurs, and relatively small amounts of lignin are lost as decay progresses (Blanchette *et al*., 1990). In advanced stages of decay, the residue is a brown mass, which mostly consists of lignin. This decayed wood is sponge-like when wet, but often cracks and checks into cubical pieces as the wood dry. Arantes *et al*. (2010) pointed out that the brown-rot fungi namely *Gloeophyllym trabeum* can destroy cellulose and hemicelluloses of wood.

Brown-rot fungi attack wood by their enzymatic systems. These enzymes penetrate the wood cell wall, alter its chemistry and break down the cell-wall polymers into constituents that can be taken up by hyphae. It has been established that the brown-rot fungi selectively decay cell-wall polysaccharides, with limited lignin degradation. The decay system in this type of fungi is based on both non-enzymic (chemical) and enzymic attacks (Eriksson *et al*., 1990).

2.6.3 Ligninolytic enzymes

Enzymes are proteins or glycoproteins that catalyze almost all biologically important reactions. Enzymes are very specific and efficient biocatalysts, resulting in much higher reaction rates as compared to chemically catalyzed reactions under ambient conditions. Microorganisms have evolved several of enzymes for degrading the different components of lignocellulosic material. These enzymes include cellulases (for degrading the cellulose), xylanases (for degrading the hemicellulose), and ligninolytic enzymes (for degrading the lignin). There are two major families of ligninolytic enzymes which are involve in lignolysis: peroxidases and laccases (Ohkuma *et al*., 2001; Sasaki *et al*., 2001; Perez *et al*., 1996). These enzymes are capable of forming radicals inside the lignin polymer, which results in damage of bonds and finally in the breakdown of the macromolecule of lignin (Hofrichter *et al*., 1999). The ligninolytic enzymes attack lignin directly and thereby are the most promising long term alternatives to lignin removal by physical and chemical processes. The ligninolytic enzymes in most basidiomycetes are highly regulated by nutrients such as nitrogen, copper and manganese. Their production is also affected by many typical factors such as medium composition, nature of carbon source, concentration of carbon source, pH of fermentation broth, fermentation temperature, amount and nature of nitrogen source and presence of inducers (Cu^{2+}, Mn^{2+}, 2,5-xylidine, ferulic acid and veratrylalcohol) (Arora and Gill, 2001; Asgher *et al*., 2010; Iqbal *et al*., 2011; Patrick *et al*., 2011).

2.6.3.1 Lignin peroxidase

Lignin peroxidase (LiP, ligninase, diarylpropane peroxidase; EC 1.11.1.14) is the first oxidative enzyme discovered in *Phanerochaete chrysosporium* (Glenn *et al*., 1986; Tien and Kirk, 1983). LiP is an extracellular monomeric glycoprotein with a heme group in its active center. LiP has a molecular mass ranges from 38 to 43 kDa and pI from 3.3 to 4.7. It is capable of catalyzing the depolymerization of the aromatic polymer lignin and a variety of non-phenolic lignin model compounds in the presence of H_2O_2 (Teunissen and Field, 1998; Haglund, 1999; Ikehata *et al*., 2004). Lignin peroxidase has the distinction of being able to oxidize methoxylated aromatic rings without a free phenolic group, generating cation radicals that can react further by a variety of pathways, oxidation of benzyl alcohols such as veratryl alcohol to corresponding aldehydes or ketones, and hydroxylation of benzylic methylene groups (Darah and Ibrahim, 1996; Haglund, 1999; Ikehata *et al*., 2004).

Ever since the discovery of LiP, veratryl alcohol has played a fundamental role in the study of the lignin biodegradation process. Veratryl alcohol is used as an assay for enzyme activity due to the easily detectable absorbance of the product veratraldehyde at 310 nm. Lignin peroxidase is capable of oxidizing non-phenolic compounds with a relatively high redox potential of the oxidized enzyme intermediates, lignin peroxidase Compound I (LiP I) or Compound II (LiP II) (Schoemaker and Piontek, 1996). LiP I will oxidize the first molecule of veratryl alcohol to the corresponding radical cation, which is liberated from the active site. Subsequently, the second substrate molecule is oxidized by LiP II to form the second molecule. In the process, LiP II is converted to native enzyme, is a very strong oxidant, which subsequently might oxidize any recalcitrant chemical present, like the lignin polymer.

2.6.3.2 Manganese peroxidase

Manganese peroxidase (MnP, manganese-dependent peroxidase; EC 1.11.1.13) was first discovered and purified from extracellular culture fluid of a basidiomycete *Phanerochaete chrysosporium* in the mid-1980s (Tien and Kirk, 1983; Glenn and Gold, 1985). Manganese peroxidase is the major enzyme involved in lignin degradation by white rot fungi (Perie and Gold, 1991). Liew *et al*. (2011) observed that manganese peroxidase produced is far more compared to lignin peroxidase, suggesting that MnP might be the predominating enzymes causing lignin degradation in wood chips. The presumed role of MnP is to oxidize Mn^{2+} to Mn^{3+} which then oxidizes the various phenolic structures in lignin (Gold *et al*., 1989).

The natural function of MnP is the degradation of the complex lignin polymer providing strength and rigidity to all higher plants. The enzyme catalyzes the H_2O_2-dependent oxidation of Mn^{2+} into highly reactive Mn^{3+} (Asgher *et al*., 2008). According to Sundaramoorthy *et al*. (1994), MnP is unique in its ability to catalyze the one-electron oxidation of Mn(II) to Mn(III) in a multi-step reaction cycle illustrated below;

$$MnP + H_2O_2 \longrightarrow \text{MnP compound I} + H_2O \qquad \text{(Reaction 1)}$$

$$\text{MnP compound I} + Mn(II) \longrightarrow \text{MnP compound II} + Mn(III) \qquad \text{(Reaction 2)}$$

$$\text{MnP compound II} + Mn(II) \longrightarrow MnP + Mn(III) + H_2O \qquad \text{(Reaction 3)}$$

Manganese peroxidases are like LiP in that they consist of multiple acidic isoenzymes encoded by multiple structural genes whose expression is nutrient regulated; in the case of MnP, regulation by Mn^{2+} also occurs. MnP are slightly larger than LiPs, but exhibit the same basic peroxidase catalytic cycle. Interestingly, MnP, in the presence of reducing agents such

as glutathione, transfers electrons to molecular oxygen, generating H_2O_2. Like LiP, MnP has been crystallized and its three-dimensional structure determined (Sundaramoorthy *et al.*, 1994). Lignin peroxidase can display MnP activity; in the presence of H_2O_2, O_2, and metabolizes veratryl alcohol and oxalate, LiP oxidizes Mn^{2+} to Mn^{3+} (Popp *et al.*, 1990).

2.6.3.3 Laccases

Laccases (Lac, benzenediol:oxygenoxidoreductase, EC 1.10.3.2) are multicopper blue oxidase which are able to oxidize polyphenols with oxygen as final electrons acceptor. Moreover, they are also able to oxidize ortho- and para-diphenols and aromatic amines by removing an electron and a proton from a hydroxyl group to form a free radical. Their active site is constituted by four copper atoms and they utilize molecular oxygen as an oxidant for the oxidation of varieties of phenols and other aromatic compounds to corresponding reactive quinines (Coll *et al*., 1993; Ikehata *et al*., 2004). The action mechanism of Lac, especially the role of the metallic centre remains unknown. Two steps mechanism have been proposed by Coll *et al*. (1993): firstly, copper T1 extracts one electron from the substrate; secondly, electron is transferred to the T2 or T3 centre. After complete reduction of the trinuclear centre, the molecular oxygen reduction occurs.

Laccases are widespread in nature; they have been found in many plants and fungal species (Mayer, 1987). The first laccase was discovered more than one century ago in the Japanese tree *Rhus venicifera* (Yoshida, 1883). The majority of the characterized laccases are from fungi, especially from the white-rot basidiomycetes species (e.g., *Trametes spp.*) in which they are involved in lignin degradation (Thurston, 1994). Laccase is a monomeric glycoprotein containing 6.5% carbohydrate and having a molecular weight of 64,000 (Coll *et*

al., 1993). It has an isoelectric point of 3.6. It is stable in a pH range from 3 to 9, and its optimum pH is 4.5. The laccase optimal reaction temperature is 80 °C, the laccase is stable for 1 h at 60 °C, and its activity increases with temperature (Coll *et al*., 1993).

Although the contribution of laccase to lignin degradation by white-rot fungi had long been speculated, its role in ligninolysis was less clear than those of lignin peroxidase (LiP) and manganese peroxidase (MnP), partly because its low redox potential did not seem to be suitable for the oxidation of nonphenolic lignin structures (Ikehata *et al*., 2004). Moreover, laccase has the capability of both polymerization and depolymerization of lignin model compounds (Haglund, 1999; Ikehata *et al*., 2004), which made this issue more complicated. Laccase has been detected in white rot fungi (Perie and Gold, 1991). Kirk and Farrell (1987) reported that Lac provides the phenoxyradicals and quinines from lignin decomposition which play a key role in the decomposition of cellobiose by cellobiose dehydrogenase.

2.7 Rubberwood *(Hevea brasiliensis)*

Hevea brasiliensis (rubberwood) is now widely cultivated in Asia for the latex as well as timber (Akhter, 2005). Rubberwood is a light-coloured hardwood with a density ranging from 435 to 626 kg m^{-3} at 12% moisture content (Rubber Board, 2005). Rubber trees reach their prime in 25 years, after which it is no longer economical to use them for producing latex (Hong *et al*., 1994). After 25 years, rubber trees normally have a clear bole of 3 to 10 meters in heights, depending on the clone, and the diameter can reach up to 50 centimeters at breast heights. Several decades ago, old rubber trees were simply burned in the field prior to planting new stock, or used as firewood for brick making and for the production of charcoal briquettes. In recent years, due to the acute shortage of forest timbers and the pressure of

seeking new wood resources, rubberwood has become an important source of timber, particularly for furniture manufacturing. It is also used extensively to manufacture wood composites and panel products such as particleboard, block board and medium density fiberboard (Hong and Sim, 1994). It has physical and mechanical properties comparable with timbers like teak, but is rather susceptible to attack by organisms. The sapwood is not differentiated from the heartwood. Ready and low-cost availability, light colour or natural colour, easy machining and staining properties have all contributed to the establishment of rubberwood as an important wood product. Today, aside from the traditional uses, rubberwood is used primarily for furniture, furniture parts and wood-based panels. Rubberwood stumpage prices have generally been very low or negative when compared to other wood species, due largely to the fact that rubberwood is an agricultural by-product. However, non durability like high susceptibility to insects and fungi attacks is the major weakness of rubberwood, caused by the high starch content that attracts a range of insects and fungal diseases, especially blue stain (Hong and Wong, 1994 a).

Rubberwood is classified as non-durable which means that under tropical conditions it would not last more than two years when use externally especially in ground contact. Jusoh and Kamdem (2001) studied the natural decay resistance and the efficacy of CCA-pressure treatment of rubberwood using soil block test according to AWPA E10-91 (1991). They found the moisture content of test cubes exposed to *Irpex lacteus* and *Trametes versicolor* increased with weight loss increase, while that exposed to *Gloeophyllum trabeum* and *Postia placenta* decreased. After a 12-week incubation period the average weight loss by white rot and brown rot fungi was about 1.5 times higher than that of soft rot fungi. They also observed that CCA retention of 4.1 kg m^{-3} reduced weight loss to between 8% and 10%, retention of 14.5 kg m^{-3} protected weight loss by all test fungi from exceeding 2%.

The natural resistance of rubberwood to decay and its protection have been studied by Hong *et al*. (1982) and Hong and Liew (1989). The performance of preservative treated rubberwood in Indian coastal waters (Rao *et al*., 1993; Edwin and Pillai, 2004) and the mechanical properties of rubberwood (Gnanaharan and Dhamodaran, 1993; Matan and Kyokong, 2003; Edwin and Pillai, 2004) have also been studied.

2.8 Pulai (*Alstonia scholaris*)

Alstonia scholaris is a medium to large tree that grows up to 40 m tall under the family of Apocynaceae. The bark is greyish. It is called pulai in Malaysia. *Alstonia scholaris* is found throughout South East Asia, southern China, northern Australia, the Bismarck Archipelago and the Solomon Island (Rudjiman *et al*., 1994).

Alstonia is named after Dr. C. Alston (1685-1760), a professor of botany at Edinburgh University. The specific name scholaris is derived from the use of the wood for school boards in Myanmar. It contains three alkaloids, namely Ditamine, Echitamine or Ditaine, and Echitenines, and several fatty and resinous substances - the second is the strongest base and resembles ammonia in chemical characters (Lemmens *et al*., 1995). In Taiwan, this species is a good timber tree in moist, protected environments. *Alstonia scholaris* has been recommended as a fuel wood species for the patana lands of Sri Lanka. The bark yields a fibre, and the wood is regarded as suitable for pulp and paper production. *Alstonia scholaris* is the most important source of pulai timber (Soerianegara and Lemmens, 1993). The density of the wood is 270-490 kg m^{-3} at 15% moisture content. The wood is also used for making

coffins in Sri Lanka. Wood charcoal is used as gun powder. *Alstonia scholaris* is a nondurable and mainly used for internal applications. The wood is not resistant to decay and prone to attack by termites, pin-hole and marine borers. The wood is permeable to preservatives treatment.

2.9 Mahang (*Macaranga triloba*)

The genus *Macaranga* under the family Euphorbiaceae comprises over 300 species that occur in the tropics of the Old World with the center of distribution being tropical Asia and the Pacific (Fiala, 1996). Mahang (*Macaranga triloba*) is a fast growing, soft wooded, ever green and medium sized tree up to 25 m tall under the family of Euphorbiaceae (Feldhaar *et al*., 2000). Wood density is low 360 kg m^{-3}. Low density restricts the use of timber only in non structural or composite uses. It is widely distributed in Malaysia.

Mahang is a light density hardwood from a pioneer tree species which emerge large quantity in logged-over forest in Southeast Asia (Helmer *et al*., 2000). The wood is non-durable and permeable to preservatives treatment. It has a potential as resources to augment the depleting supply of logs from natural and plantation forests. Due to its poor properties in nature, it is still underutilized, but this wood can have value added by being made into wood suitable for different applications like flooring, paneling and furniture through proper treatment by environmentally friendly preservatives (Norimoto and Gril, 1993).

2.10 Microdistribution of wood preservatives in treated wood

Various chemicals are impregnated into wood during preservative treatment. The effectiveness of preservatives depends not only upon the amount of uptake or retention, but also upon its uniform distribution in the wood cells (Zhang and Kamdem, 2000). The efficacy of preservatives is also the function of its performance in wood affected by preservative microdistribution. The performance of CCA in protecting softwoods both in laboratory evaluation and field tests is well established. The excellent performance of CCA-treated softwood, particularly the *Pinus* species, is attributed to deep penetration of preservatives into the cell wall of tracheids, preservative loading, uniform preservative distribution at cell level and even preservative distribution within the cell wall (Ryan and Drysdale, 1988; Bodner and Pekny, 1991; Newman and Murphy, 1996). In hardwoods, the poor performance of CCA is associated with failure to obtain even distribution and difficulty in achieving a desired level of chemical retention (Kamdem and Chow, 1999). Hence, when new preservatives are developed research is invariably conducted to examine their microdistribution in treated wood.

Marienfeld *et al*. (2000) used laser mass spectrometry to examine the distribution of inorganic elements in plant tissues, but electron microscopy in combination with energy dispersive analysis of X-rays (EDX) is the method of choice for examining the distribution and cell wall penetration of biocides, particularly metals, in preservative-treated wood. Scanning or transmission electron microscopy in combination with EDX has been used to assess whether chlorine, copper, chromium, titanium, silicon and zinc in different wood preservative systems are able to penetrate wood cell walls (Kamdem and Mcintyre, 1998; Matsunaga *et al*., 2004; Cao and Kamdem, 2005; De Vetter *et al*., 2006). Some of these

studies have also looked at differences in the distribution of such elements in earlywood and latewood in softwoods, and the vessels, fibres and rays of hardwoods (Petric *et al*., 2000; Matsunaga *et al*., 2004). Electron induced X-ray emission (EIXE) imaging was used to analysis of copper-chrome-arsenic (CCA) preservatives pressure-impregnated into wood tissues by Wong *et al*. (1999). Using higher degree of analytical sensitivity a novel particle induced X-ray emission (PIXE) system has also been used to analyze the distribution of copper, chromium and arsenic of CCA treated some Malaysian hardwoods (Wong *et al*., 1996). They illustrated a relatively higher concentration of copper, chromium and or arsenic in ray cells, apo- and paratracheal parenchyma, vasicentric parenchyma and vessel lumina than in fibre.

Energy dispersive X-ray can be used to detect elements at a particular location in a sample (point analysis) or map the distribution of elements in a selected area. The majority of studies of the distribution of metals in preservatives-treated wood have used point analysis to detect variations in the concentrations of metal elements within cell walls and between different tissue types. Some of these studies have also included dot maps of treated woods, which show higher concentrations of metals in wood cell walls compared to lumina or in certain tissue types (Cao and Kamdem, 2005; De Vetter *et al.*, 2006). Electron microscope and EDX system is easier to obtain X-ray maps at high magnifications, and these have found application in the mapping of low concentrations of metals in doped nano-scale semi-conductor devices (Huang *et al*., 2005). Matsunaga *et al.* (2008) observed the metals in pine treated with copper and iron nanoparticles. They used conventional and field-emission scanning-electron-microscopy and energy-dispersive analysis of X-rays (EDX) for treated southern pine.

The location and distribution of metal elements in wood treated with copper-based preservative may influence wood performance (Bodner and Pekny, 1991). The poor distribution of copper was argued to be the main reason for the inadequate protection of CCA-treated hardwood against soft-rot. Greaves and Nilsson (1982) suggested that uneven distribution of copper in S_2 layers of fiber is responsible for soft-rot in CCA-treated wood. However, some studies have shown that evenly distributed copper in wood cell of hardwoods remain susceptible to soft-rot decay (Drysdale *et al*., 1980; Ryan and Drysdale, 1988).

Detailed X-ray microanalysis to determine the microdistribution of preservative in wood cells can be achieved with transmission electron microscope fitted with energy dispersive X-ray analysis (TEM-EDXA). TEM-EDXA is used due to its high spatial resolution thus detail X-ray microanalytical studies on wood cell wall layer (Daniel and Nilsson, 1987) can be done and the X-ray generated outside the region of interest can be eliminated (Ryan, 1986). However, sample preparation for TEM examination is tedious and time consuming. To overcome this problem, scanning electron microscope coupled with energy dispersive X-ray analysis (SEM-EDXA) of semi thin section have shown to be useful in analyzing the microdistribution of preservative elements in wood cell walls (Matsunaga *et al*., 2004; Cao and Kamdem, 2005). Although the spatial resolution with conventional SEM may not be close to that of TEM, sufficient resolution can be obtained to analyze many cell wall regions without risk of overlapping the region of interest such as S_2 layers of fiber, ray and vessel, cell corner, and middle lamella. Due to the limited SEM resolution S_1 and S_3 layers cannot be analyzed for microdistribution of CCA components. However, for routine CCA microdistribution studies of large specimen areas, SEM-X-ray microanalysis of semi-thin sections provides a convenient alternative to TEM-X-ray microanalysis (Daniel and Nilsson, 1987). Jusoh and Kamdem (2009) also used SEM-EDXA to examine the

microdistribution of chromate copper arsenate (CCA) preservative in treated rubberwood. They observed high accumulation of chromium, copper and arsenic in the vessels and lower concentration of the three preservative elements in fibres. Studies on the distribution of waterborne chromated copper arsenate (CCA) preservative wood using SEM-EDXA employed bulk sections (Greaves *et al*., 1982; Daniel and Nilsson, 1987). SEM-EDXA bulk analysis would not reflect real loading in the S_2 cell wall layer due to the low spatial resolution (Ryan, 1986). However, the SEM-EDXA of bulk analysis is useful in evaluating the penetration pathways of preservatives in treated woods (Ryan and Drysdale, 1988).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Three non-durable tropical wood species were chosen in this study. *Alstonia scholaris* (Pulai) wood samples were collected from an old secondary forest in Kota Samarahan, Sarawak, Malaysia. *Hevea brasiliensis* (Rubberwood) was obtained from an old plantation adjacent to Universiti Malaysia Sarawak's arboretum. *Macaranga triloba* (Mahang) wood samples were collected from Malaysian Palm Oil Board (MPOB) plantation area in Sungai Asap, Belaga, Sarawak. *Alstonia scholaris* and *Macaranga triloba* are found in abundance in secondary forests throughout Sarawak.

3.2 Preparation of wood sample

The logs were quarter-sawn to 25 mm x 25 mm x 25 mm boards and kiln dried. The boards were further planed, ripped and cut into 19 mm cubes (Figure 3.1) according to the AWPA standard E10-91 (1991). The cubes were conditioned at 60°C and 70% relative humidity for four days until they reached a constant weight.

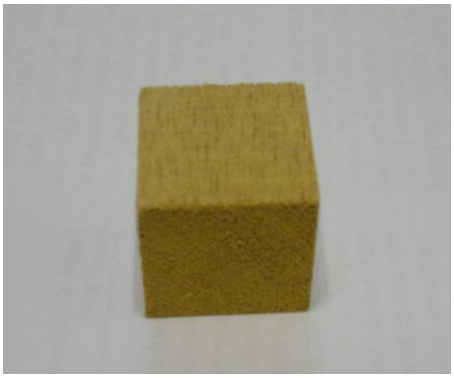


Figure 3.1. Wood cubes (19 mm x 19 mm x 19 mm) for chemical treatment.

3.3 Wood treatment

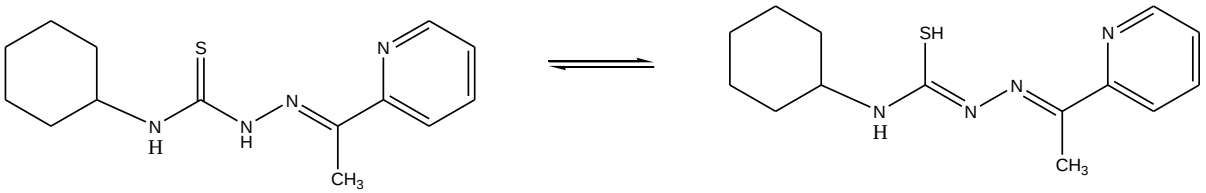
3.3.1 Preparation of wood preservatives organotin(IV) complexes

Five newly synthesized organotin(IV) compounds were used as wood preservatives. The five compounds used were 2 monosubstituted and 3 disubstituted organotin(IV) compounds as shown in Table 3.1;

Table 3.1. Monosubstituted and Disubstituted organotin(IV) compounds used for treatment.

Compound	Formula	Abbreviated name	Molecular weight	Tin (%)
Monosubstituted organotin(IV) compounds				
Monomethyltin	$MeSnCl_2$(APCT)	MMT	472.83	25.11
Monophenyltin	$PhSnCl_2$(APCT)	MPT	534.89	22.19
Disubstituted organotin(IV) compounds				
Dimethyltin	Me_2SnCl(APCT	DMT	469.42	25.29
Diphenyltin	Ph_2SnCl(APCT)	DPT	593.55	19.99
Dibutyltin	Bu_2SnCl(APCT)	DBT	553.57	21.44

Details of organotin(IV) compound synthesis can be found in Affan *et al.* (2011). In this study, 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (APCT) was used as chelating ligand which is white in colour. All organotin(IV) complexes are yellow in colour except DBT. DBT is light reddish in colour. Structure of 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (APCT) is given in Figure 3.2.

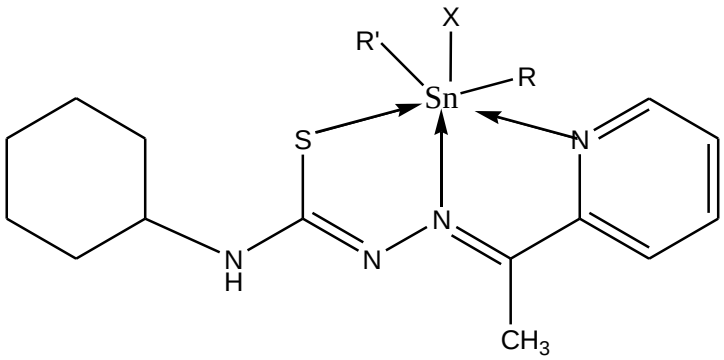


Thione form

Thiol form

Figure 3.2. Structure of 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (APCT) ligand (Affan *et al*., 2011; Salam *et al*., 2013).

Structure of mono- and disubstituted organotin(IV) complexes with 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (APCT) as demonstrated by Affan *et al*. (2011) is given in Figure 3.3.



R = Cl, R' = Cl, X = Me (MMT)
R = Cl, R' = Cl, X = Ph (MPT)
R = Me, R' = Me, X = Cl (DMT)
R = Bu, R' = Bu, X = Cl (DBT)
R = Ph, R' = Ph, X = Cl (DPT)

Figure 3.3. Structure of mono- and disubstituted organotin(IV) complexes with 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (APCT) ligand (Affan *et al*., 2011).

3.3.2 Preparation of organotin(IV) solution

Three levels of concentration (0.1, 0.5 & 1%) of organotin(1V) complexes were prepared for treatment. The organotin(IV) complexes were dissolved in solution of 20% dimethylsulphoxide (DMSO) and 80% distilled water. Twenty percent dimethylsulphoxide (DMSO) and 80% distilled water were prepared without the organotin(1V) complexes as the treating solution for control treatments.

3.3.3 Treatment of wood cubes with organotin(IV) complexes

Production of heartwood in *H. brasiliensis*, *A. scholaris* and *M. triloba* is doubtful as these are known as sapwood species. However by removing at least three cm of the outermost portion of the board and utilizing the inner portion, the rest wood sample are considered to be comprised of heartwood. Inner wood was selected the remaining 14 cm annulus is sampled to wood cubes. Ten replicates of wood cubes were used for each treatment. Treatments were carried out according to the AWPA standard E10-91 (1991) with slight modifications. All wood cubes were placed inside the beaker containing the treating solution and soaked for two hours. The beakers containing the wood cubes were then placed inside a vacuum-pressure unit. The treatment schedule was done with an initial vacuum of 100 mm Hg for 30 minutes followed by 100 psi of pressure for 1 hour and a final vacuum of 100 mm Hg for 30 minutes. After treatment, the wood cubes were taken out and the excess treating solutions on the surface of the wood cubes were wiped with tissue paper and weighed (W_2). Treatment unit of wood samples is shown in Figure 3.4.

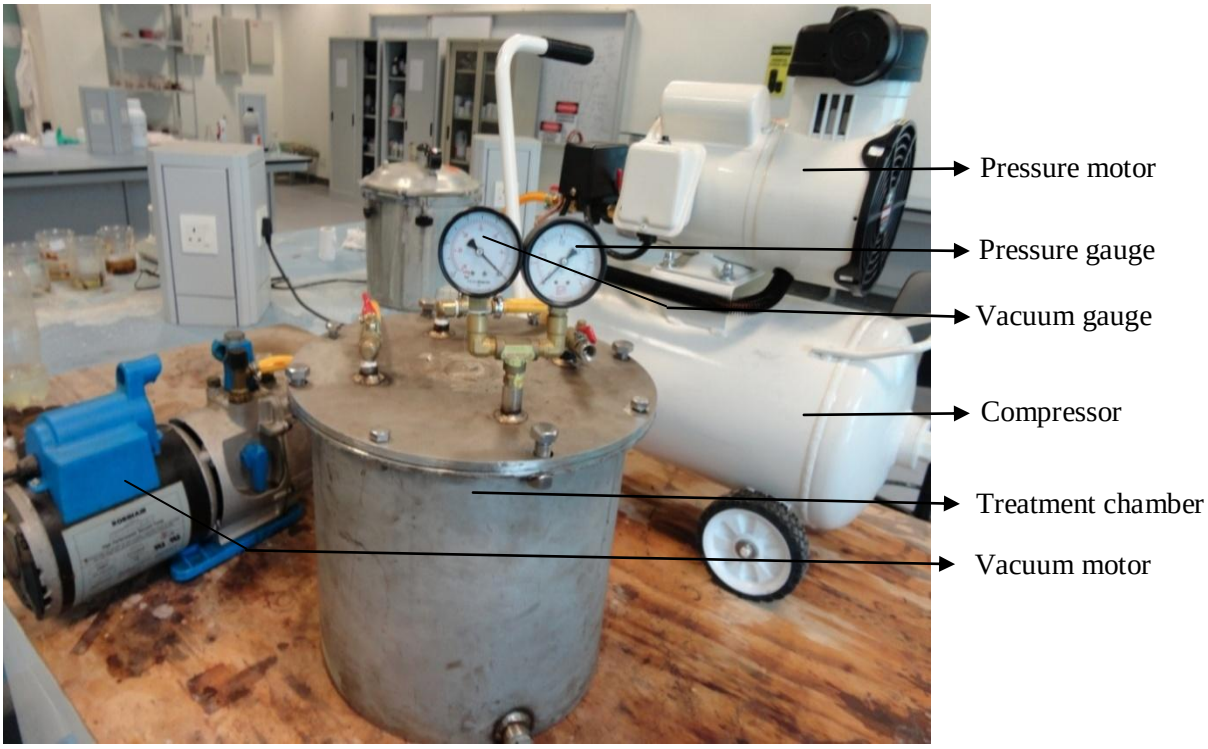


Figure 3.4. Vacuum-pressure or full-cell treatment unit for impregnation of wood cubes.

3.3.4 Determination of chemical retention

The uptake of organotin(IV) complexes by wood were obtained by using weight of wood after treatment (W_2) minus the weight of wood before treatment (W_1). The preservative retentions of organotin(IV) complexes in wood were calculated by the formula below shown in equation 1 according to AWPA standard E10-91 (1991).

$$R = \frac{GC}{V} \text{ x } 10 \qquad (1)$$

Where,

R= retention (kg m^{-3})

G = (W_2 - W_1) = net weight gain after the treatment (g)

C = concentration of treating solution (%)

V = Volume of wood cube (cm^3)

W_1 = Weight of cube before treatment (g)

W_2 = Weight of cube after treatment (g)

3.4 Decay test

3.4.1 Soil block test

Decay test was done using soil block test according to American Wood-Preservers' Association (AWPA) standard E10-91 "Standard method of testing wood preservatives by laboratory soil-block cultures" (AWPA, 1991) with few modifications. Approximately 200 g forest soils of about 130% moisture content were filled into the plastic bags. All the bags were autoclaved at 121°C for 20 minutes. The wood cubes were sterilized by exposing them to UV light for 1 hour in a laminar flow. Filter paper strips were dipped in two percent MEA to provide nutrients to fungi and placed on top of the soil. Then square-size wire mesh was placed on the filter paper strips. The treated wood cubes were placed above the wire mesh and two fungi culture plugs were placed at both sides of the wood samples on the filter paper strips. One wood cube was placed in one plastic bag. Subsequently, the plastic bags containing the treated sample cubes were kept at room temperature. *Hevea brasiliensis* blocks were used as reference to monitor decay progress. The decay test was terminated after 16 weeks when the reference blocks recorded a weight loss of more than 60%. Untreated sample cubes were used to serve as control. Soil block test is shown in Figure 3.5.

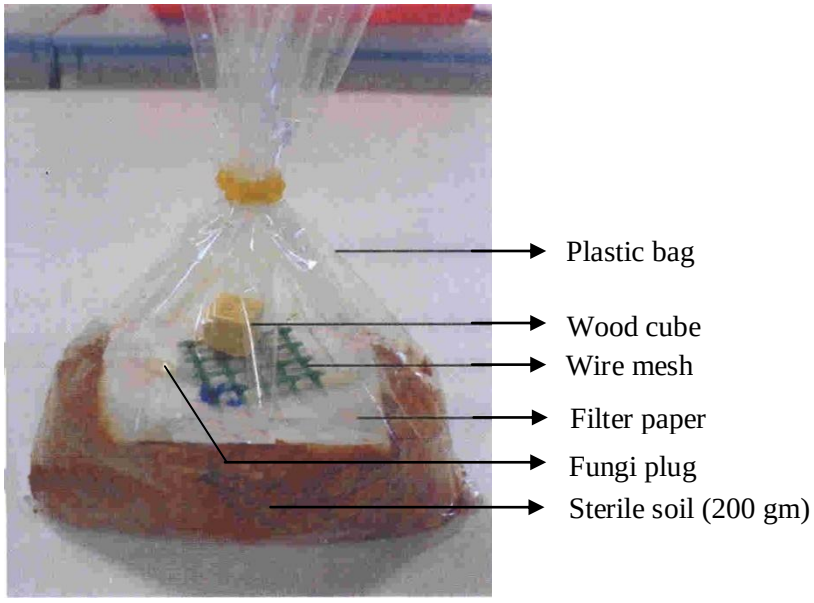


Figure 3.5. Soil block test.

3.4.2 Preparation of wood decay fungi

Two percent malt extract agar (MEA) was prepared and used as a growth media for the test fungi. Sterilization of malt extract agar was carried out by autoclaving at 121°C for 20 minutes. The cooled MEA solution was poured into petri dishes and inoculated with test fungi when the MEA was solidified.

Laboratory pure strains of *Trametes versicolor* (white rot) and *Gloeophyllum trabeum* (brown rot) were used as test fungi. Both of fungi strains were obtained from the Forest Products Technology Division, Forest Research Institute of Malaysia (FRIM). Each fungus was cultured in five petri dishes and incubated for two weeks at 25°C and 70% humidity. Actively growing fungi as shown by their rapid diameter growth were used for decay test.

3.4.3 Determination of weight loss

The degree of fungal attack was estimated by determining the weight loss after 16 weeks of incubation period. At the end of the incubation period for 16 weeks, the sample cubes in the plastic bag was cleaned from the mycelium of the fungi and weighed immediately (W_4) to determine the moisture content after decay of fungi. Test cubes were removed and the mycelium was wiped from the cube surfaces; the cleaned cubes were dried and conditioned at 60°C and 70% relative humidity until constant weight. The cubes were dried in oven at 60°C until constant weight. The weights of sample cubes after drying (W_5) were recorded to determine weight loss after decay. The weight losses of wood cubes were calculated based on the following equation 2.

$$\text{Weight loss}\,(\%) = \frac{W_3 - W_5}{W_3} \text{ x } 100 \qquad (2)$$

Where,

W_3 = Weight of cube immediately after treatment, after conditioned at 60 °C, prior exposure to fungi

W_5 = Conditioned (60 °C) weight of cube after exposure to fungi

3.4.4 Determination of wood moisture content and density

The moisture content (MC) of the test cubes were calculated based on the water contained in the cubes, expressed as a percentage of the conditioned weight of the cubes. All data were subjected to the statistical analysis. The moisture contents of wood cubes were calculated based on the following equation 3.

$$\text{Moisturecontent}\,(\%) = \frac{W_2 - W_4}{W_2} \text{ x } 100 \qquad (3)$$

Where,

W_2 = Weight of cube immediately after treatment (after wiped)

W_4 = Weight of cube immediately after exposure to fungi

Wood density was calculated using the ratio of weight per unit volume. The volume of the wood cubes was determined using water displacement method (Bowyer *et al.*, 2003). The weights (W_1) and volume (V_1) of sample cubes after conditioned at 60 °C and 70% relative humidity were recorded. Densities of wood cubes were calculated before and after exposed to decay fungi using equation 4 and expressed in kg m^{-3}.

$$\text{Density of wood}\,(\text{kg/m}^3) = \frac{\text{mass of wood}\,(\text{g})}{\text{volume of wood}\,(\text{cm}^3)} \text{ x } 1000 \qquad (4)$$

3.4.5 Threshold retention determination

The threshold value was determined according to the AWPA standard (AWPA E10-91, 1991). The weight losses of treated wood cubes were plotted against retention values to determine the DBT threshold for *H. Brasiliensis, A. Scholaris* and *M. triloba* exposed to *T.*

versicolor and *G. trabeum* following soil block test. Threshold retention point for DBT was determined as it demonstrated the greatest protection to all wood species against both fungi. Threshold were not calculated for all chemicals due to the limited number of concentration steps used in the test. Besides weight losses data for DMT, DPT, MMT and MPT were high even at the highest level concentration of 1%, which make threshold determinations not possible. The threshold value of DBT was able to be determined because the soil block test data demonstrated a broken-line model (Gezer *et al*., 1999) which consists of two lines: a straight line with negative slope as a result of weight loss due to decay decreased as retention level increased and a horizontal line due to operational weight loss. The intersection of these two lines is defined as the threshold retention point.

3.5 Fourier Transform Infrared (FTIR) spectroscopy

3.5.1 Preparation of wood samples for FTIR

Fourier transform infrared spectroscopy analysis was used to determine the chemical bonding between wood molecules and organotin(IV) complexes following treatment. Air dried untreated and treated wood cubes were ground into fine powder. Then the samples powder was compressed at pressure 9 tonne, with the ratio of 1 wood sample over 100 potassium bromide (KBr) medium.

3.5.2 FTIR spectroscopy analyses of organotin(IV)-treated wood

Fourier Transform Infrared (FTIR) analysis was performed on Perkin Elmer Spectrum GX Fourier-Transform spectrometer equipped with a micro sample holder. Potassium bromide (KBr) powder was used to establish the background. Untreated and treated wood

samples with organotin(IV) complexes were air-dried prior to mixing with KBr. Spectra of the samples were collected using diffuse Fourier transform infrared spectroscopic technique (DRIFT). Spectra were collected for a total of 64 scans on 370 to 4000 cm^{-1} wave number range with a resolution of 4 cm^{-1}. All spectra were displayed in absorbance and limited to 370 – 4000 cm^{-1} region.

3.6 Scanning electron microscopy − energy dispersive X-ray analysis (SEM-EDXA)

3.6.1 Preparation of wood samples for SEM-EDXA

Scanning electron microscopy in conjunction with energy dispersive X-ray analysis (SEM-EDXA) was performed to determine the microdistribution of organotin in wood cell following treatment. Small blocks (Figure 3.6) of approximately 3 mm sized from treated wood cubes were cut perpendicular to each other from the transverse section. A clear-cut surface of the strips was prepared by using razor blade (Exley *et al.*, 1974). The samples were mounted on aluminum stub using double-sided adhesive tape and then sputter-coated with platinum for 60 seconds. JEOL JFC-1600 Auto Fine Coater was used for coating the sample.

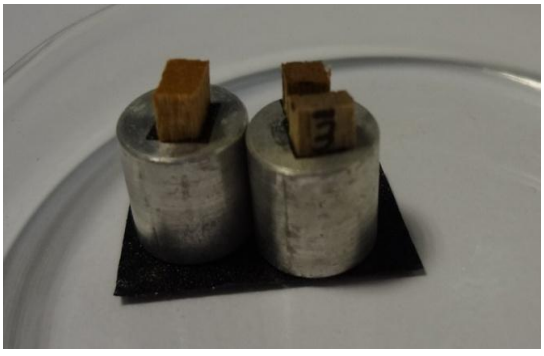


Figure 3.6. Wood sample for SEM-EDXA.

3.6.2 SEM-EDXA for microdistribution of tin in wood cells

SEM-EDXA was carried out to provide information on the distribution of organotin(IV) in treated wood cells. Wood cubes treated with 1% organotin(IV) complexes were selected for SEM-EDXA. Three small stripes of 3 mm cut from the transverse section using razor blade (Exley *et al.*, 1974). The samples were mounted on aluminum stub using double-sided adhesive tape and sputter coated with platinum and examined using a JEOL JSM-6390LA analytical scanning electron microscope (MP 14400035) fitted with Noran Vantage Energy dispersive X-ray system. Operating and analyses conditions were standardized using a light element detector capable of detecting elements to atomic number 5. A 20 kV accelerating voltage with 128 x 96 resolution pixels were used to examine the samples. Gross distribution pattern of tin was obtained by X-ray mapping. The map consists of bright dots and dot density is used as qualitative measure of the concentration of tin. Linescan analyses were performed on two adjacent fibre cell walls. The analysis provides information on the distribution of tin within a fibre cell wall. This study confined to examine organotin distribution in fibre cell wall, since fibre make up the bulk of tropical wood. However future studies should include all cell types. X-ray intensities were measured by counting number of X-ray detected per second.

3.7 Leaching test

3.7.1 Evaluation of the release of tin during water leaching

Leaching tests were carried out on cubes treated with 1% MPT, DMT and DBT. Six 1%-treated wood cubes were air-dried in laboratory ambient condition to reach about 10% moisture content prior to the water leaching exposure. Evaporation tests were not carried out

due to time constraint and it should be done in future studies. The six cubes were leached with 300 ml deionized water according to AWPA standard E11-97 (1997) procedure. After six hours, the leachate water was removed from each sample and replaced with 300 ml fresh deionized water. The leaching water was exchanged after 1 day, 3 days, 5 days, 7 days, 9 days, 11 days and 13 days intervals for a total of 14 days.

3.7.2 Determination of tin in leachates

The tin level in leachates was analyzed by Flame operation - Atomic Absorption Spectrometer (iCE 3500 Spectrometer, Flame type-nitrous oxide/acetylene). Each leachates were analyzed immediately upon collection. The Spectrometer was adjusted to atomic no. 50, primary wavelength 224.6 nm, emission wavelength 284.0 nm, flame characteristics concentration 0.5 mg/L, fuel flow rate 4.5 L/min and burner height 3 mm for detection of tin.

3.8 Bioassay for enzymes activities

3.8.1 Microorganisms, culture condition and wood cubes biodegradation

Two hundred milliliter of malt extract broth (MEB) was prepared and used as a growth media for the test fungi. Sterilization of MEB was carried out by autoclaving at 121°C for 20 minutes. The cooled MEB solution was used as growth media for test fungi.

Laboratory pure strains of *T. versicolor* and *G. trabeum* used in the decay test were used in the enzyme bioassay. Twenty mycelium blocks from each fungus was cultured and maintained unshaken for two weeks at 25 °C. Grown mycelium mat were separated from broth using centrifuge. Centrifuge was adjusted at 3000 rpm under 4 ºC for 15 minutes. Wood

cubes were kept under UV light for 1 hour before exposing to test fungi. Separated mycelium was used to inoculate the selected wood cubes in flask (de Souza-Cruz *et al.*, 2004).

Ten replicates for untreated and six replicates for treated wood cubes (19 mm x 19 mm x 19 mm) were used to test the enzyme assay. Each beaker was loaded with one wood cube and 10 mg of homogenate mycelium. Cultures were maintained static at 25 ºC for period of 16 weeks. Untreated inoculated wood cubes were used to serve as controls (de Souza-Cruz *et al.*, 2004).

3.8.2 Enzymes extraction from wood cubes

After 16 weeks incubation, the wood cubes were subsequently extracted with 25 ml of extraction solution in 100 ml Erlenmeyer flask (50 mM sodium acetate buffer (pH 5.0) and 0.01% Tween 80) at 140 rpm (Ferraz *et al.*, 2003). Extraction was performed for 2 hrs at 20 ºC ± 2 ºC. The crude extract was recovered by centrifuge at 8000 rpm under 4 ºC for 15 mins. Extracts obtained were then subjected to oxidative enzymes assays.

3.8.3 Enzymes activities

Lignin peroxidase (LiP) activity was measured as the oxidation of veratryl alcohol (VA) to veratryl aldehyde with an increased absorbance at 310 nm. Reaction mixtures contained 50 mM sodium tartrate buffer, pH 2.5, 2.0 mM VA, 450 µl samples and 0.4 mM H_2O_2. Reaction was initiated by the addition of H_2O_2. An extinction coefficient of 9300 $M^{-1}cm^{-1}$ for veratryl alcohol was used for calculation of enzyme turnover number. One unit

activity was defined as the amount of enzyme oxidizing one μmol of substrate per minute (Tien and Kirk, 1988).

Manganese peroxidase (MnP) activity was determined by monitoring the oxidation of 2,6-dimethoxyphenol (DMP) and measured as the oxidation of Mn^{2+} to Mn^{3+} by following the formation of Mn^{3+}-tartrate complex at 469 nm. Reaction mixtures contained 1 mM 2,6 dimethoxyphenol (DMP), 50 mM sodium tartrate buffer, pH 4.5, 1 mM $MnSO_4.H_2O$, 600 μl samples and 0.4 mM H_2O_2. An extinction coefficient of 10,000 M^{-1} cm^{-1} for Mn^{3+}-tartrate complex will be used for calculation of enzyme turnover number. One unit activity is defined as the amount of enzyme oxidizing one μmol of substrate per minute (Paszczynski *et al*., 1988).

Laccase (Lac) activity was determined using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as substrate at 420 nm. The reaction mixture contained 100 mM sodium acetate buffer, pH 5, 0.5 mM ABTS and 400 μl enzyme supernatant. An extinction coefficient of 36,000 $M^{-1}cm^{-1}$ for ABTS was used for calculation of enzyme turnover number. One unit activity is defined as the amount of enzyme oxidizing one μmol of substrate per minute (Dittmer *et al*., 1997).

3.9 Bending and compression strength

3.9.1 Preparation of wood samples for bending and compression test

Bending and compression test were carried out to investigate the mechanical properties of selected tropical wood species namely *A. scholaris, M. triloba* and *H. brasiliensis* treated with novel organotin(IV) complexes. Wood samples treated with 1%

organotin(IV) complexes were selected for bending and compression test. Prior to treatment, clear, defect-free planks were ripped and cut to obtain sample size of 300 mm (L-longitudinal), 19 mm (T-tangential) and 19 mm (R-radial) for three point bending test. Same planks were cut to obtain sample size of 60 mm (L-longitudinal), 19 mm (T-tangential) and 19 mm (R-radial) for compression parallel to grain test. Both the tests were carried out according to ASTM D-143 (1996). All samples both untreated and treated with organotin(IV) complexes were kept in the testing room environment prior to testing and test was done under air-dry condition. The moisture content of the test samples were 15%.

3.9.2 Bending and compression parallel to grain test

Untreated and treated wood samples with 1% organotin(IV) complexes were tested for bending and compression parallel to grain strength. Three-point bending test was carried out according to ASTM D-143 (1996) using a mechanical testing apparatus AUTOGRAPH, Shimadzu Universal Testing Machine having a loading capacity of 300 kN. The modulus of elasticity (MOE) and modulus of rupture (MOR) the three-point bending test were calculated using equations 5 and 6, respectively.

$$MOE = \frac{L^3 m}{4bd^3} \quad (5)$$

$$MOR = \frac{1.5PL}{bh^2} \quad (6)$$

Where

L = span length of sample, 200 mm

b = width of sample, 19 mm

d = thickness of sample, 19 mm

m = slope of tangent to the initial line of the force displacement curve

P = maximum breaking load

h = depth of the beam

Compression parallel to grain test was conducted as specified in ASTM D-143 (1996) in an attempt to measure compressive Young's modulus of wood samples. Only compressive strength was determined using the uniaxial compression test. A mechanical testing apparatus AUTOGRAPH, Shimadzu Universal Testing Machine having a loading capacity of 300 kN was used for the test with the cross head speed of 2 mm/min. The compressive strength was calculated using the following equation.

$$E = \frac{Stress}{Strain} \qquad (7)$$

$$E = \frac{F/A}{\Delta L/L}$$

Where

F = force

A = cross sectional area

ΔL = displacement

L = length of sample

$$F = \frac{(EA)}{L} \times \Delta L \qquad (8)$$

The gradient of graph F vs ΔL, $m = \frac{EA}{L}$

3.10 Data analysis

3.10.1 Chemical retention

One-way analysis of variance (ANOVA) was used to determine the differences between mean values of chemical retention of different wood species using different concentrations of chemicals. Analyses were done using statistical program SPSS-18.0 for windows. One-way ANOVA was appropriate in this study because the experiment was carried out in batches. The first batch was experimentation with DPT followed by DBT, DMT, MMT and MPT. In each batch, chemical concentrations and experimental conditions were kept the same. Further analyses of mean comparisons were done using Tukey Multiple Comparison test. The factors which were analyzed are types of chemicals and wood species. The dependent variable was chemical retention.

3.10.2 Weight loss, moisture content and density

Analysis of variance on weight loss, moisture content and density of wood cubes were used to determine the mean differences among the wood species and different concentrations of chemicals using SPSS 18.0 for windows. The factors which were analyzed are chemical concentrations and wood species.

3.10.3 Lignin peroxidases, manganese peroxidases and laccases

One-way analysis of variance was used to determine the differences between mean values of lignin peroxidases, manganese peroxidases and laccases of different wood species using different concentrations of chemicals. The SPSS-18.0 was used for analysis. Further

analyses of mean comparisons were done using Tukey Multiple Comparison test. The factors which were analyzed are concentrations of chemicals and wood species.

3.10.4 MOE, MOR and compressive strength

Analysis of variance was performed to determine the differences among mean values of modulus of elasticity (MOE), modulus of rupture (MOR) and compressive strength (CS) of wood samples following treatment of different organotin(IV) complexes. Further analyses of mean comparisons were done using Tukey Multiple Comparison test. The factors analyzed were chemical types.

CHAPTER FOUR

RESULTS

4.1 Preservative retention

The preservative retention of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood using monosubstituted of organotin(IV) (namely MMT & MPT) and disubstituted of organotin(IV) (namely DMT, DPT & DBT) compound of each three levels of concentration (0.1, 0.5 & 1%) was statistically analyzed to determine the treatability of wood species and the results are shown in Table 4.1.

Table 4.1. Average chemical retention values (kg m^{-3}) in *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* heartwood following treatment with mono- and disubstituted of organotin(IV) at three levels of concentration.

Treating chemicals		*H. brasiliensis*	*M. triloba*	*A. scholaris*
Monosubstituted organotin(IV)	N	Chemical retention values (kg m^{-3})		
MMT				
0.1%	10	0.58* (0.04) a	0.77 (0.02) b	0.84 (0.02) c
0.5%	10	3.27 (0.17) a	3.62 (0.14) b	4.59 (0.10) c
1%	10	5.62 (0.13) a	7.31 (0.14) b	7.48 (0.17) c
MPT				
0.1%	10	0.57 (0.04) a	0.77 (0.02) b	0.89 (0.02) c
0.5%	10	3.33 (0.14) a	3.59 (0.18) b	4.39 (0.02) c
1%	10	5.76 (0.12) a	7.70 (0.11) b	7.90 (0.14) c

Continue Table 4.1

Disubstituted organotin(IV)				
DMT				
0.1%	10	0.47 (0.06) a	1.00 (0.02) b	1.00 (0.03) b
0.5%	10	2.80 (0.63) a	4.62 (0.15) b	5.65 (0.21) c
1%	10	5.65 (0.14) a	9.58 (0.17) b	10.59 (0.15) c
DPT				
0.1%	10	0.52 (0.08) a	0.97 (0.02) b	0.96 (0.03) b
0.5%	10	2.58 (0.11) a	4.52 (0.15) b	4.62 (0.15) b
1%	10	5.34 (0.11) a	7.61 (0.12) b	7.94 (0.13) c
DBT				
0.1%	10	0.62 (0.03) a	0.83 (0.02) b	0.81 (0.02) b
0.5%	10	3.35 (0.13) a	4.37 (0.12) b	4.91 (0.15) c
1%	10	6.05 (0.23) a	8.06 (0.16) b	9.49 (0.20) c

Values in parenthesis are the standard deviation.

MMT- Monomethyltin(IV) complex, MPT-Monophenyltin(IV) complex, DMT- Dimethyltin(IV) complex, DPT- Diphenyltin(IV) complex, DBT- Dibutyltin(IV) complex.

*Means followed by a different letter within a row are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

n – Number of sample

The mean preservative retentions varied significantly among three wood species (Table 4.1). *Alstonia scholaris* gained higher chemical retention uptake than *Macaranga triloba* and *Hevea brasiliensis* wood for all level of preservatives concentration. *Hevea brasiliensis* showed least chemical retention uptake than *Macaranga triloba* and *Alstonia scholaris*. Differences in preservatives uptake indicated that treatability differ among wood species. The highest (10.59 kg m^{-3}) and lowest (0.47 kg m^{-3}) retention uptake was observed in *Alstonia scholaris* and *Hevea brasiliensis* wood, respectively.

Results also showed that increased in concentration from 0.1 to 1% resulted in increased retention in all wood species. The mean preservatives retention following increased

in treating concentration varied from 0.81 kg m^{-3} to 10.59 kg m^{-3} in *Alstonia scholaris* for all treating chemicals.

The retention following treatment with 1% organotin(IV) in each wood species is illustrated in Figure 4.1. In this study higher retention was observed with disubstituted preservatives (DMT and DBT) in *Alstonia scholaris* and *Macaranga triloba* than the monosubstituted preservatives. Among monosubstituted 1% MPT showed significantly higher retention than MMT in *Alstonia scholaris* and *Macaranga triloba*. However in *Hevea brasiliensis* the difference of retention between MPT and MMT was not significant.

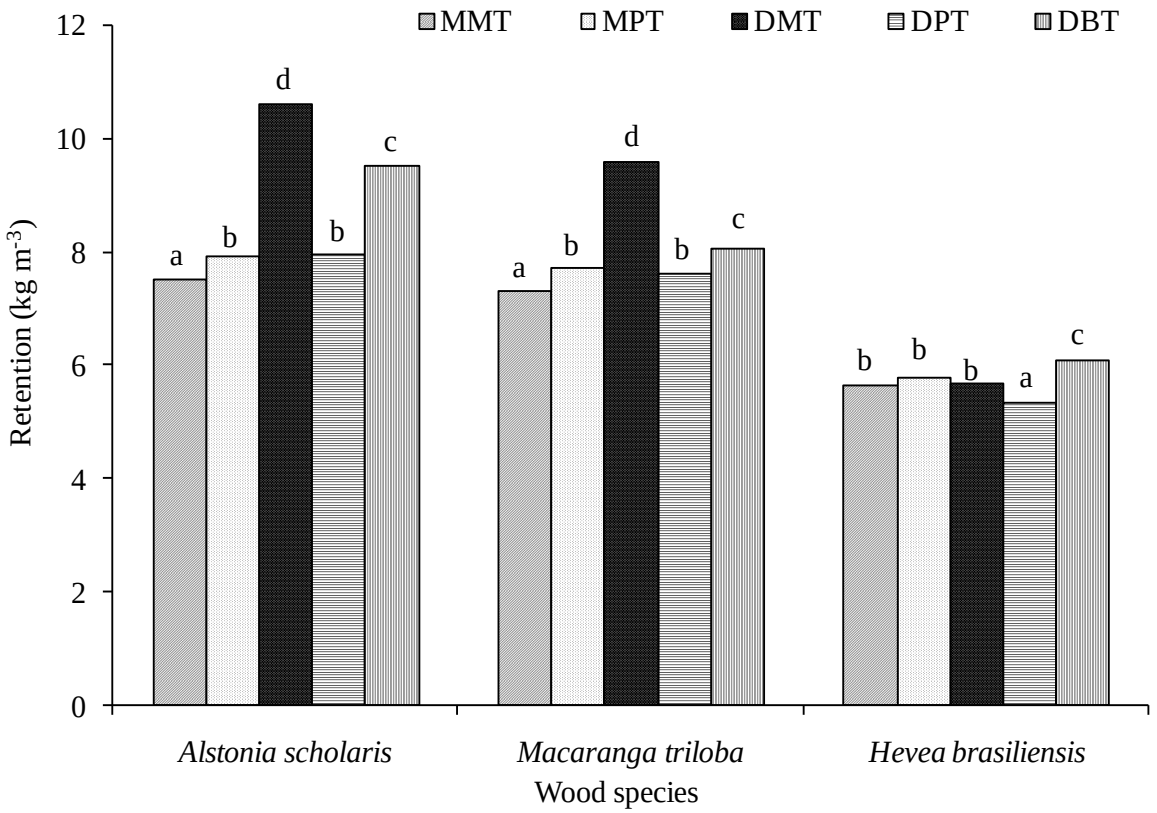


Figure 4.1. Chemical retention of 1% organotin(IV) treated wood species.

Among disubstituted of 1% organotin(IV), DMT gave significantly higher retention than DPT and DBT in *Alstonia scholaris* and *Macaranga triloba*. But in *Hevea brasiliensis* DBT gave significantly higher retention than DMT and DPT.

4.2 Decay test

4.2.1 Decay of untreated wood cubes

Weight loss due to decay of untreated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood cubes that were exposed to *Trametes versicolor* and *Gloeophyllum trabeum* for 16 weeks are summarized in Table 4.2.

Table 4.2. Mean weight loss (WL), moisture content (MC) and density (D) of untreated wood cubes after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Wood species	n	*Trametes versicolor*				*Gloeophyllum trabeum*			
		WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})	WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})
Alstonia scholaris	10	53* a (2.53)	64 b (1.20)	361 a (5.37)	173 a (3.87)	52 a (2.99)	45 a (1.45)	362 a (3.83)	174 a (5.25)
Macaranga triloba	10	51 a (3.20)	62 a (1.30)	410 b (5.24)	194 b (3.23)	51 a (3.21)	45 a (1.90)	408 b (4.99)	192 b (6.49)
Hevea brasiliensis	10	65 b (2.76)	68 c (0.69)	658 c (4.93)	227 c (4.84)	61 b (2.95)	51 b (2.03)	659 c (4.19)	225 c (3.55)

Values in parentheses are the standard deviation
*Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.
**Density of wood cubes before exposure to decay fungus
***Density of wood cubes after exposure to decay fungus
n – Number of sample

The untreated wood cubes weight losses varied from 51 to 65% after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*. Mean weight loss of untreated *Hevea brasiliensis* wood cubes was significantly higher than *Macaranga triloba* and *Alstonia scholaris*. The lowest (51%) and highest (65%) weight losses of untreated wood cubes due to decay recorded with *Gloeophyllum trabeum* in *Macaranga triloba* and *Trametes versicolor* in *Hevea brasiliensis*, respectively.

Mean moisture content (MC) of untreated wood cubes ranged from 45 to 68% after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*. Mean MC was significantly different among wood species after exposure to *Trametes versicolor* while exposure to *Gloeophyllum trabeum* mean MC of *Alstonia scholaris* and *Macaranga triloba* was similar (Table 4.2). Mean wood density before and after exposure to decay fungi were significantly different among the wood species (Table 4.2). Mean densities of untreated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* were 362, 409 and 658 kg m^{-3}, respectively.

4.2.2 Decay of organotin(IV)-treated wood cubes

Data for mean percentage weight loss, moisture content and density of treated wood cubes with mono- and disubstituted organotin(IV) complexes after exposure to decay fungi for 16 weeks are summarized in Tables 4.3 and 4.4, respectively.

Table 4.3. Mean weight loss (WL), moisture content (MC) and density (D) of treated wood cubes with monosubstituted organotin(IV) complexes at three different retentions after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*.

Treating chemicals	Wood species	N	Retention (kg m^{-3})	*Trametes versicolor*				*Gloeophyllum trabeum*			
				WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})	WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})
MMT	*Alstonia scholaris*	6	0.84	23* c (1.55)	53 b (3.20)	357 a (3.57)	256 a (5.65)	25 c (1.71)	50 c (4.04)	355 a (4.31)	251 a (5.64)
		6	4.59	18 b (1.93)	48 ab (4.01)	360 a (5.17)	275 b (7.03)	22 b (2.35)	44 b (2.34)	359 a (6.13)	268 b (6.80)
		6	7.48	14 a (1.98)	44 a (3.94)	364 a (6.04)	289 c (5.81)	18 a (1.68)	38 a (2.78)	362 a (4.49)	288 c (6.34)
	Macaranga triloba	6	0.77	26 c (1.23)	52 a (4.01)	409 a (5.50)	306 a (7.66)	26 c (2.04)	39 b (2.32)	410 a (4.13)	299 a (6.94)
		6	3.62	21 b (1.11)	48 a (3.85)	414 a (5.39)	314 a (3.92)	21 b (1.72)	41 b (2.40)	413 a (4.75)	308 b (4.53)
		6	7.31	17 a (1.40)	50 a (4.89)	414 a (4.62)	330 b (6.73)	19 a (1.89)	31 a (4.64)	414 a (5.43)	323 c (5.20)
	Hevea brasiliensis	6	0.58	23 c (2.77)	50 c (3.36)	661 a (3.43)	511 a (6.59)	25 c (2.84)	41 b (1.75)	660 a (5.95)	509 a (5.46)
		6	3.27	19 b (1.87)	45 b (2.04)	662 a (7.26)	553 b (6.92)	19 b (1.75)	38 b (3.55)	661 a (5.68)	551 b (6.11)
		6	5.62	14 a (2.44)	38 a (2.64)	663 a (3.84)	581 c (7.34)	15 a (1.77)	32 a (5.14)	665 a (3.34)	579 c (6.50)

Continue Table 4.3

		6	0.89	20 c (2.78)	39 a (3.68)	358 a (4.81)	260 a (4.14)	23 c (4.24)	42 b (3.45)	357 a (5.94)	254 a (7.57)
	Alstonia scholaris	6	4.39	15 b (1.85)	45 a (4.46)	360 a (4.77)	284 b (4.51)	17 b (4.46)	42 b (4.05)	359 a (6.94)	270 b (3.58)
		6	7.90	11 a (1.92)	40 a (4.86)	363 a (5.48)	292 c (5.21)	11 a (2.14)	28 a (2.84)	363 a (5.27)	285 c (5.83)
		6	0.77	23 c (2.26)	39 a (3.93)	410 a (5.52)	308 a (4.36)	22 c (2.87)	39 c (2.63)	413 a (4.24)	310 a (4.85)
MPT	*Macaranga triloba*	6	3.59	16 b (2.01)	45 a (5.40)	415 a (5.26)	318 b (7.16)	16 b (2.23)	43 b (3.32)	414 a (4.81)	315 a (7.99)
		6	7.70	10 a (2.35)	39 a (3.39)	416 a (2.94)	331 c (7.02)	10 a (1.55)	29 a (2.56)	414 a (4.78)	330 b (6.73)
		6	0.57	24 c (2.21)	44 b (4.84)	660 a (3.63)	513 a (6.06)	20 b (2.75)	39 b (3.93)	660 a (3.73)	515 a (4.30)
	Hevea brasiliensis	6	3.33	13 b (2.11)	42 b (3.69)	661 a (5.91)	558 b (3.08)	16 b (2.95)	43 b (3.61)	663 a (5.91)	556 b (7.04)
		6	5.76	10 a (1.24)	31 a (2.61)	664 a (3.83)	586 c (9.03)	10 a (2.21)	29 a (5.84)	665 a (3.75)	581 c (6.12)

Values in parentheses are the standard deviation. MMT- Monomethyltin(IV) complex, MPT- Monophenyltin(IV) complex. *-Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test. **-Density of wood cubes before exposure to decay fungus. ***-Density of wood cubes after exposure to decay fungus. n = Number of sample

Table 4.4. Mean weight loss (WL), moisture content (MC) and density (D) of treated wood cubes with disubstituted organotin(IV) complexes at three different retentions after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*.

Treating chemicals	Wood species	n	Retention (kg m^{-3})	*Trametes versicolor*				*Gloeophyllum trabeum*			
				WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})	WL (%)	MC (%)	D** (kg m^{-3})	D*** (kg m^{-3})
DMT	*Alstonia scholaris*	6	1.00	18* c (2.29)	38 ab (3.41)	359 a (2.26)	258 a (5.87)	19 c (3.32)	39 b (2.96)	361 a (3.26)	261 a (6.90)
		6	5.65	13 b (1.46)	43 b (3.04)	361 a (5.32)	283 b (7.96)	14 b (1.30)	38 b (4.31)	362 a (2.92)	280 b (7.65)
		6	10.59	9 a (1.89)	36 a (4.04)	364 a (1.97)	297 c (5.48)	8 a (1.29)	37 a (4.06)	364 a (4.63)	293 c (4.87)
	Macaranga triloba	6	1.00	19 c (2.02)	37 b (2.90)	412 a (2.53)	307 a (6.77)	20 c (2.39)	37 b (2.37)	412 a (1.61)	304 a (6.08)
		6	4.62	14 b (2.07)	36 b (4.95)	413 a (4.88)	318 b (5.60)	14 b (2.19)	36 b (3.79)	415 a (2.72)	317 b (2.71)
		6	9.58	8 a (1.04)	29 a (5.25)	414 a (4.13)	331 c (4.38)	8 a (1.22)	26 a (2.59)	415 a (4.82)	328 c (6.52)
	Hevea brasiliensis	6	0.47	18 c (2.65)	38 b (1.86)	661 a (3.52)	551 a (7.17)	19 c (1.72)	34 b (2.47)	662 a (4.05)	548 a (5.32)
		6	2.80	12 b (1.63)	35 b (2.04)	662 a (5.22)	561 b (6.45)	15 b (2.36)	37 b (3.16)	663 a (7.02)	562 b (5.05)
		6	5.65	8 a (1.35)	27 a (3.95)	663 a (5.21)	589 c (5.49)	8 a (1.21)	25 a (3.11)	665 a (2.37)	586 c (5.14)
	Alstonia scholaris	6	0.96	20 c (1.07)	45 c (2.12)	359 a (2.61)	267 a (3.15)	19 c (1.87)	47 c (3.65)	360 a (3.01)	269 a (5.32)
		6	4.62	13 b (1.71)	35 b (1.86)	363 a (4.48)	287 b (3.98)	15 b (1.56)	34 b (2.73)	362 a (6.25)	287 b (3.98)
		6	7.94	5 a (1.31)	26 a (1.69)	364 a (5.32)	307 c (8.53)	6 a (1.09)	26 a (2.20)	363 a (4.92)	302 c (5.39)

Continue Table 4.4

DPT		6	0.97	19 c (1.50)	41 b (4.14)	414 a (2.17)	316 a (3.83)	21 c (4.56)	40 b (4.15)	412 a (3.19)	310 a (3.57)
	Macaranga triloba	6	4.52	13 b (1.82)	32 a (5.53)	415 a (2.72)	322 a (6.27)	15 b (2.63)	29 a (4.78)	414 a (4.73)	319 a (6.33)
		6	7.61	6 a (1.27)	28 a (1.15)	415 a (6.36)	333 a (5.62)	6 a (2.02)	27 a (2.38)	414 a (4.78)	329 b (6.64)
		6	0.52	20 c (2.53)	44 c (2.14)	661 a (4.59)	552 a (6.77)	17 b (3.63)	47 b (2.33)	664 a (4.33)	554 a (7.54)
	Hevea brasiliensis	6	2.58	8 b (1.01)	35 b (2.64)	663 a (5.63)	565 b (5.34)	8 a (3.06)	30 a (6.37)	664 a (6.19)	570 b (5.17)
		6	5.34	7 a (1.08)	27 a (4.59)	666 a (3.25)	592 c (5.67)	6 a (1.49)	27 a (4.48)	665 a (3.23)	591 c (6.51)
DBT		6	0.81	13 c (2.06)	40 b (4.64)	362 a (4.22)	271 a (5.25)	16 c (2.29)	38 b (4.01)	361 a (3.71)	270 a (4.97)
	Alstonia scholaris	6	4.91	9 b (1.86)	31 a (3.48)	362 a (2.39)	290 b (4.88)	11 b (1.40)	34 b (2.74)	365 a (2.39)	287 b (3.98)
		6	9.49	4 a (1.18)	26 a (1.94)	365 a (4.65)	311 c (7.71)	4 a (1.01)	24 a (2.17)	365 a (4.13)	310 c (6.92)
		6	0.83	15 b (2.88)	36 b (2.95)	412 a (3.25)	331 a (2.75)	17 c (2.09)	35 b (4.83)	413 a (2.27)	322 a (5.53)
	Macaranga triloba	6	4.37	7 a (1.60)	28 a (4.60)	415 a (2.73)	337 a (5.23)	8 b (2.57)	27 a (5.16)	415 a (2.19)	337 b (5.23)
		6	8.06	5 a (1.47)	25 a (2.81)	415 a (5.11)	349 b (5.44)	4 a (1.24)	24 a (2.55)	415 a (3.47)	349 c (7.66)
		6	0.62	13 c (2.17)	36 b (3.14)	661 a (5.38)	555 a (7.51)	14 c (2.22)	35 b (3.62)	662 a (4.17)	553 a (7.10)
	Hevea brasiliensis	6	3.35	7 b (2.27)	27 a (3.15)	664 a (6.29)	576 b (7.59)	7 b (1.28)	24 a (2.24)	664 a (3.42)	577 b (6.96)
		6	6.05	4 a (1.37)	25 a (3.35)	664 a (3.63)	598 c (5.52)	4 a (1.38)	24 a (1.84)	666 a (2.66)	596 c (6.93)

Values in parentheses are the standard deviation. DMT- Dimethyltin(IV) complex, DPT- Diphenyltin(IV) complex, DBT- Dibutyltin(IV) complex. *Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test. **Density of wood cubes before exposure to decay fungus. ***Density of wood cubes after exposure to decay fungus. n=Number of sample

Moisture content in mono- and disubstituted organotin(IV) treated wood cubes varied from 28 to 53% and 24 to 47%, respectively, after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*. Following treatment mean densities of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* varied from 355 to 365, 409 to 416 and 660 to 666 kg m^{-3}, respectively. Whereas after 16 weeks exposure to decay fungi, the mean densities of treated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* ranged from 251 to 311, 299 to 349 and 509 to 598 kg m^{-3}, respectively.

The weight losses of treated wood cubes varied significantly with the types and concentrations of treating organotin(IV) compounds following 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*, but overall, decreased with increased chemical concentrations. This indicates that high concentration provide better protection of wood against *Trametes versicolor* and *Gloeophyllum trabeum*. Among monosubstituted of organotin(IV) complexes, the weight losses of MMT and MPT-treated wood cubes ranged from 14 to 26 and 10 to 24%, respectively (Table 4.3), whereas using disubstituted organotin(IV) complexes, DMT, DPT and DBT-treated wood cubes weight loss recorded from 8 to 20, 5 to 21 and 4 to 17%, respectively (Table 4.4).

Further analyses of weight loss were done to determine whether there are differences among chemicals. Weight loss (%) of untreated and treated wood cubes with 1%-organotin(IV) complexes after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum* among wood species are shown in Figures 4.2 and 4.3, respectively.

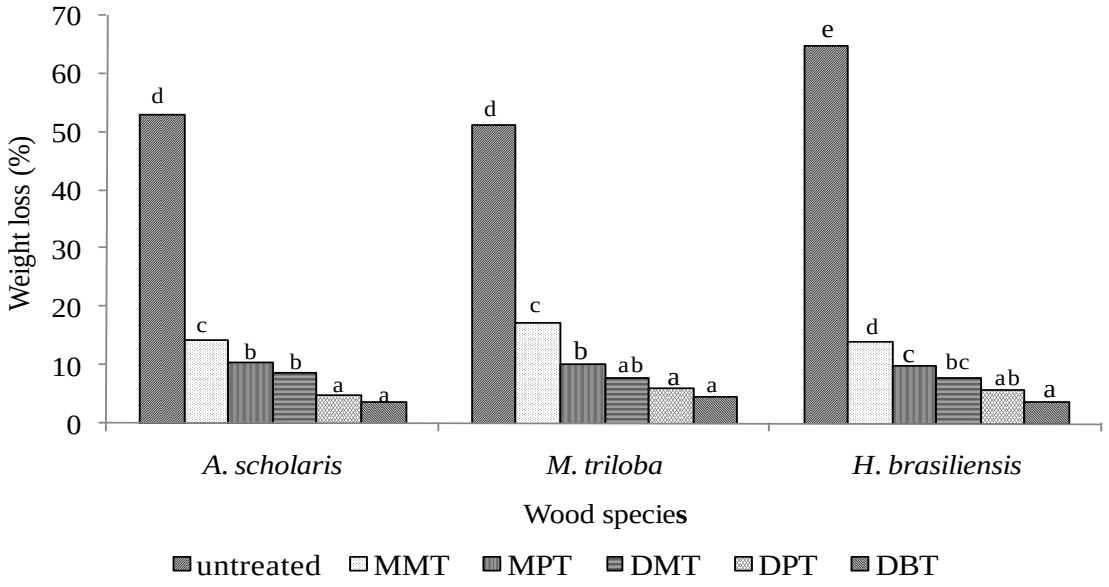


Figure 4.2. Weight loss (%) of untreated and treated wood cubes with 1% organotin(IV) complex after 16 weeks exposure to *T. versicolor*. Different letter within wood species showed that they are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

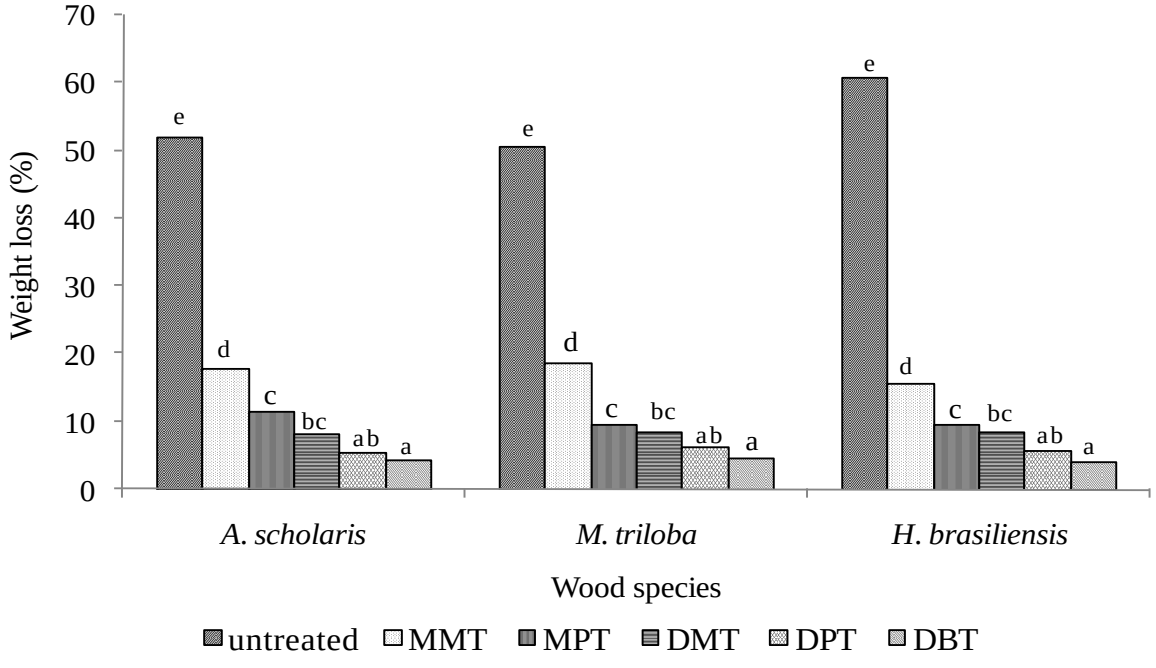


Figure 4.3. Weight loss (%) of untreated and treated wood cubes with 1% organotin(IV) complex after 16 weeks exposure to *G. trabeum.* Different letter within wood species showed that they are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

The results of 1%-treated cubes is presented here because it is the most effective among three level of concentrations. DBT showed that it can protect the wood cube against both *Trametes versicolor* and *Gloeophyllum trabeum* with the lowest weight loss. Further analyses showed that the effect of DBT and DPT is similar (Figures 4.2 and 4.3). The least effective against both *Trametes versicolor* and *Gloeophyllum trabeum* was MMT.

4.2.3 Threshold determination

The approximate threshold for unleached DBT-treated *H. Brasiliensis, A. Scholaris* and *M. triloba* exposed to *T. versicolor* and *G. trabeum* are shown in Figures 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9, respectively.

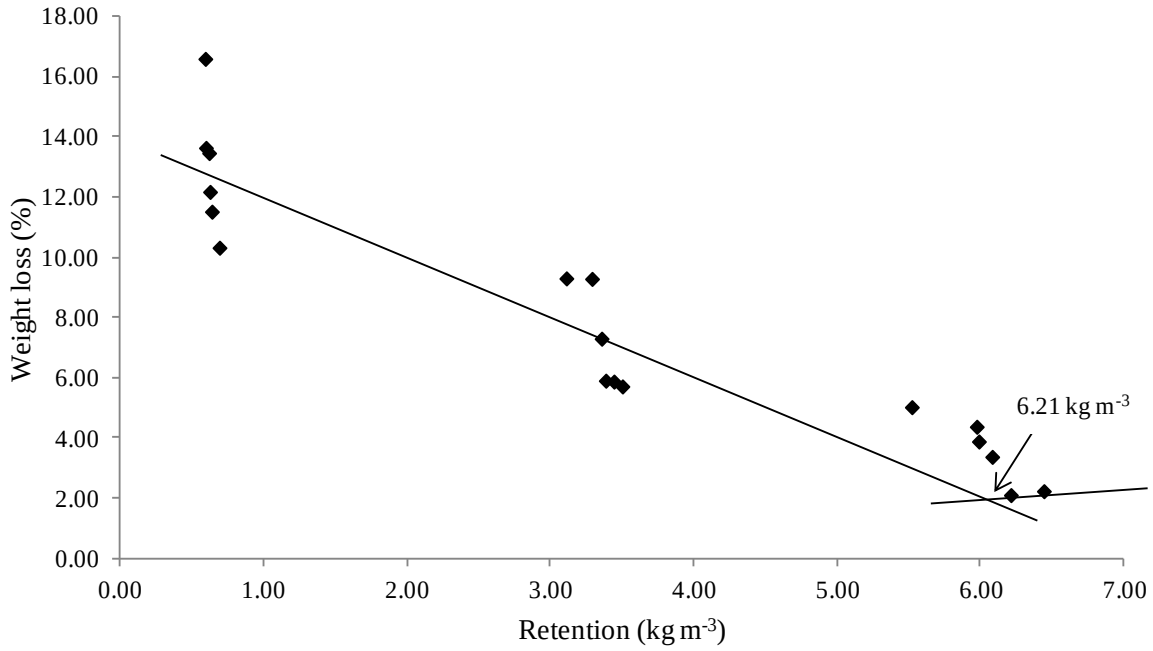


Figure 4.4. Approximate threshold for unleached DBT treated *H. brasiliensis* through soil block test exposed to *T. versicolor*. DBT- Disubstituted butyltin(IV) complex.

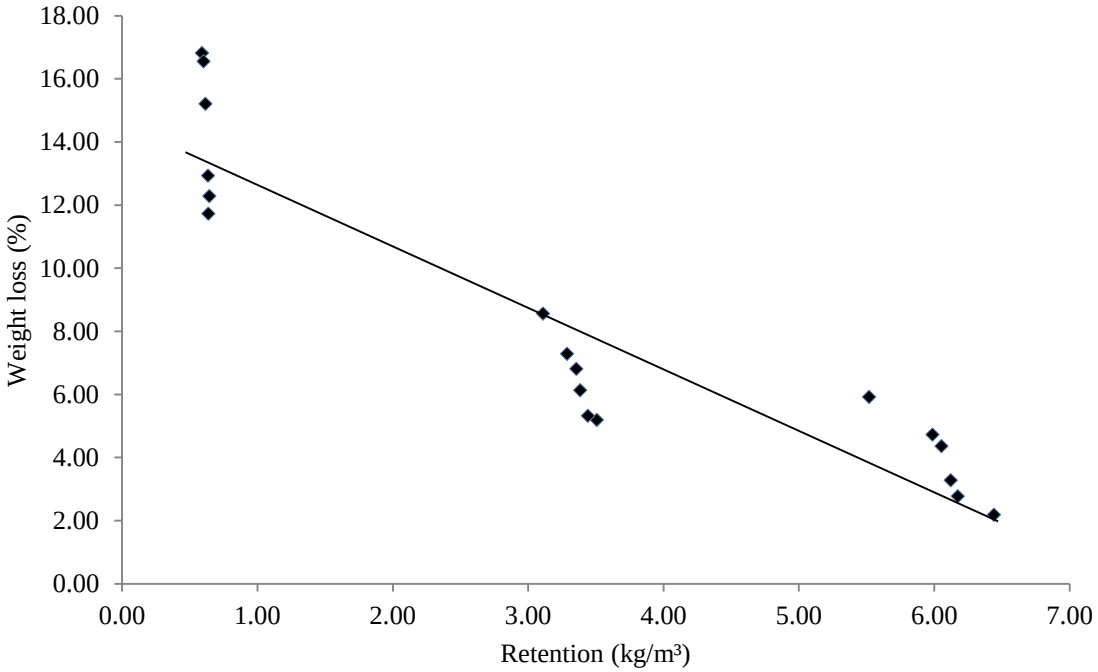


Figure 4.5. Approximate threshold for unleached DBT treated *H. brasiliensis* through soil block test exposed to *G. trabeum*. DBT- Disubstituted butyltin(IV) complex.

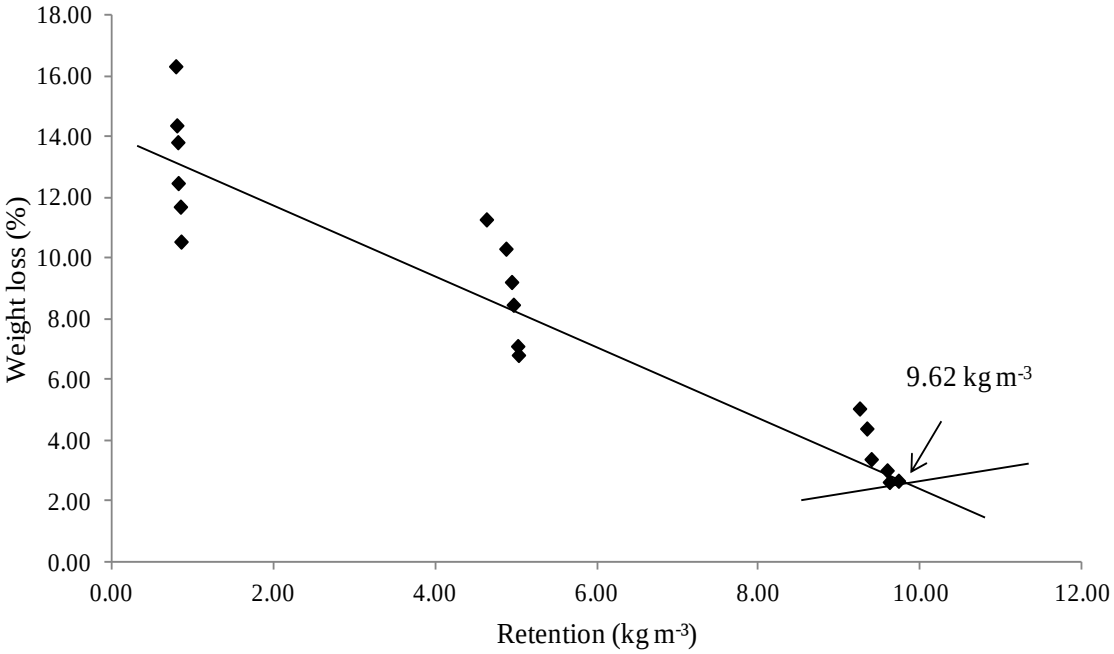


Figure 4.6. Approximate threshold for unleached DBT treated *A. scholaris* through soil block test exposed to *T. versicolor*. DBT- Disubstituted butyltin(IV) complex.

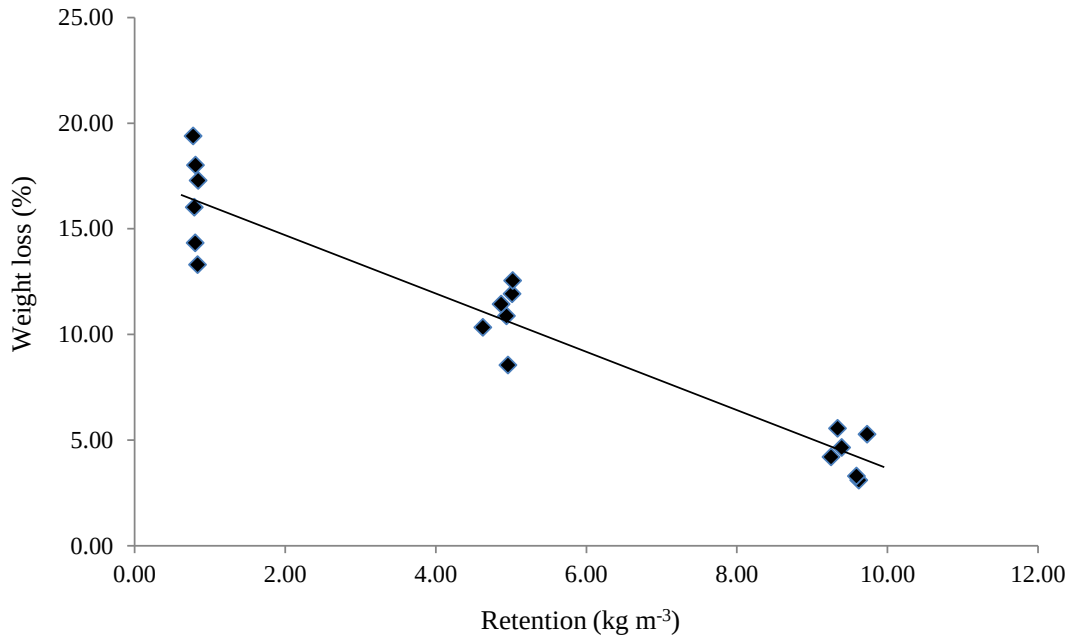


Figure 4.7. Approximate threshold for unleached DBT-treated *A. scholaris* through soil block test exposed to *G. trabeum*. DBT- Disubstituted butyltin(IV) complex.

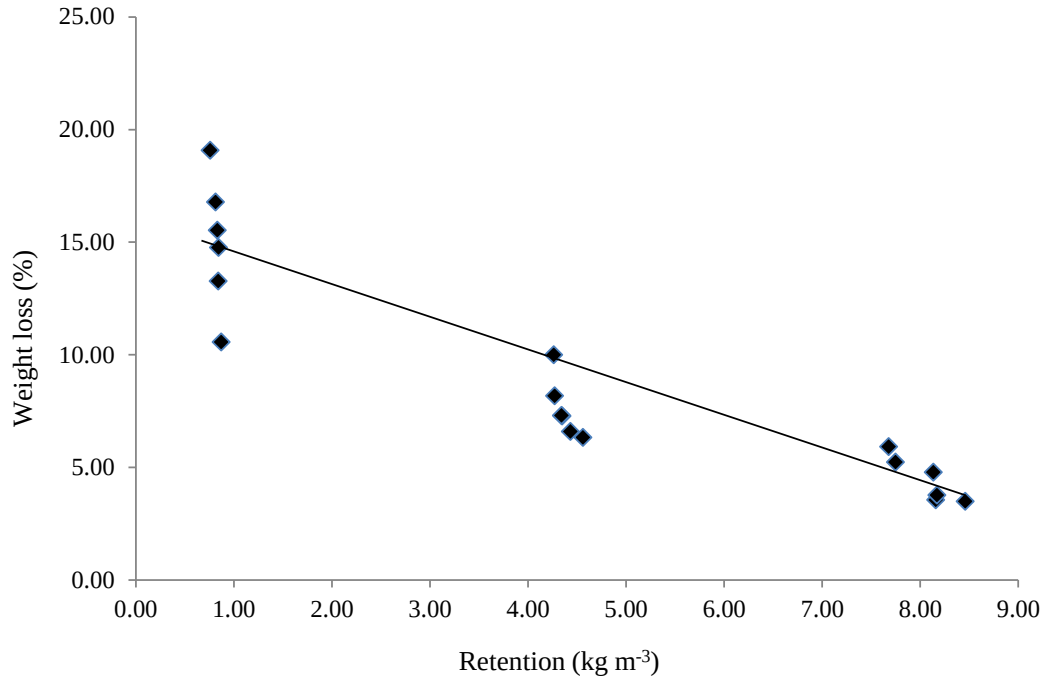


Figure 4.8. Approximate threshold for unleached DBT treated *M. triloba* through soil block test exposed to *T. versicolor*. DBT- Disubstituted butyltin(IV) complex.

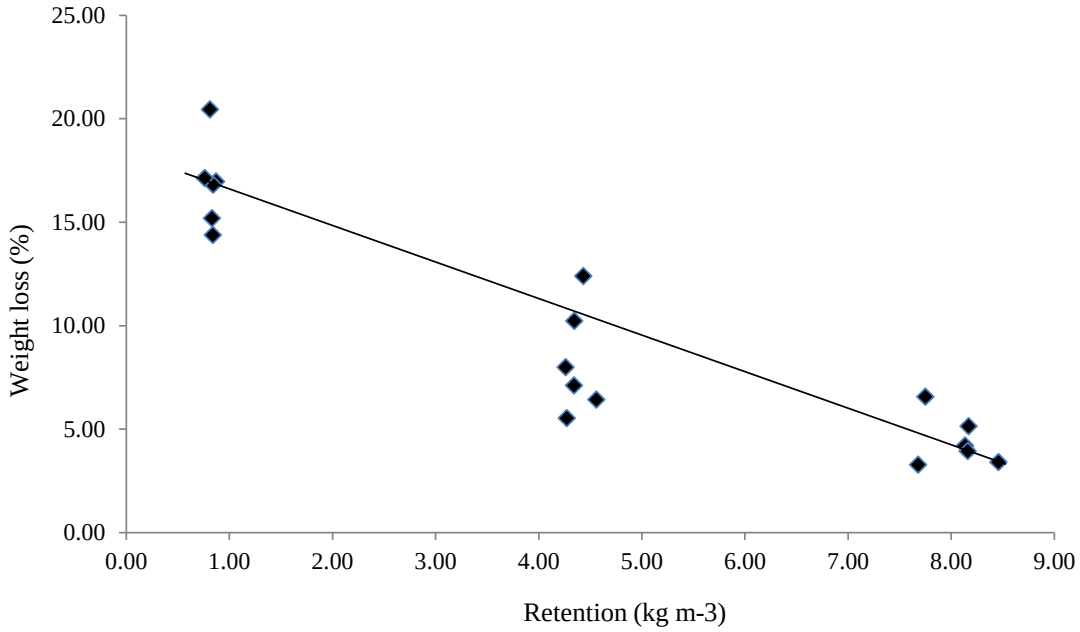


Figure 4.9. Approximate threshold for unleached DBT treated *M. triloba* through soil block test exposed to *G. trabeum*. DBT- Disubstituted butyltin(IV) complex.

The approximate threshold value for DBT-treated *H. brasiliensis* was 6.21 kg $m^{-3.}$ It was observed the threshold value for DBT-treated *A. scholaris* was 9.62 kg m^{-3}. At 6.21 and 9.62 kg m^{-3} retention the weight loss of DBT-treated *H. brasiliensis* and *A. scholaris*, respectively is less than 5%. For more accurate threshold determination more retention should be used in the test. Nevertheless, this test showed that DBT provided good protection to *Hevea brasiliensis* with weight losses of less than 5% at the retention of 5.97 and 6.44 kg m^{-3} exposed to *Trametes versicolor*. Result showed that higher retention value (9.62 kg m^{-3}) was needed to protect *A. scholaris*. The estimated amount of tin (% g/g) in 1% DBT-treated wood species exposed to *T. versicolor* and *G. trabeum* is shown in Table 4.5. The amount of tin (% g/g) in 1% DBT-treated *H. brasiliensis* and *A. scholaris* were 0.02 and 0.06 % g/g, respectively.

Other results for DBT-treated *H. brasiliensis* and *A. scholaris* exposed to *G. trabeum* did not showed threshold values (Figures 4.5 and 4.7). Similar results were obtained for *M.*

triloba exposed to *T. versicolor* and *G. trabeum* (Figures 4.8 and 4.9). These results indicate that 1% DBT was not able to protect *H. brasiliensis* and *A. scholaris* against *G. trabeum* and *M. triloba* against *T. versicolor* and *G. trabeum.*

Table 4.5. Approximate threshold and per cent of tin of 1% DBT-treated wood species with different fungi

Fungus	Wood species	Approximate threshold (kg/m^3)	Tin (% g/g)
T. versicolor	*H. brasiliensis*	6.21	0.02
	M. triloba	ND	0.04
	A. scholaris	9.62	0.06
G. trabeum	*H. brasiliensis*	ND	0.02
	M. triloba	ND	0.04
	A. scholaris	ND	0.06

ND- Not detectable

4.3 Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

Alstonia scholaris, *Macaranga triloba* and *Hevea brasiliensis* treated wood cubes were analyzed by FT-IR spectroscopy to determine whether the treating chemicals were bonded within the wood cell. The reaction of organotin(IV) complexes with wood cellulose or cellulose derivatives occurred during treatment in vacuum-pressure unit. The reaction of tin in organotin(IV) complex make a bonding with hydrogen of functional hydroxyl group of wood, resulting form a stable new bond tin-oxide (Sn-O) which is stable and confirmed by the FTIR spectroscopy analyses. The main characteristics of FT-IR absorption data of all treated wood samples are presented in Table 4.6.

Table 4.6. IR Spectra data of organotin(IV) treated wood species.

Wood Species	Treating Compound	Bonding								
		ν(NH) cm^{-1}	ν(cyclohexyl) cm^{-1}	ν(C=N-N=C) cm^{-1}	ν(N-N) cm^{-1}	ν(C-S) cm^{-1}	ν(Pyridin in plane) cm^{-1}	ν(Sn-C) cm^{-1}	ν(Sn-O) cm^{-1}	ν(Sn-N) cm^{-1}
Alstonia scholaris	MMT	3172	2930, 2854	1557	1028	1252, 831	649	603	568	441
	MPT	3178	2932, 2854	1556	1033	1255, 830	649	606	561	449
	DMT	3176	2936, 2851	1559	1034	1250, 831	622	596	567	449
	DPT	3176	2929, 2852	1542	1021	1251, 829	628	602	569	456
	DBT	3173	2936, 2851	1542	1018	1249, 836	634	596	565	448
Macaranga triloba	MMT	3179	2930, 2854	1557	1028	1252, 831	649	603	568	450
	MPT	3170	2932, 2853	1557	1020	1254, 830	650	605	567	448
	DMT	3177	2937, 2851	1542	1016	1250, 832	633	596	567	449
	DPT	3177	2930, 2852	1542	1021	1251, 829	636	596	569	451
	DBT	3175	2936, 2851	1542	1016	1249, 836	634	598	566	452
Hevea brasiliensis	MMT	3173	2930, 2854	1557	1027	1242, 831	649	603	568	451
	MPT	3179	2932, 2854	1556	1017	1254, 830	631	606	569	454
	DMT	3178	2936, 2851	1542	1013	1249, 832	622	596	567	450
	DPT	3171	2929, 2851	1542	1021	1250, 829	633	594	569	457
	DBT	3175	2936, 2851	1542	1011	1249, 835	621	595	561	453

Data of Table 4.6 showed that the cyclohexyl and C-S bond of organotin(IV) treated wood were at two different spectra are 2929-2937 & 2851-2854 and 1242-1255 & 829-836 cm^{-1}, respectively. The most notable new linkage of organotin(IV) treated wood spectra at 594-606, 561-569 and 441-457 cm^{-1} are assigned to the stretching mode of ν(Sn-C), ν(Sn-O) and ν(Sn-N), respectively.

The characterizations were performed on all species of wood samples and similar results were obtained. Thus only the results corresponding to MMT & DPT, MPT & DBT, MMT & DMT treated and untreated *Alstonia scholaris*, *Macaranga triloba* & *Hevea brasiliensis* wood spectra are presented as shown in Figures 4.10, 4.11 and 4.12, respectively.

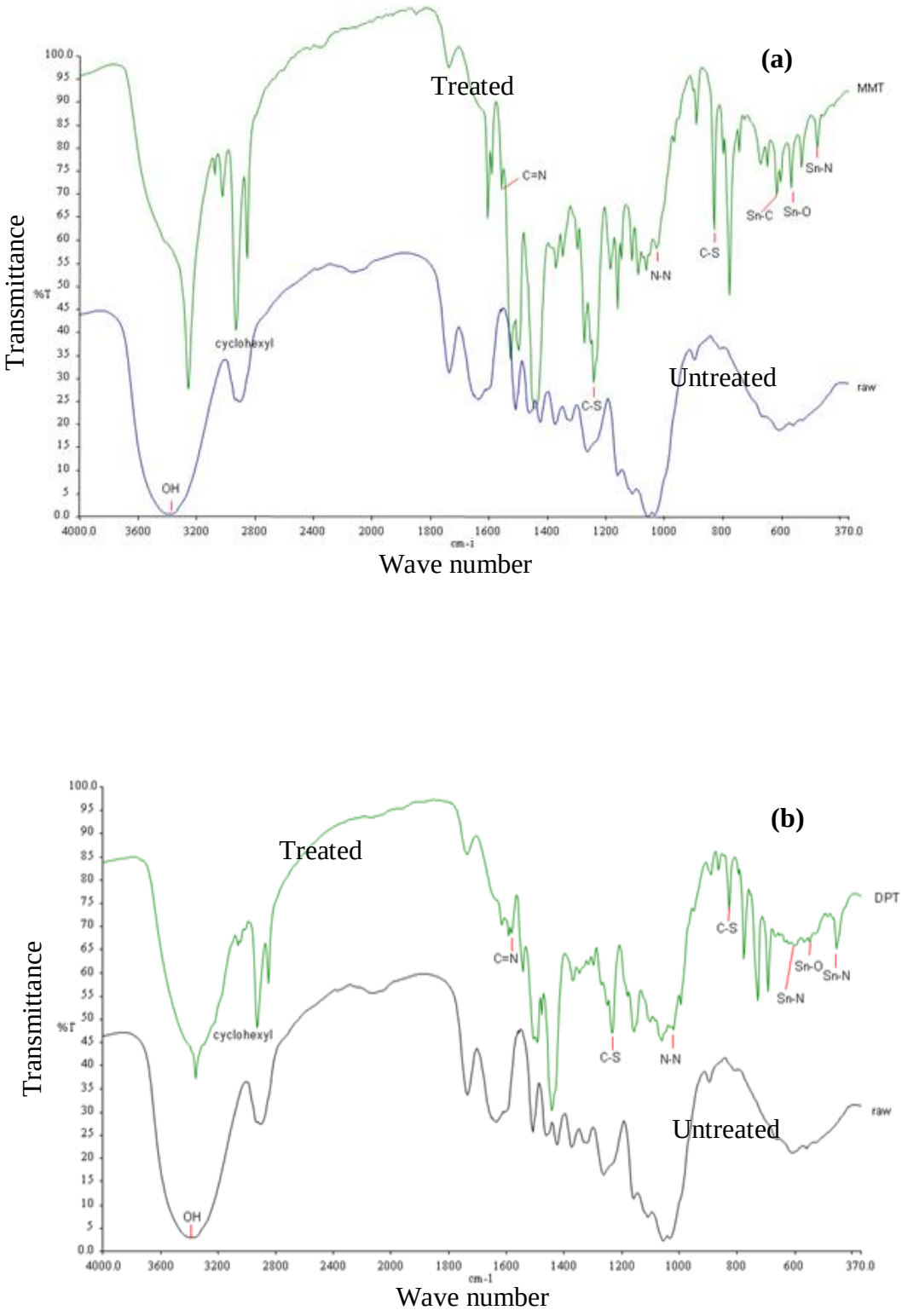


Figure 4.10. IR spectra of untreated and treated *Alstonia scholaris* wood with **(a)** monomethyltin(IV) complex and **(b)** diphenyltin(IV) complex.

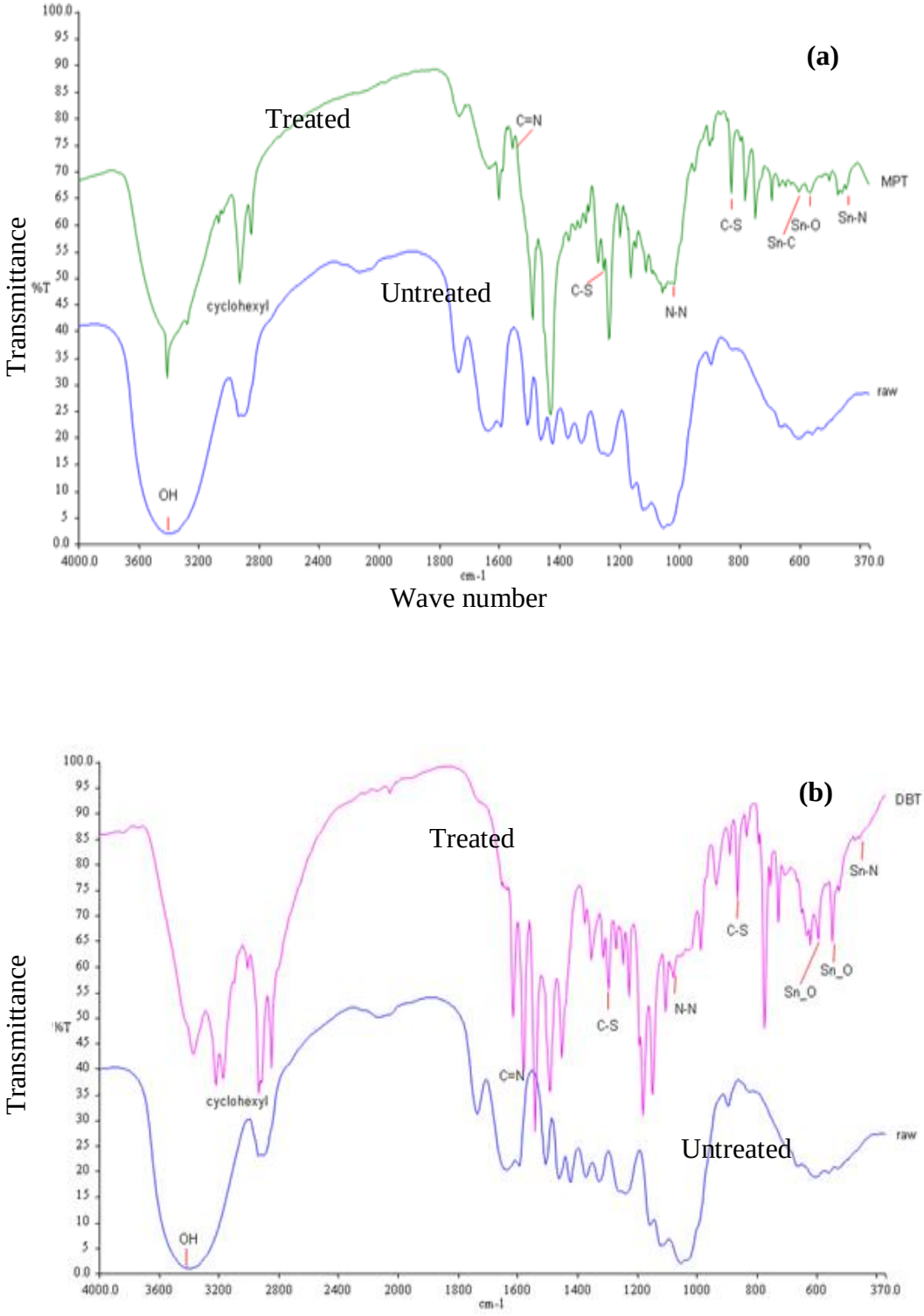


Figure 4.11. IR spectra of untreated and treated *Macaranga triloba* wood with **(a)** monophenyltin(IV) complex and **(b)** dibutyltin(IV) complex.

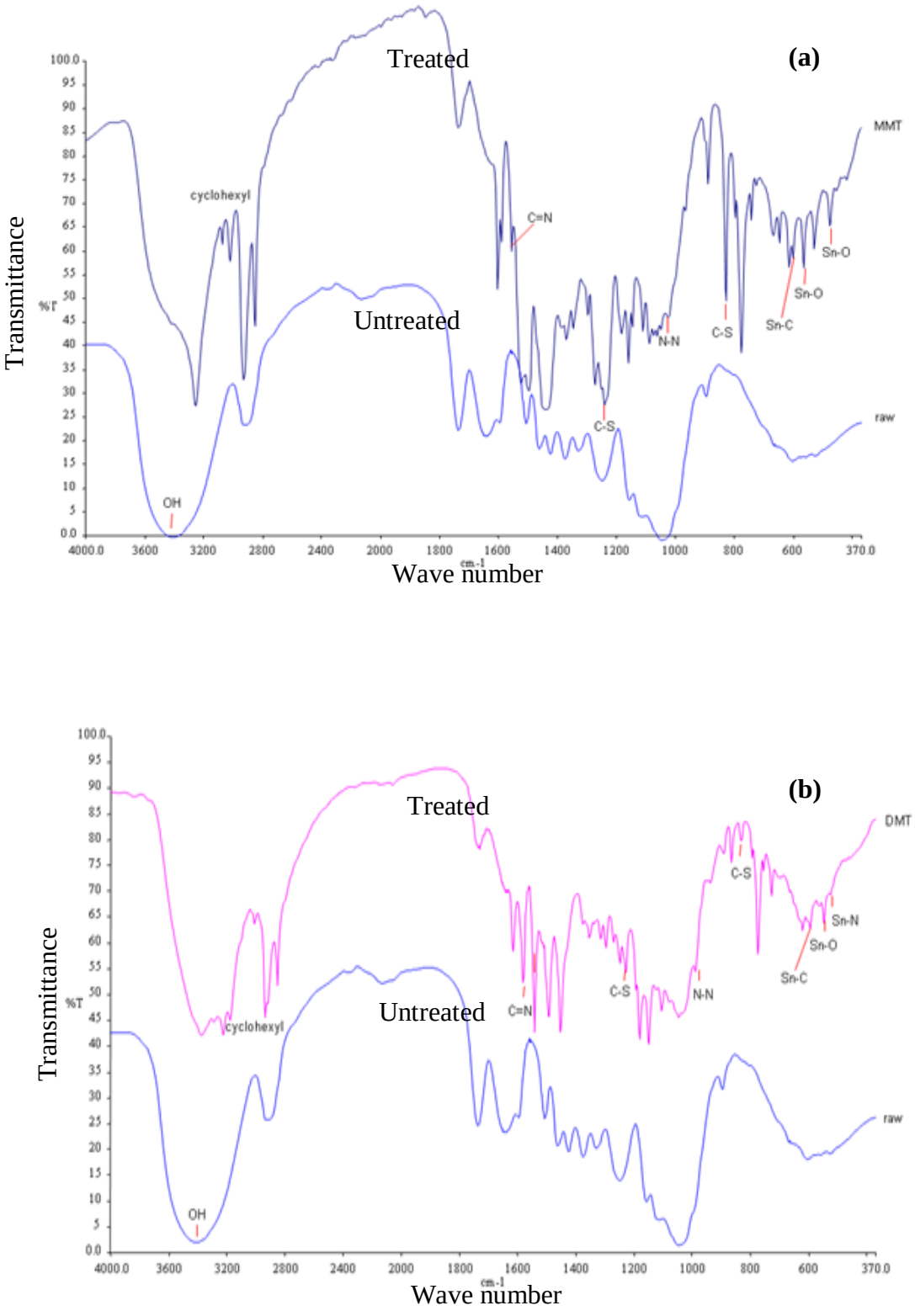


Figure 4.12. IR spectra of untreated and treated *Hevea brasiliensis* wood with **(a)** monomethyltin(IV) complex and **(b)** dimethyltin(IV) complex.

Figures 4.10, 4.11 and 4.12 showed that the distinguishable different peak spectra were present between the treated and untreated wood samples in *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood species for all tested organotin(IV) complexes. Peak assigned for hydroxyl group in organotin(IV) treated wood spectra became narrowed and shifted towards lower wave number than untreated wood spectra. The new linkage tin-oxide were observed in organotin(IV) treated wood spectra which was not present in untreated wood samples spectra. Moreover the new bond namely tin-carbon, tin-nitrogen and tin-oxide was detected in organotin(IV) treated wood spectra.

4.4 Microdistribution of tin in wood cell through SEM-EDXA

Scanning electron microscope in conjunction with energy dispersive X-ray analysis (SEM-EDXA) was conducted to provide information on the distribution of tin following treatment with organotin(IV) complexes. The X-ray distribution map of tin and EDX spectrum of organotin(IV) treated *Alstonia scholaris*, *Hevea brasiliensis* wood samples are shown in Figures 4.13 and 4.14, respectively.

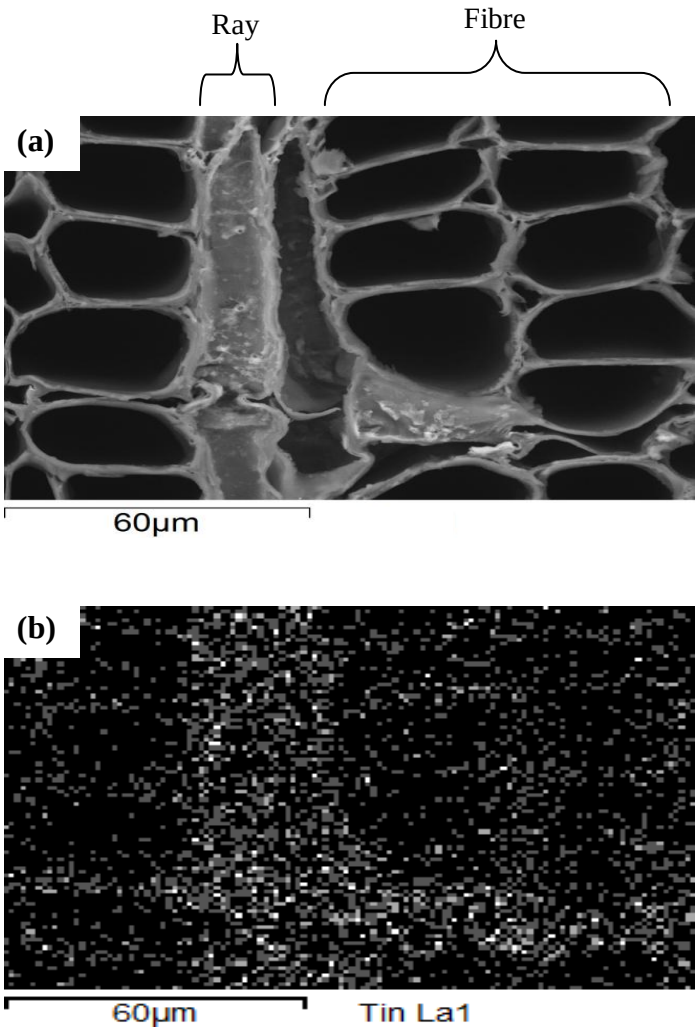


Figure 4.13. (a) Transverse section of wood sample and **(b)** X-ray distribution map of tin in *Alstonia scholaris*.

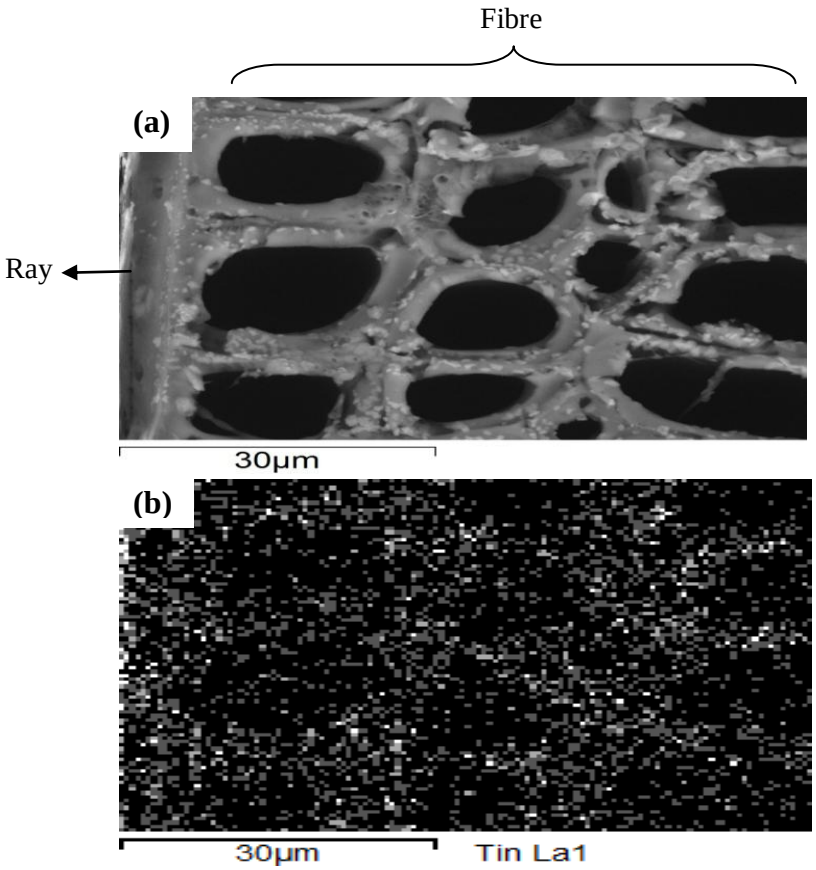


Figure 4.14. (a) Transverse section of wood sample and **(b)** X-ray distribution map of tin in *Hevea brasiliensis*.

The X-ray distribution maps of organotin(IV)-treated *A. scholaris* and *H. brasiliensis* wood showed that high accumulation of tin was observed in ray and fibre cell wall. The X-ray distribution map indicated that tin was deposited in the cell wall (Figures 4.13 and 4.14). The white dots indicate the presence of tin, the brighter the dot the higher the concentration of tin. Results showed that the high concentration of tin was detected in ray cell and fibre cell wall.

Linescan analyses on organotin(IV)-treated *Hevea brasiliensis* was carried out to detect the presence of tin within fibre cell wall. The result of a linescan analysis of *Hevea brasiliensis* wood sample treated with organotin(IV) is shown in Figure 4.15.

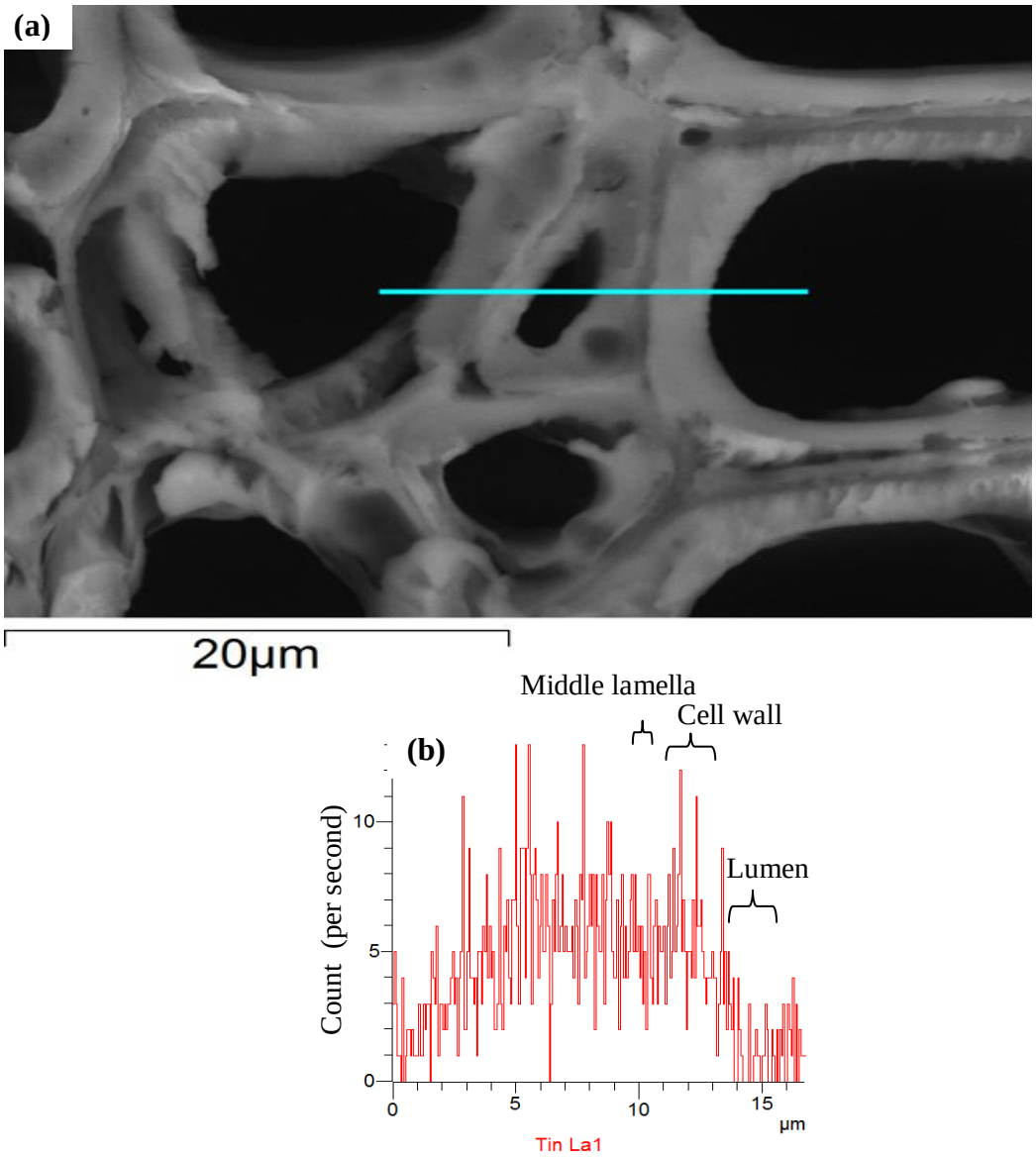


Figure 4.15. SEM-EDXA linescan analysis of *Hevea brasiliensis* treated with organotin(IV) complex. **(a)** SEM micrograph of transverse surface showing the linescan and **(b)** Microdistribution of tin in fibre cell wall.

Linescan analysis showed that tin was detected in fibre cell walls (Figure 4.15). The linescan showed that count rates increases from the lumen area towards the lumen surface then decreases across the S2 layer and then increases again in the middle lamella region.

4.5 Leaching rate of organotin(IV)

Leaching rates were determined to estimate the release of preservative component from 1% organotin(IV)-treated *Alstonia scholaris, Macaranga triloba* and *Hevea brasiliensis*. The release rates of tin at each leaching interval from wood cubes treated with 1% MPT, DMT and DBT are shown in Figures 4.16, 4.17 and 4.18, respectively.

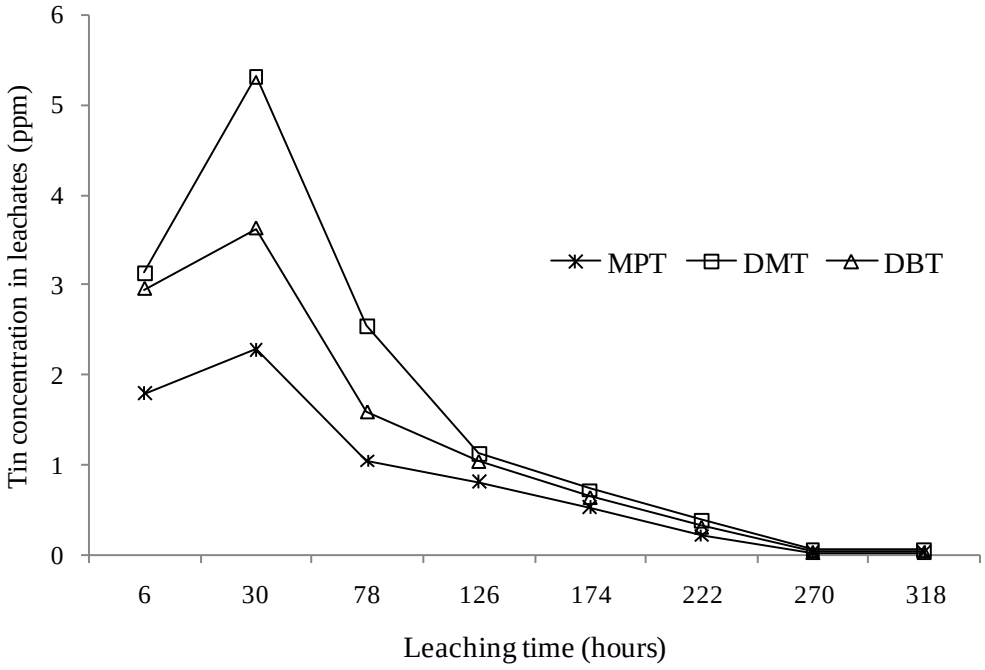


Figure 4.16. Tin concentration at each leaching interval from *Alstonia scholaris* wood cubes treated with organotin(IV) complexes. The retention of MPT, DMT and DBT are 7.90, 10.59 and 9.49 kg m^{-3}, respectively.

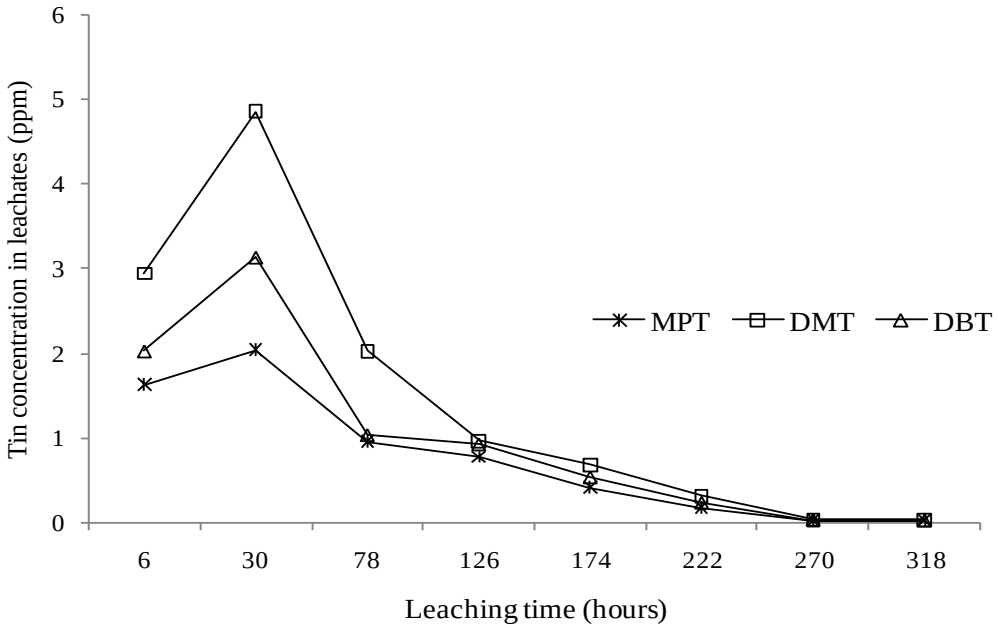


Figure 4.17. Tin concentration at each leaching interval from *Macaranga triloba* wood cubes treated with organotin(IV) complexes. The retention of MPT, DMT and DBT are 7.70, 9.58 and 8.06 kg m^{-3}, respectively.

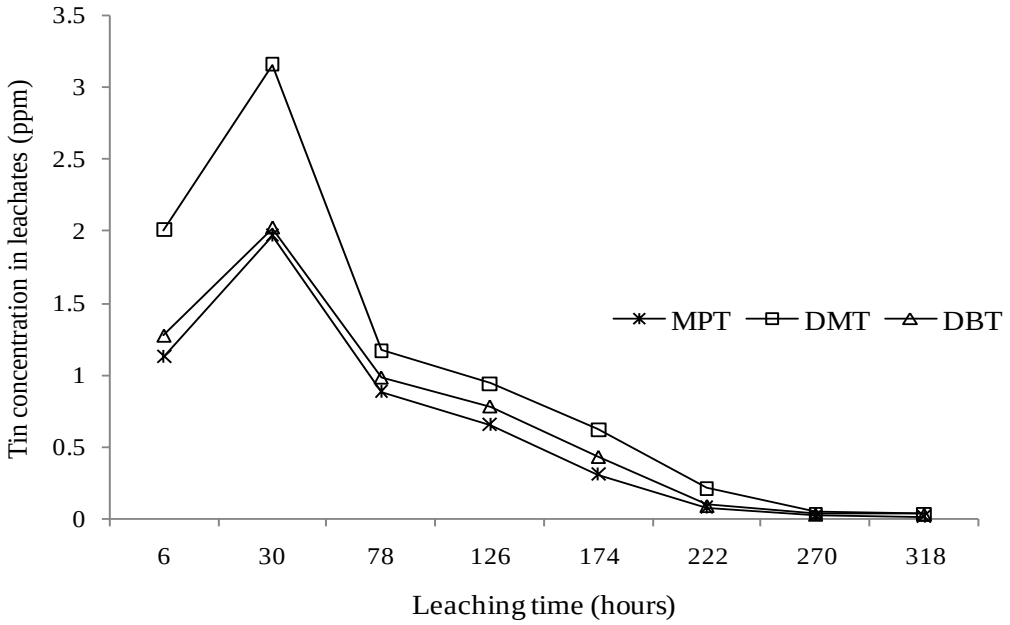


Figure 4.18. Tin concentration at each leaching interval from *Hevea brasiliensis* wood cubes treated with organotin(IV) complexes. The retention of MPT, DMT and DBT are 5.76, 5.65 and 6.05 kg m^{-3}, respectively.

Leaching result showed that, among all tested chemicals DMT treated wood cubes demonstrated the greatest tin release rate followed by DBT and MPT for the entire leaching cycle. Wood cubes treated with MPT showed the lowest tin release among three tested chemicals. The maximum and minimum tin was determined from 2nd and 8th (last) leachates sample among tested chemicals.

Among three wood species, *Alstonia scholaris* showed the highest leaching of tin followed by *Macaranga triloba* and *Hevea brasiliensis*. The highest (5.32 ppm) and lowest (0.01 ppm) release of tin was determined from DMT treated *Alstonia scholaris* and MPT treated *Hevea brasiliensis* wood cubes, respectively.

4.6 Bioassay for enzyme activity

4.6.1 Enzyme activities of untreated *A. scholaris, M. triloba and H. brasiliensis*

Determination of lignolytic enzymes produced under the same conditions was carried out to evaluate the actual role of each enzyme in wood decay. *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood cubes were exposed for 16 weeks to *Trametes versicolor* and *Gloeophyllum trabeum* under semi-solid state cultivation. Fungal growth was shown to be homogeneous in this study. After 16 weeks of exposure, lignolytic enzymes were extracted with buffer solution from inoculated wood cubes. Lignolytic enzymes namely lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) of extracted buffers are summarized in Table 4.7.

Table 4.7. Mean lignin peroxidase (LiP), manganese peroxidase (MnP), Laccase (Lac), weight loss (WL) and density (D) of untreated wood cubes after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Wood species	n	*Trametes versicolor*						*Gloeophyllum trabeum*				
		LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})
Alstonia scholaris	10	5.39* a (0.69)	16.23 a (3.49)	2.72 a (0.13)	50.52 a (4.52)	356.27 a (8.51)	179.69 a (7.85)	2.88 a (0.49)	7.88 a (1.53)	48.40 a (4.28)	357.82 a (11.03)	184.15 a (8.91)
Macaranga triloba	10	4.84 a (0.39)	13.94 a (4.69)	2.61 a (0.14)	50.48 a (3.46)	410.28 b (11.54)	201.37 b (8.18)	2.45 a (0.43)	6.21 a (1.25)	47.21 a (3.21)	410.65 b (13.89)	210.94 b (8.97)
Hevea brasiliensis	10	6.57 b (0.51)	26.72 b (3.11)	3.17 b (0.19)	61.66 b (3.56)	654.99 c (12.93)	246.85 c (10.55)	4.21 b (0.57)	15.47 b (2.62)	60.13 b (5.25)	655.93 c (11.35)	249.87 c (9.97)

Values in parentheses are the standard deviation

*Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

**Density of wood cubes before exposure to decay fungus

***Density of wood cubes after exposure to decay fungus

n = Number of sample

Wood cubes degradaed by *Trametes versicolor* showed LiP, MnP and Lac activities but *Gloeophyllum trabeum* did not show any Lac activity. LiP, MnP and Lac activities of *Hevea brasiliensis* varied significantly from *Alstonia scholaris* and *Macaranga triloba*. The LiP, MnP and Lac activities of untreated wood varied from 2.45 to 6.57, 6.21 to 26.72 and 2.61 to 3.17 U/ml, respectively. *Trametes versicolor* showed higher enzyme activity than *Gloeophyllum trabeum*. The highest (6.57 U/ml) and lowest (2.45 U/ml) LiP activity was recorded from *Hevea brasiliensis* exposed to *Trametes versicolor* and *Macaranga triloba* exposed to *Gloeophyllum trabeum*. Similarly the highest (26.72 U/ml) and lowest (6.21 U/ml) MnP activity was recorded from *Hevea brasiliensis* exposed to *Trametes versicolor* and *Macaranga triloba* exposed to *Gloeophyllum trabeum*. The highest (3.17 U/ml) Lac activity was recorded from *Hevea brasiliensis* and lowest (2.61 U/ml) Lac activity was observed from *Macaranga triloba* exposed to *Trametes versicolor* (Table 4.7).

Mean weight loss of untreated wood cubes varied from 47 to 62% after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum* (Table 4.7). Mean weight loss of untreated *Hevea brasiliensis* wood cubes was significantly higher than that of *Macaranga triloba* and *Alstonia scholaris*.

Mean wood densities before and after exposure to fungi were significantly different among wood species (Table 4.7). Mean densities of untreated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* were 357, 410 and 655 kg m^{-3}, respectively. After 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum* the densities ranged from 180 to 184, 201 to 211 and 247 to 250 kg m^{-3}, respectively.

4.6.2 Enzyme activities for organotin(IV)-treated wood

Mean values of LiP, MnP and Lac activities of treated wood with mono- and disubstituted organotin(IV) complexes each three level of concentration after exposure to *Trametes versicolor* and *Gloeophyllum trabeum* for 16 weeks are summarized in Table 4.8 and 4.9, respectively.

Table 4.8. Mean lignin peroxidase (LiP), manganese peroxidase (MnP), Laccase (Lac), weight loss (WL) and density (D) of treated wood cubes with monosubstituted organotin(IV) complexes at three different retentions after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Treating chemicals	Wood species	n	Retention (kg m^{-3})	*Trametes versicolor*						*Gloeophyllum trabeum*				
				LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})
MMT		6	0.84	2.06* b (0.19)	7.99 b (0.47)	1.01 b (0.11)	22.31 c (2.06)	357.66 a (8.09)	260.61 a (8.04)	1.91 b (0.19)	5.09 c (0.55)	25.05 c (2.21)	359.22 a (9.49)	254.61 a (9.02)
	Alstonia scholaris	6	4.59	1.79 ab (0.33)	7.53 b (0.41)	0.89 ab (0.12)	18.24 b (1.76)	359.02 a (8.31)	279.51 b (7.25)	1.71 ab (0.17)	4.01 b (0.58)	20.11 b (4.01)	360.61 a (8.69)	270.73 b (6.44)
		6	7.48	1.51 a (0.20)	6.04 a (0.47)	0.81 a (0.05)	14.38 a (2.55)	361.52 a (7.66)	291.90 c (7.20)	1.49 a (0.19)	3.02 a (0.56)	16.12 a (2.40)	362.40 a (7.61)	291.24 c (6.34)
		6	0.84	2.01 b (0.15)	7.01 b (0.60)	1.01 b (0.10)	25.63 c (4.05)	409.53 a (12.83)	310.37 a (8.32)	1.91 b (0.18)	5.09 c (0.54)	25.78 b (3.01)	410.05 a (14.91)	304.44 a (10.15)
	Macaranga triloba	6	4.59	1.71 ab (0.26)	6.53 ab (0.49)	0.85 ab (0.13)	19.92 b (1.51)	410.16 a (11.14)	318.45 ab (7.70)	1.60 a (0.22)	3.99 b (0.60)	20.61 ab (3.96)	413.28 a (11.47)	314.34 a (9.69)
		6	7.48	1.40 a (0.19)	5.88 a (0.28)	0.74 a (0.09)	15.48 a (2.68)	413.68 a (9.77)	334.07 b (8.21)	1.32 a (0.16)	3.01 a (0.59)	17.56 a (4.44)	414.73 a (12.73)	331.41 b (8.03)
		6	0.58	3.57 c (0.21)	15.07 b (1.91)	2.02 c (0.33)	23.31 b (3.65)	656.03 a (14.96)	509.81 a (12.66)	2.52 c (0.15)	7.44 c (0.54)	24.13 c (6.50)	657.41 a (13.35)	502.88 a (13.01)
	Hevea brasiliensis	6	3.27	3.06 b (0.17)	13.33 b (1.08)	1.51 b (0.13)	18.43 b (4.02)	658.22 a (13.10)	530.01 b (7.89)	2.02 b (0.37)	6.04 b (0.39)	19.94 b (3.63)	659.39 a (12.62)	525.51 b (8.16)
		6	5.62	2.51 a (0.16)	10.23 a (1.65)	1.10 a (0.17)	13.01 a (2.77)	661.88 a (13.98)	551.14 c (11.15)	1.48 a (0.24)	4.99 a (0.41)	14.62 a (3.48)	661.21 a (14.30)	541.15 c (9.39)

Continue Table 4.7

		6	0.89	1.92 b (0.29)	7.01 b (0.51)	0.99 b (0.13)	19.83 c (3.18)	358.27 a (13.11)	265.21 a (10.64)	1.84 b (0.21)	4.05 c (0.48)	22.88 c (3.05)	358.72 c (10.77)	259.11 a (11.61)
	Alstonia scholaris	6	4.39	1.69 ab (0.31)	6.11 ab (0.81)	0.89 ab (0.14)	14.58 b (3.78)	359.22 a (11.98)	287.12 b (7.74)	1.65 ab (0.22)	3.07 b (0.36)	16.61 b (3.86)	359.44 b (7.01)	274.84 b (7.87)
		6	7.90	1.32 a (0.24)	5.54 a (0.66)	0.79 a (0.09)	10.35 a (2.17)	361.81 a (10.59)	298.07 b (8.21)	1.30 a (0.28)	2.08 a (0.46)	10.13 a (2.10)	361.06 a (5.80)	296.24 c (7.81)
		6	0.77	1.82 b (0.33)	6.13 b (0.73)	0.91 a (0.32)	23.81 c (4.30)	409.66 a (12.95)	314.04 a (10.97)	1.02 a (0.25)	3.18 b (0.72)	24.78 b (5.19)	411.72 a (12.58)	309.94 a (9.55)
MPT	*Macaranga triloba*	6	3.59	1.51 ab (0.38)	5.08 a (0.65)	0.85 a (0.27)	16.75 b (2.67)	412.99 a (13.35)	325.95 b (9.33)	0.83 a (0.31)	2.53 ab (0.47)	16.44 b (5.82)	413.78 a (11.07)	319.18 ab (8.84)
		6	7.70	1.29 a (0.32)	4.26 a (0.68)	0.62 a (0.23)	10.68 a (1.98)	416.34 a (11.98)	340.53 c (5.43)	0.63 a (0.24)	1.91 a (0.34)	9.96 a (1.68)	415.90 a (13.92)	329.91 b (11.52)
		6	0.57	3.07 b (0.51)	14.41 b (2.76)	1.44 a (0.41)	21.14 c (4.95)	655.86 a (12.61)	521.47 a (5.22)	2.08 b (0.44)	6.44 c (0.68)	23.96 c (3.90)	656.08 a (14.58)	519.22 a (11.01)
	Hevea brasiliensis	6	3.33	2.51 ab (0.46)	12.51 ab (2.72)	1.29 a (0.40)	16.09 b (3.19)	657.55 a (14.83)	544.35 b (12.16)	1.53 a (0.28)	5.09 b (0.51)	17.44 b (4.39)	658.59 a (11.41)	537.85 b (10.72)
		6	5.76	2.09 a (0.45)	9.73 a (1.75)	0.91 a (0.38)	10.35 a (2.14)	659.88 a (12.39)	560.81 c (8.07)	1.02 a (0.34)	4.19 a (0.51)	11.66 a (2.49)	660.45 a (10.01)	555.99 c (8.71)

Values in parentheses are the standard deviation

MMT- Monomethyltin(IV) complex, MPT- Monophenyltin(IV) complex

*Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

**Density of wood cubes before exposure to decay fungus

***Density of wood cubes after exposure to decay fungus

n = Number of sample

Table 4.9. Mean lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac), weight loss (WL) and density (D) of treated wood cubes with disubstituted organotin(IV) complexes at three different retentions after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Treating chemicals	Wood species	n	Retention (kg m^{-3})	*Trametes versicolor*						*Gloeophyllum trabeum*				
				LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg m^{-3})	D*** (kg m^{-3})
DMT	*Alstonia scholaris*	6	1.00	1.10* b (0.44)	5.96 b (1.13)	0.84 a (0.33)	16.83 c (4.16)	357.16 a (11.21)	276.21 a (10.79)	0.93 b (0.45)	3.02 b (0.49)	19.38 c (5.68)	359.72 a (12.25)	268.27 a (12.62)
		6	5.65	0.85 ab (0.36)	4.07 a (0.57)	0.67 a (0.32)	11.92 b (4.17)	359.88 a (9.63)	294.62 b (11.03)	0.82 ab (0.34)	2.56 ab (0.45)	12.56 b (2.61)	362.06 a (6.18)	291.34 b (11.71)
		6	10.59	0.68 a (0.26)	2.94 a (0.44)	0.46 a (0.17)	9.18 a (2.73)	362.08 a (12.52)	318.07 c (9.83)	0.60 a (0.35)	2.08 a (0.46)	9.46 a (2.31)	364.44 a (10.04)	312.57 c (12.61)
	Macaranga triloba	6	1.00	1.58 b (0.51)	6.03 b (0.66)	0.79 a (0.40)	17.63 c (5.81)	409.83 a (12.41)	336.37 a (11.71)	0.91 b (0.21)	3.06 b (0.88)	19.11 c (3.31)	409.59 a (11.19)	331.44 a (11.80)
		6	4.62	1.24 ab (0.33)	4.94 ab (0.71)	0.58 a (0.28)	14.42 b (3.26)	411.34 a (12.88)	344.12 b (6.90)	0.75 ab (0.32)	2.43 ab (0.52)	13.94 b (3.32)	410.40 a (10.45)	338.18 ab (12.99)
		6	9.58	1.05 a (0.31)	4.16 a (0.75)	0.51 a (0.25)	8.21 a (2.28)	414.66 a (13.96)	358.91 c (8.24)	0.49 a (0.24)	1.51 a (0.51)	8.29 a (2.14)	413.44 a (12.95)	356.07 b (9.82)
	Hevea brasiliensis	6	0.47	2.09 b (0.64)	10.41 b (2.02)	1.06 a (0.42)	20.48 c (3.94)	657.23 a (13.42)	548.31 a (10.37)	1.03 b (0.26)	3.99 b (0.61)	21.46 c (5.22)	659.75 a (12.65)	539.05 a (12.32)
		6	2.80	1.45 ab (0.34)	6.90 a (1.94)	0.87 a (0.26)	13.46 b (2.49)	660.92 a (13.56)	576.68 b (9.44)	0.85 ab (0.39)	2.40 a (0.63)	15.27 b (2.08)	661.92 a (11.21)	570.68 ab (9.82)
		6	5.65	1.06 a (0.42)	6.56 a (1.74)	0.59 a (0.27)	9.06 a (1.78)	664.51 a (14.81)	596.97 c (9.85)	0.53 a (0.22)	2.34 a (0.59)	9.96 a (2.42)	664.62 a (12.19)	583.32 b (8.15)
	Alstonia scholaris	6	0.96	0.77 b (0.32)	3.04 c (0.73)	0.29 b (0.18)	16.33 c (5.12)	357.36 a (12.74)	286.21 a (9.72)	0.54 b (0.28)	1.90 b (0.52)	18.05 c (3.77)	357.05 a (12.78)	281.11 a (12.36)
		6	4.62	0.48 ab (0.25)	1.94 b (0.69)	0.18 ab (0.14)	10.92 b (2.89)	361.24 a (12.93)	311.62 b (9.93)	0.36 ab (0.27)	1.43 ab (0.39)	13.51 b (2.28)	361.11 a (8.67)	301.34 a (12.92)
		6	7.94	0.28 a (0.21)	0.96 a (0.29)	0.08 a (0.06)	7.18 a (2.60)	364.38 a (11.23)	334.24 c (12.61)	0.18 a (0.12)	0.89 a (0.46)	8.26 a (2.64)	363.73 a (8.52)	329.24 b (11.15)
		6	0.97	1.24 b (0.32)	4.03 c (0.67)	0.54 b (0.29)	17.31 c (4.89)	411.01 a (13.32)	337.37 a (11.34)	0.67 b (0.25)	1.99 b (0.53)	18.78 c (4.48)	410.22 a (11.75)	326.77 a (13.97)

Continue Table 4.8

DPT	*Macaranga triloba*	6	4.52	0.99 ab (0.21)	3.02 b (0.63)	0.43 ab (0.21)	11.42 b (3.10)	412.99 a (12.47)	357.45 ab (12.65)	0.48 ab (0.23)	1.53 ab (0.29)	13.49 b (2.02)	412.11 a (12.96)	352.01 b (10.47)
		6	7.61	0.78 a (0.27)	1.92 a (0.59)	0.18 a (0.11)	6.15 a (1.47)	415.51 a (11.19)	374.57 b (11.56)	0.31 a (0.12)	1.01 a (0.42)	7.49 a (2.51)	414.23 a (11.70)	368.24 b (13.73)
	Hevea brasiliensis	6	0.52	1.46 b (0.32)	6.41 b (1.46)	0.81 b (0.23)	18.14 b (6.03)	661.56 a (12.09)	580.97 a (8.41)	0.76 b (0.31)	3.09 b (0.67)	18.46 b (5.07)	659.10 a (12.24)	577.22 a (7.53)
		6	2.58	1.25 ab (0.29)	5.00 ab (0.92)	0.59 ab (0.21)	9.26 a (1.78)	662.59 a (12.01)	588.68 ab (12.76)	0.52 ab (0.29)	2.31 ab (0.49)	10.57 a (1.27)	663.59 a (11.71)	582.51 ab (9.88)
		6	5.34	0.92 a (0.25)	4.19 a (0.83)	0.39 a (0.27)	7.02 a (2.20)	664.51 a (11.21)	601.97 b (12.36)	0.34 a (0.15)	2.09 a (0.79)	7.45 a (1.57)	666.45 a (11.82)	595.99 b (12.16)
DBT	*Alstonia scholaris*	6	0.81	0.54 b (0.26)	1.97 b (0.73)	0.24 a (0.21)	13.49 c (3.48)	360.52 a (11.94)	332.87 a (10.53)	0.42 b (0.23)	1.51 b (0.28)	15.55 c (4.36)	360.39 a (10.64)	328.27 a (9.01)
		6	4.91	0.33 ab (0.23)	1.43 ab (0.31)	0.13 a (0.11)	9.42 b (2.55)	361.41 a (12.83)	345.79 ab (12.54)	0.22 ab (0.20)	1.30 ab (0.37)	10.27 b (3.12)	362.78 a (10.65)	341.18 ab (11.27)
		6	9.49	0.16 a (0.12)	0.93 a (0.32)	0.06 a (0.03)	3.28 a (1.14)	363.21 a (13.14)	353.24 b (9.44)	0.11 a (0.06)	0.81 a (0.18)	4.28 a (1.15)	365.40 a (8.35)	347.40 b (10.32)
	Macaranga triloba	6	0.83	0.86 b (0.29)	2.11 b (0.61)	0.24 a (0.19)	15.97 c (3.06)	410.66 a (13.26)	388.54 a (10.23)	0.43 b (0.25)	1.13 b (0.44)	15.95 c (3.59)	412.39 a (12.71)	385.11 a (10.36)
		6	4.37	0.63 ab (0.37)	1.49 ab (0.51)	0.15 a (0.06)	8.42 b (2.54)	412.49 a (11.36)	397.12 ab (11.63)	0.24 ab (0.22)	0.92 ab (0.38)	9.44 b (3.12)	413.78 a (12.46)	393.18 ab (10.47)
		6	8.06	0.35 a (0.15)	1.03 a (0.41)	0.08 a (0.04)	4.15 a (1.67)	414.34 a (11.95)	404.39 b (10.67)	0.12 a (0.06)	0.60 a (0.09)	4.71 a (1.95)	415.06 a (12.83)	402.07 b (9.86)
	Hevea brasiliensis	6	0.62	0.72 b (0.27)	2.08 b (0.54)	0.21 a (0.18)	14.81 c (3.38)	661.40 a (11.97)	593.31 a (15.14)	0.44 b (0.25)	1.08 b (0.24)	14.80 c (3.55)	660.76 a (7.19)	585.55 a (10.42)
		6	3.35	0.51 ab (0.31)	1.50 ab (0.29)	0.15 a (0.14)	7.43 b (2.30)	663.59 a (11.02)	599.01 a (14.21)	0.31 ab (0.21)	0.82 ab (0.42)	8.44 b (2.23)	663.26 a (9.70)	596.18 a (9.37)
		6	6.05	0.34 a (0.16)	1.13 a (0.64)	0.07 a (0.07)	3.55 a (0.97)	664.51 a (10.81)	622.31 b (10.01)	0.13 a (0.06)	0.61 a (0.21)	4.18 a (1.66)	666.62 a (10.91)	615.49 b (9.34)

Values in parentheses are the standard deviation. DMT- Dimethyltin(IV) complex, DPT- Diphenyltin(IV) complex, DBT- Dibutyltin(IV) complex
*Means followed by a different letter within a column are statistically different at $P < 0.05$ using Tukey Multiple Comparison test. **Density of wood cubes before exposure to decay fungus. ***Density of wood cubes after exposure to decay fungus. n=Number of sample.

Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) activities of treated wood varied significantly with the concentration of treating organotin(IV) compounds following 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum* (Table 4.8 and 4.9). Results showed that enzyme activity decreased with the increased organotin(IV) concentrations indicate high concentration of organotin(IV) complex resist the activity of enzyme or enzyme production. LiP, MnP and Lac activity of monosubstituted organotin(IV) treated wood varied from 0.63 to 3.57, 1.91 to 15.07 and 0.62 to 2.02 U/ml, respectively (Table 4.8) whereas disubstituted organotin(IV) treated wood ranged from 0.11 to 2.09, 0.60 to 10.41 and 0.06 to 1.06 U/ml, respectively (Table 4.9). Disubstituted organotin(IV) complexes showed lower enzyme activities than that of monosubstituted organotin(IV) complexes.

Weight losses of organotin(IV) treated wood varied significantly with the concentration of chemicals following 16 weeks exposure to fungi. Weight losses decreased with the increased chemical concentrations indicate higher concentration provides better protection against decay. Among monosubstituted organotin(IV) complexes, the weight losses of MMT and MPT-treated wood cubes varied from 13 to 26 and 10 to 25%, respectively (Table 4.8), whereas using disubstituted organotin(IV) complexes, DMT, DPT and DBT-treated wood cubes weight loss varied from 8 to 21, 6 to 19 and 3 to 16%, respectively (Table 4.9).

Densities of treated wood after exposure to *Trametes versicolor* and *Gloeophyllum trabeum* varied significantly among the chemical concentrations (Table 4.8 and 4.9). Following treatment mean densities of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* varied from 357 to 365, 410 to 416 and 656 to 667 kg m^{-3}, respectively. Results

showed that non-inoculated mean densities of *A. scholaris*, *M. triloba* and *H. brasiliensis* did not differ significantly. Results also showed that densities of treated wood did not change significantly with increased in retention. Whereas after 16 weeks exposure to fungi, the decreased mean densities of treated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* ranged from 255 to 353, 304 to 404 and 503 to 622 kg m^{-3}, respectively, which showed a reduction of 3 to 27%.

Further analyses to determine differences of lignin peroxidase, manganese peroxidase and laccase activities due to chemicals effect with each wood species were performed. Lignin peroxidase, manganese peroxidase and laccase activities of untreated and treated wood cubes with 1%-organotin(IV) complexes after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum* among wood species are shown in Figures 4.19, 4.20, 4.21, 4.22 and 4.23, respectively.

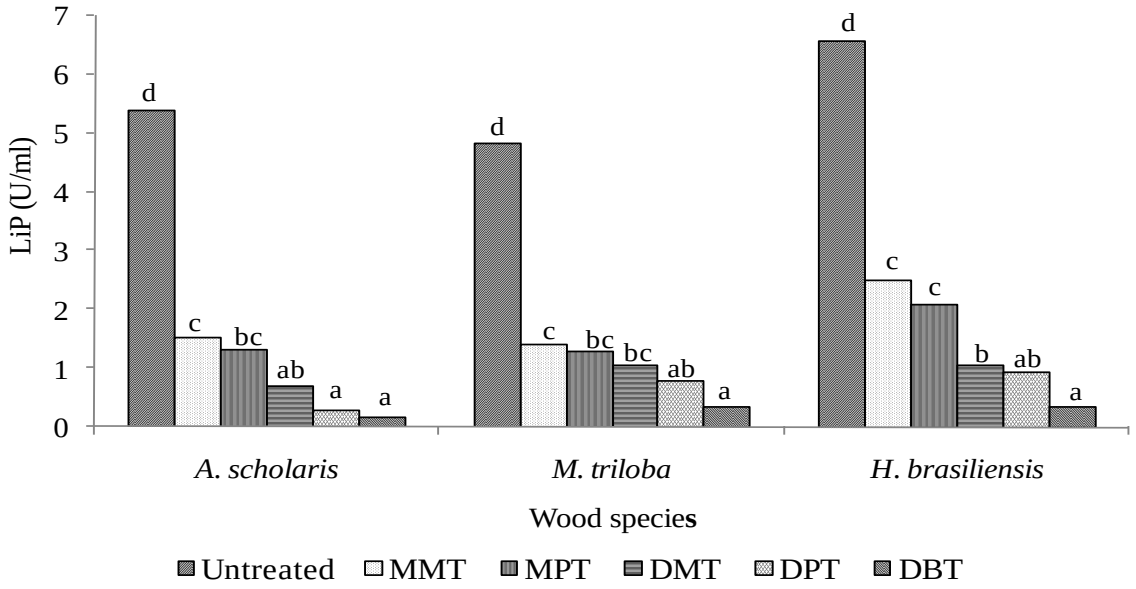


Figure 4.19. Lignin peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

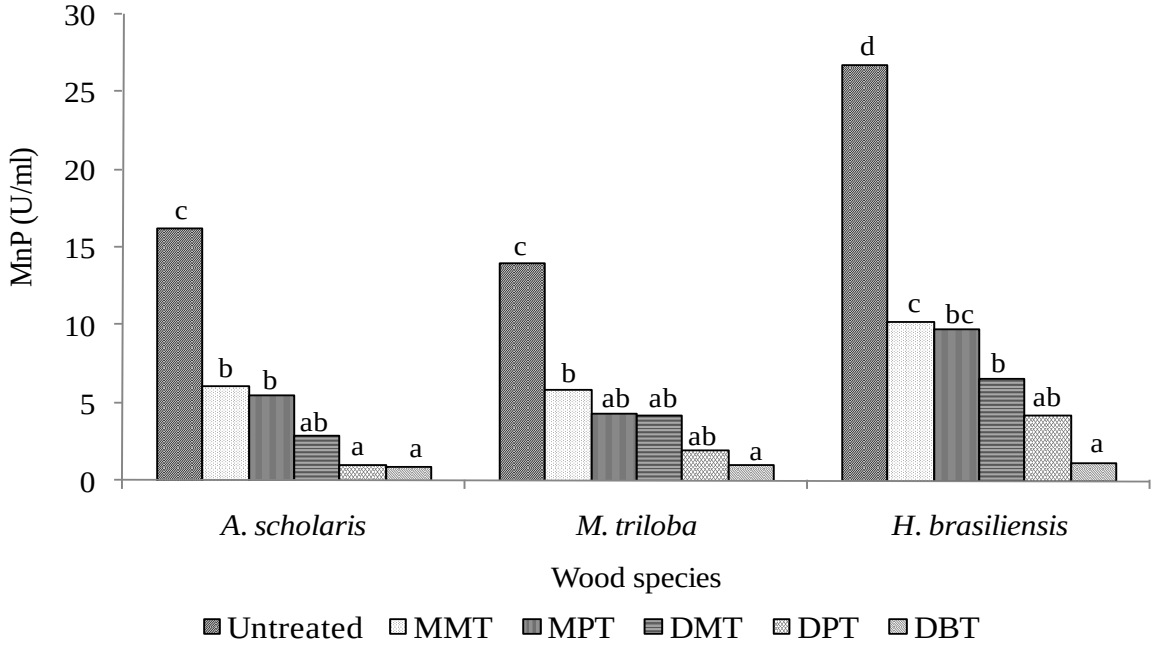


Figure 4.20. Manganese peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

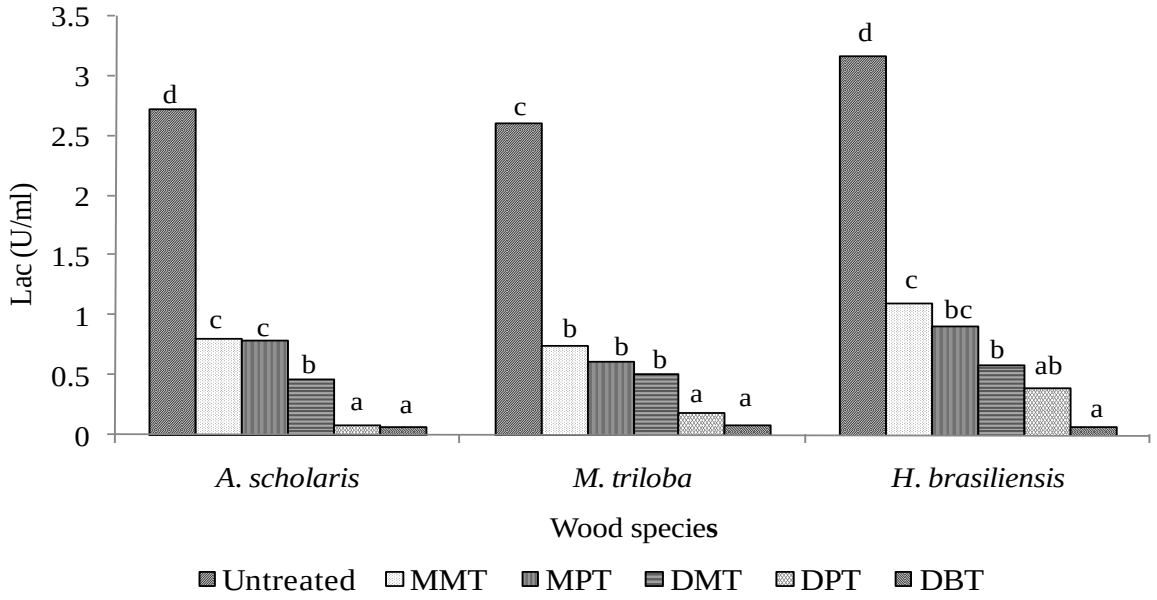


Figure 4.21. Laccase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

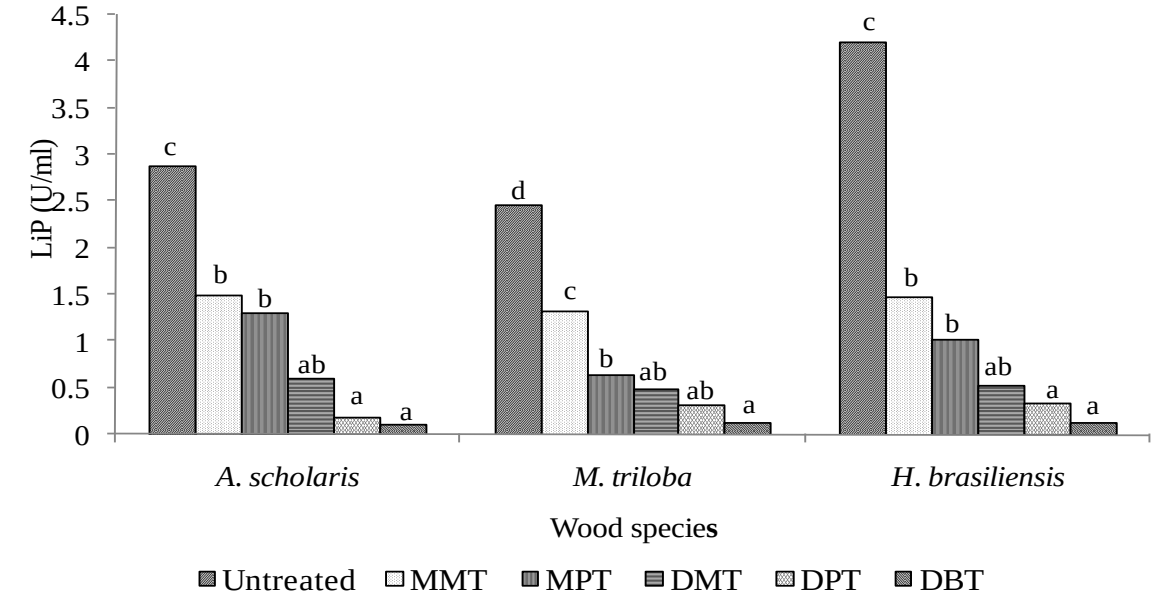


Figure 4.22. Lignin peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Gloeophyllum trabeum*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

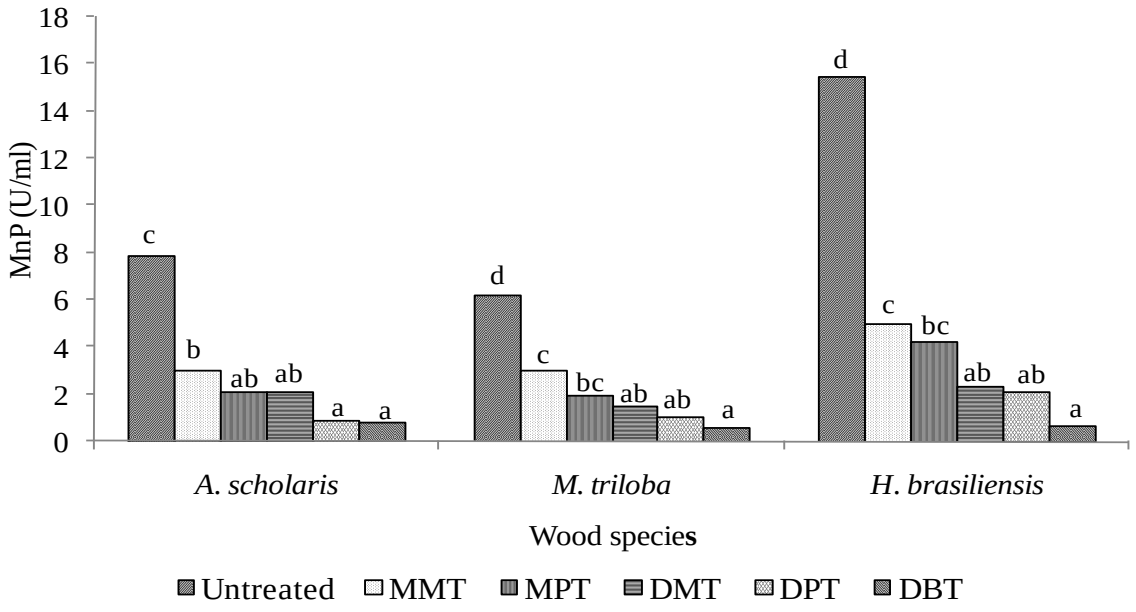


Figure 4.23. Manganese peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Gloeophyllum trabeum.* Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

Results showed that for each wood species, untreated wood enzyme activities is significantly higher than treated wood in both *Trametes versicolor* and *Gloeophyllum trabeum*. Among monosubstituted organotin(IV) treated wood, MMT showed higher enzyme activities than MPT. However for disubstituted organotin(IV) treated wood, the lowest and highest enzyme activity was determined from DBT and DMT, respectively.

Figures 4.19 and 4.22 show that lignin peroxidase activity by *Trametes versicolor* was higher than that of *Gloeophyllum trabeum*. The activities of LiP overall in untreated wood is significantly higher than that of treated wood samples with organotin(IV) complexes. Manganese peroxidase activity was almost two fold higher than that of lignin peroxidase. Laccase activities of untreated wood by *Trametes versicolor* was signifintly higher than treated wood. *Gloeophyllum trabeum* did not show any laccase activity. DBT-treated wood

sample showed significantly lower LiP, MnP and Lac activities than that of other organotin(IV) complexes in all wood species.

4.7 Mechanical properties of Organotin(IV)-treated Wood

4.7.1 Bending Strength of Organotin(IV)-treated Wood

The results of bending test of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* following organotin(IV) complexes treatment are illustrated in Figures 4.24 and 4.25.

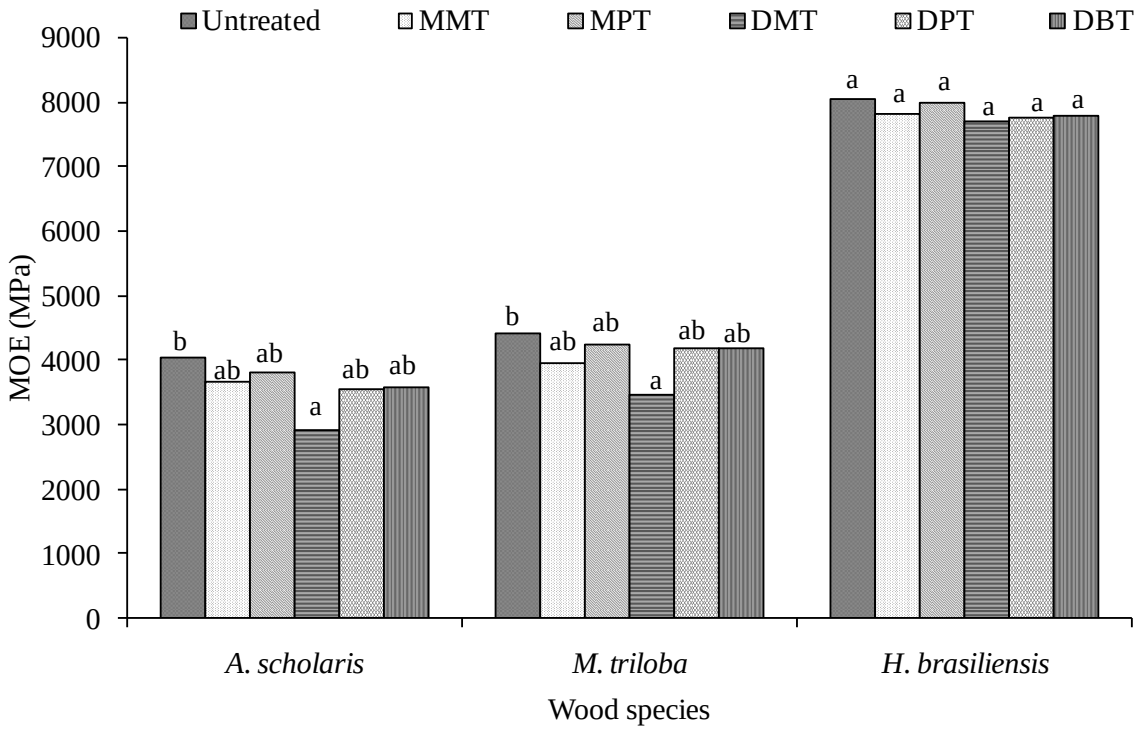


Figure 4.24. Modulus of elasticity (MOE) of untreated and treated wood samples. Different letter within a wood species are statistically different at $P<0.05$ using Tukey Multiple Comparison tests.

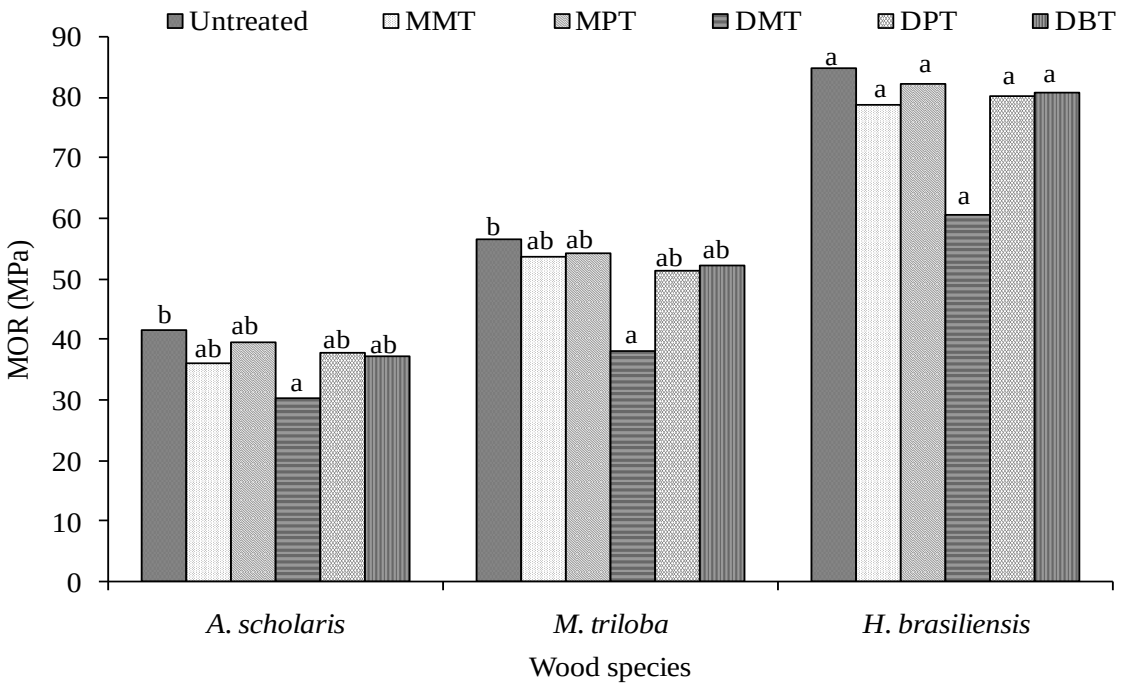


Figure 4.25. Modulus of rupture (MOR) of untreated and treated wood samples. Different letter within a wood species are statistically different at P<0.05 using Tukey Multiple Comparison tests.

Mean MOE of untreated *A. scholaris*, *M. triloba* and *H. brasiliensis* were 4021, 4400 and 8026 MPa, respectively, and that for MOR were 41, 56 and 85 MPa, respectively. It was observed that MOE and MOR of treated samples reduced from 0.7 to 27.6% and 2.9 to 32.7%, respectively compared to untreated samples. However the reduction was not significant except for samples treated with DMT. The MOE of *A. scholaris* and *M. triloba* DMT-treated samples recorded a significant reduction of 27.6 and 21.2%, respectively. As for MOR a significant reduction of 27.1 and 32.7% was recorded for *A. scholaris* and *M. triloba*, respectively. No significant reduction of bending strength of treated samples was observed for *Hevea brasiliensis*.

4.7.2 Compressive strength of organotin(IV)-treated wood

The compressive strength parallel to the grain for treated and untreated wood samples are presented in Figure 4.26. Mean compressive strength of untreated *A. scholaris*, *M. triloba* and *H. brasiliensi* were 22, 26 and 35 MPa, respectively.

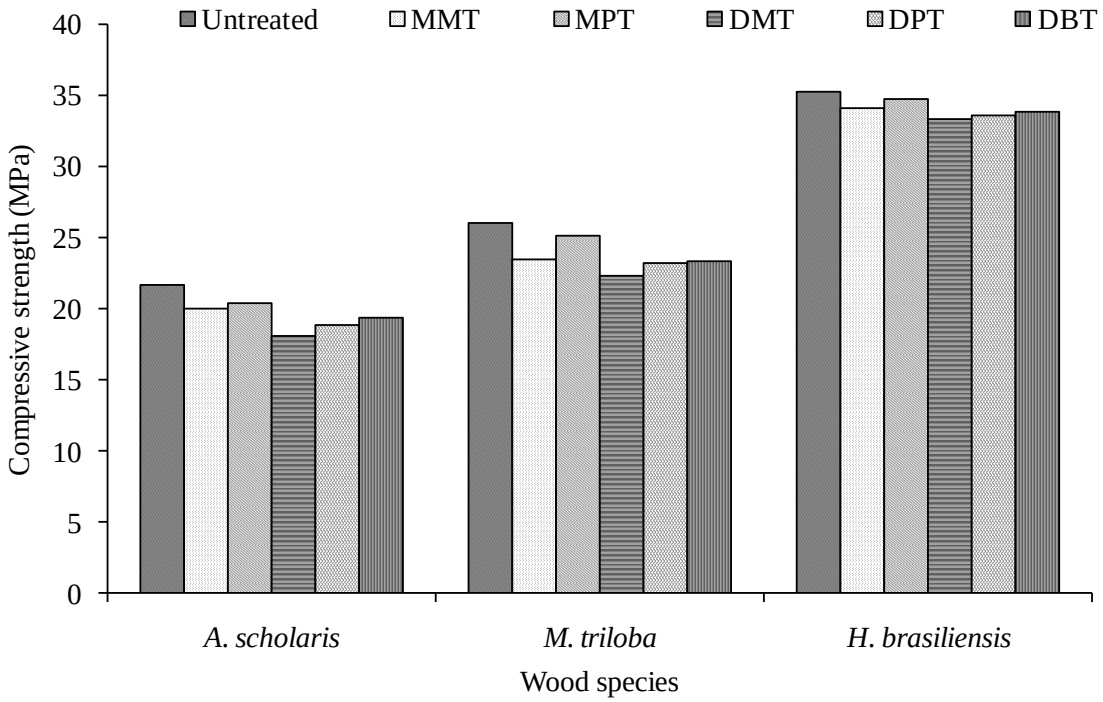


Figure 4.26. Compressive strength of untreated and treated wood samples.

The compressive strength of treated wood sample was lower than untreated wood for all treating organotin(IV) complexes in all wood species but did not show any significant effect following organotin(IV) treatment. The highest (35 MPa) and lowest (18 MPa) compressive strength was observed respectively in MPT-treated *H. brasiliensis* and DMT-treated *A. scholaris* wood. Disubstituted organotin(IV)-treated wood samples showed higher decreasing tendency than that of monosubstituted organotin(IV)-treated in all wood species. Insignificant reduction of 1.7 to 17.0% in compressive strength was recorded of organotin(IV)-treated wood samples compare to untreated wood samples.

CHAPTER FIVE

DISCUSSION

It should be noted that this is preliminary study on newly synthesized organotin(IV) compounds and it is merely an academic exercise. I was recently made aware of the status of tin-based preservatives in wood protection, that global research in organotin as wood preservative is discouraged due to environmental concern with some forms of wood preservatives. Here the author attached some world scientist opinion relevant to tin based wood preservatives-

1. Yes, tin is discouraged mainly for environmental concerns but also as it does not last long-term. The main concerns first arose when used in antifouling paint and TBT caused deformities and toxicity to marine life. But I think those concerns have also transfered to wood preservation and other uses. So the thesis would be more of academic value only. (Dr. Laurie Cookson, formerly, CSIRO Australia).

2. The ultimate test for environmentally sound technology and sustainability is the following. The end of the service life of the product is questionable. Recyling might not be appopriate. Method for extraction of tin is not known. (Prof Dr. Pascal Kamdem, Michigan State University, USA).

3. It has not been used here for at least 15 years- probably closer to 20 year- because of the potential effects on shellfish. As I recall, it was also volatilized from the wood in outdoor exposures and did not do well in field tests. (Prof. Dr. Jeff Morrell, Oregon State University, USA).

4. Tin is a heavy metal not wanted in nature. Tin systems were phased out in Denmark 20 yrs ago and IPBC/tebuconazole/propiconazole systems used instead (Dr. Thomas Venas, Danish Technological Institute, Denmark).

5.1 Treatability of organotin(IV) complexes

Penetration and retention extents of treating organotin(IV) complexes in wood sample crucial to assess the treatability of tropical wood before exposure to wood decay fungi. This study explored whether three selected tropical wood species namely *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* could be treated successfully with newly synthesized organotin(IV) complexes as wood preservatives.

Results presented in Table 4.1 and Figure 4.1 suggest organotin(IV) can successfully treat *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis*. Treatability results showed that preservative retention for *Alstonia scholaris* was almost twice that of *Hevea brasiliensis*. Retention values indicate the treatability of wood species is in the decreasing order of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis*. This is expected because lower density wood species gain higher amounts of preservatives and vice-versa (Yap *et al*., 1990). *Hevea brasiliensis* is a treatable timber (Hong and Wong, 1994; Mohd. Dahlan *et al.*, 1994; Hiziroglu, 1997; Jusoh and Kamdem, 2000). This study showed that non-durable tropical wood namely *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* are treatable with organotin(IV) preservatives and highest retention (10.59 kg m^{-3}) was

recorded in *Alstonia scholaris* treated with 1% dimethyltin(IV) complex. This is comparable to the retention in *Hevea brasiliensis* treated with 2% copper chrome arsenic type C (CCA-C) after two hours of pressure by full cell method which was 13.0 kg m^{-3} (Jusoh and Kamdem, 2000).

The Malaysian Standard MS 360: 1991 did not specify the use of organotin(IV) to treat wood. However, as a point of reference the specified retention of 8 kg m^{-3} and 12 kg m^{-3} of the CCA preservative is required in the treated timber for above ground use. Retention results from this study (Table 4.1) indicated that disubstituted organotin(IV) treated wood showed higher retention than that of monosubstituted organotin(IV) complexes. Apparently retention increased with the increase of organotin(IV) concentration. Greaves *et al*. (1982) observed that treating *Pinus radiata* using 0.1% TBTO resulted in retention of 1.2 kg m^{-3}.

Fourier transform infrared (FTIR) spectroscopy analysis was an attempt to authenticate that the preservative tin was incorporated within the wood cell. From Figures 4.10, 4.11 and 4.12, the IR spectra of the untreated wood showing the absorption band at 3406-3415, 2903-2917 and 1730-1742 cm^{-1} due to OH, CH and CO stretching vibrations, respectively, are due to the hydroxyl group in cellulose, carbonyl group in hemicellulose and carbonyl aldehyde in lignin (Ismail *et al*., 2002). Zhang and Kamdem (2000) also observed that hemicellulose and lignin are the bonding sites for copper of a preservative. In this thesis, the formation of new bonds such as tin-carbon (Sn-C), tin-oxygen (Sn-O) and tin-nitrogen (Sn-N) by the fixation of organotin(IV) compound within the wood cell was confirmed by the

FT-IR spectroscopy analysis of treated wood. In the spectra of organotin(IV) treated *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood, a new absorption band at 594-606 cm^{-1}, 561-569 cm^{-1} and 441-457 cm^{-1} are assigned to the stretching mode of ν(Sn-C), ν(Sn-O) and ν(Sn-N), respectively (Table 4.6). A new ν(Sn-O) linkage indicating the tin(IV) coordinated with oxygen of OH after deprotination in wood cell suggests the fixation of organotin(IV) within the wood cell (Yin *et al.*, 2007; Mendes *et al.*, 2006).

Moreover IR spectra of treated wood clearly showed the presence of the characteristics of cyclohexyl, C=N, C-S and N-N bond at 2929-2937 and 2851-2854; 1542-1559; 1242-1255 and 829-836; and 1011-1034 cm^{-1}, respectively (Rebolledo *et al*., 2005; Elvy *et al*., 1995; Haque *et al*., 2009; Covolan *et al*., 1997). Figures 4.10, 4.11 and 4.12 show that the absorption band of OH group also shifted towards lower wavenumbers from 3415 to 3375-3395 cm^{-1} with narrowed band intensity, which gives further evidence of the reaction of cellulose OH groups with organotin(IV) compound and formed Sn-O bond (Hortling *et al*., 1997; Tolvaj and Faix, 1995). This study established by FTIR that preservative tin (Sn) incorporated within the non-durable tropical wood cell and formed new linkage tin-oxide (Sn-O). Based on the IR spectra results, it can be confirmed that the organotin(IV) compounds were incorporated within the wood cell which makes tropical woods treatable with synthesized organotin(IV) compounds.

5.2 Decay of untreated wood cubes

The mean weight loss of untreated *Hevea brasiliensis* wood cubes was significantly higher than *Macaranga triloba* and *Alstonia scholaris* both in soil block (Table 4.2) and enzymes assay (Table 4.7) test. The presence of carbohydrates in *Hevea brasiliensis* is high which makes it highly susceptible to decay (Florence *et al*., 2005; Kadir and Sudin, 1989). Higher percent weight loss recorded from both soil block and enzyme assay tests were attributed to adaptability, survivability of fungi (Nilsson, 1997), moisture content (Nan *et al*., 1991), temperature (Rowan *et al*., 1999). Low relative humidity and extreme temperatures can inhibit growth and spore germination of fungi (Harrison *et al*., 1994). However in a natural environment fungal abundance and richness are positively related to soil organic carbon, nitrogen, clay, and silt and negatively related to soil pH and high sand content (Talley *et al*., 2002). In this study weight losses due to *Trametes versicolor* were slightly higher than those of *Gloeophyllum trabeum* after 16 weeks of incubation possibly due to wood species, fungal adaptability (Anagnost and Smith, 1997; Nilsson, 1997) and genetic factors (Campbell and Clark, 1960).

Mean moisture content (MC) of untreated wood cubes ranged from 45 to 68% after 16 weeks exposure to *Trametes versicolor* and *Gloeophyllum trabeum*, which is in agreement with Scheffer (1973). Scheffer (1973) reported that optimal moisture levels for growth of most basidiomycete decay fungi in laboratory evaluations are between 40 and 80%. Mean MC was significantly different among wood species after exposure to *Trametes versicolor*.

After exposure to *Gloeophyllum trabeum* mean moisture content of *Alstonia scholaris* and *Macaranga triloba* was similar at 45% MC (Table 4.2). Fungal colonization of wood begins when at moisture content about 20% (Zabel and Morrell, 1992) and moisture critically affects the growth and development of decay fungi which is directly related to weight loss of decayed wood (Eaton and Hale, 1993).

5.3 Efficacy of organotin(IV)-complexes

Results showed that weight loss decreased with the increased in chemical retention as expected. This indicates that high retention provide better protection of wood against *T. versicolor* and *G. trabeum*. However, the protection magnitude differ between mono- and disubstituted organotin(IV). After decay, higher density reduction of monosubstituted organotin(IV)-treated wood cubes compare to disubstituted organotin(IV)-treated wood cubes indicate that disubstituted organotin(IV) provide higher decay resistance than monosubstituted organotin(IV).

The approximate threshold value for DBT was 6.21 and 9.62 kg m^{-3} indicating that at 6.21 and 9.62 kg m^{-3} DBT is effective in protecting *H. brasiliensis* and *A. scholaris*, respectively against *Trametes versicolor*. The amount of tin in 1% DBT-treated *H. brasiliensis* and *A. scholaris* were estimated to be 0.02 and 0.06 % g/g, respectively. The DBT was used for estimating the threshold because it was the most effective among the tested organotin(IV) complexes. Further examination showed that DBT provided good protection to *H. brasiliensis* with weight losses of less than 5% at retention between 5.97 and

6.44 kg m^{-3} exposed to *T. versicolor*. This suggests that the toxic limit for DBT-treated *H. brasiliensis* was 5.97 to 6.44 kg m^{-3}. The threshold values for DBT-treated *M. triloba* against *T. versicolor* and *G. trabeum* were not detected. The toxic limit is the interval between the retention of preservatives chemical which just permits decay and the next highest in the series which inhibits decay. A weight loss of below 5% was considered as showing no decay (Hill *et al*., 1983). The toxic limits for unleached tributyltin ethanesulphonate were 0.53 to 1.07 kg m^{-3} for protecting ponderosa pine and alder wood blocks against *T. versicolor* (Hill *et al*., 1983). Since trisubstituted organotin is more toxic than disubstituted organotin (Richardson, 1993) thus it can be expected that low tributyltin retention will provide good protection against wood decay fungi.

Organotin(IV)-substituted thiosemicarbazone compounds used in this study is likely to be stable due to the presence of five-membered chelate rings in the coordination compounds as illustrated in Affan *et al*. (2011). Due to the presence of chelate ring there is less chance to leach out into the environment which make these compounds could be more effective compare to other tin based wood preservatives like TBTO and TBTN (Affan *et al*., 2012). Besides, 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone ligand itself is considered as toxic to the fungi. Therefore organotin(IV)–substituted thiosemicarbazone complexes are possibly more biologically effective. Furthermore the presence of S atom in the compounds might enhance its biological activity (Affan *et al*., 2011). In addition, these complexes could be comparatively safe compare to the CCA due to the absence of arsenic. According to Hoch (2001), the toxicity of organotin(IV) compounds against fungi varies widely, depending first on the number of organic groups attached to tin and second on the

nature of the organic groups. Therefore, changing the organic groups play a significant role on growth inhibitory activity of the compounds.

Among the tested organotin(IV) complexes, DBT was found with the best protection against decay fungi. This is possibly due to the bulkiness and also due to more electron delocalization as pointed by Li *et al*. (2011). On the other hand, methyltin(IV) complex showed less toxicity compared to others. This is most likely due to the presence of two chlorides which makes the complexes more polar and enhances decreases permeability through the wood cell (Teoh *et al*., 1999). Antifungal activity of the organotin(IV) complexes are due to either killing of microbes or inhibiting their multiplication by blocking their active site (Nath *et al*., 2003).

Selected organotin(IV) complexes in this study showed toxicity and exhibit strong biological activities at higher retention level. Salamah *et al*. (1993) reported that CCA and CCB at 5% concentration and above gave sufficient protection to *Koompassia malaccensis* (Kempas) and *Shorea leprosula* (meranti tembaga). A 3.5% TBTN in the formulation of fungicide in a study by Zaidon *et al*. (2008) found to be effective in protecting rubberwood against *Pycnoporus sanguineus*.

Results indicated that disubstituted organotin(IV) complexes are more effective than monosubstituted organotin(IV) complexes and this is in agreement with Richardson (1993). Richardson (1993) reported that disubstituted organotin(IV) shows greater toxicity than monosubstituted organotin(IV) against decay fungi. This study showed best protection with 4% weight loss was recorded in DBT(APCT)-treated *A. scholaris* wood cubes exposed to *T.*

versicolor and lowest protection with 19% weight loss observed in MMT(APCT)-treated *M. triloba* wood cube exposed to *G. trabeum*.

5.4 Microdistribution of tin in *A. scholaris, M. triloba* and *H. brasiliensis* wood cells

Energy dispersive X-ray analysis (EDXA) was conducted to confirm and obtain better understanding about the incorporation of tin within wood cell. The SEM-EDXA results revealed that tin is deposited in the reacted wood, indicating that chemical bonds were formed as a result of the reaction of wood and tin. Distributions of tin in the *Hevea brasiliensis* wood cell were variable. Different tin counts were observed at different positions of the cell wall and cell middle lamella. From Figure 4.15 it can be observed that the count rates for tin is relatively uneven across the secondary cell walls and the X-ray intensities were lower in the S_2 cell wall layer compared to the middle lamella. Uneven distribution of copper was detected by An *et al*. (1998) in lumen surface and ray cells of CCA-treated heartwood of lodgepole pine and white spruce. Using Field Emission Scanning Electron Microscopy (FE-SEM) in combination with X-ray Microanalysis (EDX), Matsunaga *et al.* (2007) studied the southern pine wood commercially treated with micronized copper quat (MCQ) and confirmed the presence of copper in the cell wall. In addition, they concluded that the micro distribution of copper in MCQ treated wood looks like that observed in wood treated with amine soluble copper wood preservative. Stirling *et al*. (2008) conducted a study on MCQ treated wood using Environmental Scanning Electron Microscopy (ESEM) equipped with Energy Dispersive X-Ray Spectrometry (EDS), and their study revealed that there was a high amount

of copper in the lumens and small amount of copper in cell walls of wood for both MCQ treated wood and Ammine Copper Quat (ACQ) treated wood. This finding is consistent to that observed by Matsunaga *et al*. (2007). Doyle and Ruddick (1994) studied the distribution of an alkylammonium compound, iodobenzalkonium chloride, in ponderosa pine sapwood using scanning electron microscopy coupled with an energy dispersive X-ray analyzer (SEM-EDX) and detected the highest accumulation of iodine was in the compound middle lamella.

Tin appears to penetrate into the fibre cell walls as revealed by linescan analyses (Figure 4.15). The cell corner and middle lamella are rich in lignin (Fengel and Wegener, 1983; Haygreen and Bowyer, 2003). Lignin plays an important role for bonding preservatives components and it has been suggested that lignin is one of the binding sites for preservative components (Daniel and Nilsson, 1987; Ryan and Drysdale, 1988). Lee *et al*. (1992) revealed that chromium, copper and arsenic were more abundant in the compound middle lamella in wood treated with chromate-copper-arsenate (CCA). Daniel and Nilsson (1987) reported that the relative microdistribution of CCA to follow closely that of the lignin distribution and regions showing high lignin levels showed high CCA levels and vice-versa. They recorded highest CCA and lignin in the vessels, fibre and ray middle lamella cell corner regions while the lowest levels were detected in the fibre (S_2) secondary walls. They also pointed that the lignin content and fixation of preservative components varied with wood species. Another factor influencing even CCA distribution in softwoods than hardwoods may be due to the presence of G-lignins in softwoods versus higher proportions of S-lignins in many hardwoods (Kim *et al*., 2006; Nilsson *et al*., 1988). Additionally, the distribution of lignin within the cell wall and the lignin content of different parts of a tree are not uniform. For example, high

lignin amounts are characteristic for softwood branches and compression wood (Timell, 1986), whereas the gelatinous layers of the tension wood fibres in hardwoods may be almost lacking of lignin (Novaes *et al*., 2010). Scanning electron microscopy (SEM) combination with energy dispersive X-ray analysis (EDXA) technique confirmed on a high-resolution level that middle lamella portions contain distinctly more lignin than secondary walls in wood (Donaldson and Ryan, 1987). The variation of lignin content of different wood cell is most likely due to one important cause of uneven distribution of preservatives component of different wood cell. It is well known that the cell corner and middle lamella regions are constituents with the highest lignin content (Fengel and Wegener, 1983). Compound middle lamellae are generally rich in lignin with lignin and hemicelluloses accounting for more than 90% of this zone which may have caused the greater copper concentration in the compound middle lamella (Matsunaga *et al*., 2004). They observed the uneven copper concentration in Japanese cedar sapwood, although the aqueous solution penetrated the sapwood specimen entirely.

For any full cell preservative treatment like this study it is expected that the cell lumina are filled preservative solution and as the wood dries the preservative components are deposited on the lumen wall (Hedley *et al*., 1990). Conventional SEM images and EDX maps of treated southern pine revealed that both copper and iron were deposited in the lumina of rays and some tracheids and also on bordered pits (Matsunaga *et al*., 2008). Dawson-Andoh and Kamdem (1998) studied copper microdistribution in copper naphthanete treated soft maple and northern red oak. They used environmental scanning electron microscopy and detected high counts of copper in the vessels of both treated wood species. Jusoh and

Kamdem (2009) studied the microdistribution of chromate copper arsenate (CCA) preservative in rubberwood using scanning electron microscope in conjunction with energy dispersive X-ray analyzer (SEM-EDXA). They observed a high accumulation of chromium, copper and arsenic in the vessels and lower concentration of the three preservative elements in fibres. The increase of the solution strength in chromium, copper and arsenic corresponds to an increase in Cr, Cu and As level in wood cells (Jusoh and Kamdem, 2009).

5.5 Leaching of organotin(IV)

Leaching is an important part of water-borne preservative performance in treated wood and environmental contamination. Organotin(IV) preservatives contain tin which is impregnated in wood and the majority of tin react with wood substrate to form water insoluble tin oxide (Yin *et al.*, 2007; Mendes *et al.*, 2006). Tin oxide of treated wood is most likely permanent and fix in wood cell wall. Reacted tin would stay in wood cell. However there still remains partial non-reacted free tin component which is leach out to the water. Tin from vessel which is unfix or not reacted most probably leach out to the water. This explain that organotin(IV) preservatives have relatively high tin leaching rate for the first 30 hours, and then the tin leaching rate is substantially declined till 270 hours. After that no tin was detected in the leachates. Results are consistent with the findings of Brooks (2000) and Waldron *et al*. (2005). They observed that the greatest preservative losses tend to occur in the period immediately after exposure of water following treatment, then decrease with time in service. Zhang and Ziobro (2009) conducted a research study to observe the release of cupric ion ($Cu2^{+}$) from treated wood with copper preservatives. They observed that wood treated

with micronized copper preservatives release cupric ion at a higher level than CCA and leaching results indicated that the leaching rate is higher at first 24 hours.

Treated *Alstonia scholaris* and *Macaranga triloba* wood leached relatively higher amount of tin than *Hevea brasiliensis* (Figures 4.16, 4.17 and 4.18). This is expected because high density wood performs low retention and low leaching loss of preservative component. This result is comparable with the findings of Cockcroft and Laidlaw (1978) and Wilson (1971). They pointed out that high density, lower permeability species of wood tend to be more resistant to chemical leaching. Also moisture content influences the rate of preservative diffusion within the wood. Low moisture content increases the diffusion and decreases the leaching (Kaldas and Cooper, 1996; Cockcroft and Laidlaw, 1978). Results are also consistent with the observation of Yap *et al*. (1990). Chung and Ruddick (2004) pointed out some factors that influence leaching are rainfall or water exposure, temperature, exposure time and sunlight. However, Kennedy and Palmer (1994) also noted that leaching of CCA may be greater from heartwood than sapwood, possibly because the heartwood extractives interfere with the fixation process. Salim *et al*. (2012) observed that the arsenic losses were significantly higher in some Malaysian tropical hardwoods (MTHs) than in *radiata* pine, except for *Acacia*. High amount of copper and chromium losses were also observed in Geronggang (*Cratoxylon arborescens*), Meranti rambai daun (*Shorea acuminata*), Putat (*Barringtonia racemosa*) and Ramin (*Gonystylus bancanus*). The high amount of CCA components leached from the treated MTHs could be explained by the deposition of the products derived from CCA fixation reaction in the cell lumens due to higher extractive levels where they would be more readily accessible to leaching (Cooper, 1991; Srinivasan *et*

al., 1999; Stevanovic-Janezic *et al.*, 2000; Kim *et al.*, 2008). Differences of leaching losses among Malaysian tropical woods are possibly due to the differences in wood chemical composition such as extractive content and the ratio of major cell wall components including cellulose, hemicelluloses and lignin (Yamamoto and Rokova, 1991; Srinivasan *et al.*, 1999). Generally, leaching of components from preservatives treated lumber is small and primarily occurs immediately after the initial exposure of the treated member as unfixed components are removed from the wood surface (Evans, 1987). Even fully-fixed CCA will leach to some degree, depending on the exposure conditions (Ruddick, 1993), but this process is slow. Organic solvent preservative relatively insoluble in water and their permanence depends on their degree of volatility (Kaldas and Cooper, 1996; Cooper and Stokes, 1993).

Moreover the type of grain exposed can also influence leaching characteristics. Although wood properties may affect leaching in different ways, comparative studies generally agree that CCA components are more leachable from hardwoods than softwoods (Becker and Buchmann, 1966; Cooper, 1990; Yamamoto and Rokova, 1991) and CCA-treated hardwoods are more susceptible to soft-rot attack (Butcher and Nilsson, 1982; Gray, 1993). This phenomenon has been attributed to the lower content and type of lignin in hardwoods (Butcher and Nilsson, 1982; Cooper, 1990; Gray, 1993). However, this trend is not always consistent and softwood species also differ in leach susceptibility. Slow fixation and high leaching rates have been noted for Japanese cedar (Yamamoto and Rokova, 1991), and Cooper (1990) reported that CCA leaching rates from small, red pine specimens were approximately double those from lodgepole pine, Douglas Fir, and red cedar.

5.6 Lignolytic enzymes activities

The activity of recovered manganese peroxidase (MnP) was far more than that of lignin peroxidase (LiP), suggesting that MnP might be the predominating enzymes causing lignin degradation or modification (in brown rot) in tested tropical wood samples as shown in Tables 4.7, 4.8 and 4.9. The higher detectable MnP activity compared to LiP and laccase might explained that some Mn-dependent peroxidase could be present in the wood extracts if they contain enough $Mn^{2+}$to initiate MnP catalyzed reactions. This result is consistent with the findings of Husaini *et al*. (2011), where they recorded the highest MnP activity from *Acacia mangium* wood chips exposed to *Trametes versicolor* among other lignolytic enzymes. Popp *et al*. (1990) observed that LiP can display MnP activity in the presence of H_2O_2, O_2, and metabolizes veratryl alcohol and oxalate, LiP oxidizes Mn^{2+} to Mn^{3+}.

The lowest activity of laccase was recovered in all the fungi tested in all wood species. The lower level of detectable laccase activity depends on the addition of easily available carbon and nitrogen sources to the culture medium (Enoki *et al*., 1999; Ferraz *et al*., 2003). It could also be due to the low content of copper (inducer of laccases) (de Souza-Cruz *et al*., 2004) in *Acacia mangium* wood. In this study, the brown-rot fungus *Gloeophyllum trabeum* did not show any laccase activity but the white-rot fungus *Trametes versicolor* produced the least amount of Lac activity during wood decay. Laccase activity in this study is consistent with the findings of Mtui and Masalu (2008). They studied a brown-rot basidiomycetous fungus *Laetiporus sulphureus* and recorded MnP (2.5 U/mL) and LiP (1 U/mL), but showed no laccase activity. On the other hand the high rate of laccase activities

than the other lignolytic enzymes was extracted from white-rot basidiomycete fungus *Pycnoporus coccineus* in *Acacia mangium* wood chips by Husaini *et al*. (2011). This might be due to the high content of copper in *A. mangium* wood (de Souza-Cruz *et al.,* 2004). Copper had been reported to be a strong laccase inducer in the fungal species such as *Trametes versicolor* and *Pycnoporus chrysosporium* (Collins and Dobson, 1997; Dittmer *et al*., 1997**)**. The presence of copper did not affect fungal growth since the biomass dry weights at different times were the same in the presence and in the absence of copper (Palmieri *et al*., 2000).

In this study, the low levels of LiP activity detected was compared to MnP activity in the wood extracts and showed that LiP might be of minor significance in wood degradation. However, the low detectable level might be caused by interferences that could possibly occurred upon assaying LiP in extracts recovered from cultures grown on lignocellulosic materials. Archibald (1992) reported that typical LiP assay based on oxidation of veratryl alcohol suffers the interference of dissolved aromatic compounds present in wood extracts.

Weight losses were consistent with the increasing amount of ligninolytic enzymes recovered from the wood extracts. However, a direct correlation between the levels of oxidative ligninolytic enzymes and the rate of lignin removal was not expected because the lignin removal expresses the final stage of lignin degradation, which starts with the depolymerization steps. Results obtained from this study on the lignin losses was in agreement with other studies (Haddadin *et al*., 2002; Ferraz *et al.,* 2003) that *Ceriporiopsis subvermispora* and *Ganoderma* sp. also showed 20–30% of lignin breakdown at 60 days of

biodegradation periods. Liew *et al*. (2011) recorded the highest activity of MnP than LiP and Lac from *Acacia mangium* wood chips exposed to *Trametes versicolor*. It is well established that enzyme production is highly dependent on the cultivation conditions of an organism (de Souza- Cruz *et al*., 2004). Most white rot fungi started lignin degradation when nitrogen, carbon, or sulfur became limited (Gold and Alic, 1993). Dashtban *et al*. (2010) showed that white rot fungi degrade lignin efficiently using a combination of extracellular ligninolytic enzymes, organic acids, mediators and accessory enzymes.

Trametes versicolor a white rot fungus produced more enzymes during decay process than that of brown rot *Gloeophyllum trabeum*. This is expected because brown rot fungi degrade wood using both enzymatic and non-enzymatic process. It has been established that the brown-rot fungi selectively decay cell-wall polysaccharides, with limited lignin degradation (Eriksson *et al*., 1990). The decay system in this type of fungus is based on both non-enzymic (chemical) and enzymic attacks (Eriksson *et al*., 1990). On the other hand, white rot fungi have wood degrading mechanisms which mostly involve enzyme catalysis by hydrolases (i.e. cellulases and hemicellulases) and oxidizing enzymes (i.e. peroxidases and laccases) and are considered the only organisms capable of total mineralization of lignin (ten Have and Teunissen, 2001).

Analyses of lignin peroxidase, manganese peroxidase and laccase activities showed that LiP, MnP and Lac production of untreated wood was significantly different from treated with mono- and diorganotin(IV) wood. This is expected because organotin(IV) treated wood produce comparatively lower amount of lignolytic enzymes than that of untreated wood when

exposed to both *T. versicolor* and *G. trabeum*. This data on lignolytic enzyme profiling represents a significantly interesting results. The results obtained indicated that the reduction in lignolytic enzyme production, those oxidative enzymes that are responsible for lignin biodegradation are in correlation to organotin(IV) complexes pretreatment. In white-rot fungi, most attention has been focused on the metal toxicity towards extracellular enzyme produced. These involved the energy metabolism complex of cellulose and hemicellulose degrading enzymes as well as the lignolytic enzymes produced extracellularly. The enzymes produced often face high concentrations of metals, since they are not protected by the cell-associated metal-detoxication mechanisms. After entering the cell, metals can also influence the production of extracellular enzymes on the levels of transcriptional and translational regulation (Baldrian, 2003). Treated wood might be less susceptible to enzyme involved in the degradation of wood cell or less accessible to enzymes or inhibit the fungal activity by preservatives (Elisashvili *et al*., 2012). Transition metal compounds of dithiocarbamates and dithiophosphinates are known antifungal agents (Kalita *et al.*, 2002) and the mode of action being inhibition of certain vital enzymes by the sulphur donors, the Cu^{2+} also can inhibit action of several biomolecules. Dithiocompounds also inhibit the enzyme *actylcholinesterase* (Gruzdyev *et al.*, 1980).

5.7 Effect of organotin(IV) treatment on mechanical properties of wood

The bending and compressive strength results of untreated *A. scholaris* and *H. brasiliensis* are comparable with the values published by MTIB (2010). According to Malaysian Timber Industry Board (MTIB: 2010), the air-dry compression parallel to grain of *A. scholaris* and *H. brasiliensis* are 25 and 32 MPa, respectively.

Preservative treatment of wood sometimes may alter its mechanical strength. The strength decrease might be due to the depolymerization reactions which play an important role in strength properties of wood (Hillis, 1984; Yildiz *et al*., 2006). The variation of bending and compressive strength may occur within a species due to location within the tree, site condition, genetic factor and age of tree (Fearnside, 1997; Josue, 2004; Izekor *et al*., 2010).

Figures 4.24 and 4.25 showed that bending strength of treated wood sample was somewhat reduced for all treating organotin(IV) complexes in all wood species. Among the five organotin(IV) complexes, only DMT-treated *A. scholaris* and *M. triloba* wood samples were significantly ($P<0.05$) reduced than that of untreated samples. Reduction in bending strength of chemically treated wood was attributed to the hydrolysis of hemicellulose in the cell wall (Hillis, 1984). The effect of treated wood on its properties may be due to the disturbance within the wood fibre (LeVan and Winandy, 1990) and chemical interaction

following treatment. This study showed that bending strength of treated *H. brasiliensis* was not affected as much as MOE and MOR in *A. scholaris* and *M. triloba* wood samples.

Hiziroglu (1997) observed that static bending properties of CCA-treated rubberwood reduced as pressure treatment time increased compare to untreated sample. Yildiz *et al*. (2004) also reported that almost 10% decrease in MOE and 12% decrease in MOR of yellow pine wood samples treated with CCA compared to untreated one. Green *et al*. (2007) stated that wood preservatives can negatively affect on MOE and MOR of wood and may reduced the strength up to 30%.

The compressive strength of treated wood samples was reduced but was not statistically significant (Figure 4.26). Wood preservatives can affect compressive strength parallel to grains and the strength can be reduced up to 20% (Green *et al*., 2007). This study showed that disubstituted organotin(IV) treated wood samples showed higher decreasing strength tendency than that of monosubstituted organotin(IV) treated in all wood species. This effect might be due to the presence of double alkyl or aryl group in disubstituted of organotin(IV) complexes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 General conclusions

Since tin preservatives are being rapidly phased out globally this study can be regarded as preliminary and an academic exercise only. The results showed that the newly synthesized organotin(IV) compounds successfully treated *Alstonia scholaris* (pulai), *Macaranga triloba* (mahang) and *Hevea brasiliensis* (rubberwood). *Alstonia scholaris* recorded the highest preservative retention followed by *Macaranga triloba* and *Hevea brasiliensis*. Obviously preservative retention in wood increased with increased in preservative concentration treatment for all wood species. A newly formed Sn-O bond was observed in all treated wood sample. SEM-EDX analysis suggests the bonding of tin within the wood cell. Linescan analysis showed that the highest tin may be found in fibre wood cells and middle lamella. FTIR and SEM-EDXA showed tin compounds bind with wood cell suggest that the tropical wood species are treatable with synthesized organotin(IV) complexes. Leaching test showed that the least tin was detected from MPT leachate followed by DBT and DMT.

Soil block test revealed that dibutyltin(IV) complex showed the best protection against *Trametes versicolor* and *Gloeophyllum trabeum* after 16 weeks of exposure. The moisture content of cubes exposed to decay fungi increased with weight loss increased. The density of wood cubes decreased with the weight loss increase. Laboratory soil block test showed that novel organotin(IV) compounds are effective in protecting *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* against *Trametes versicolor* and *Gloeophyllum*

trabeum decay fungi. The approximated threshold value for unleached DBT-treated *Hevea brasiliensis* was 6.21 kg/m^3 exposed to *Trametes versicolor*.

This study observed that density of all treated wood samples was comparable to untreated wood sample. Disubstituted organotin(IV) treated wood density was higher than monosubstituted organotin(IV) treated wood density in all wood species. The decreased in MOE, MOR and CS of the wood treated with disubstituted organotin(IV) complexes was much higher than those of monosubstituted organotin(IV) treated wood. The MOE and MOR of DMT treated *A. scholaris* and *M. triloba* wood was significantly lower compare to untreated wood samples. In contrast, MPT treated wood samples showed least effect on MOE, MOR and CS among all tested organotin(IV) complexes.

This study had successfully characterized the lignolytic enzymes namely lignin peroxidase, manganese peroxidase and laccase from the extract of wood cubes after 16 weeks exposure to white rot fungus *T. versicolor* and brown rot fungus *G. trabeum*. Least amount of laccase activity was detected only from *T. versicolor*. However, highest manganese peroxidase activity was detected among three lignolytic enzymes from both fungi where *T. versicolor* showed almost double from *G. trabeum*. The reduction in the production of important oxidative enzymes that plays a major role in lignin degradation are shown to be significantly affected by the organotin(IV) complexes pretreatment in comparison to the control, untreated wood cubes. Therefore, it can be concluded that newly synthesized organotin(IV) complexes had an adverse effect on lignolytic enzymes production and are effective as wood preservatives where DBT complex is the best wood preservative among all the tested organotin(IV) complexes.

6.2 Recommendations

Although some new insight has been gained with respect to the new preservative treatment of tropical wood, some issues still need to be resolved. Therefore it is recommended that the following future work to be carried out (noting that this is an academic exercise, as future use of tin preservatives globally is threaten, see page 108);

(1) Above ground field tests and evaporation test of organotin(IV)-treated wood should be carried out.

(2) More nondurable tropical wood species should be treated with organotin(IV) complex to evaluate fully it's potential as wood preservative.

(3) More level of concentrations to be used to determine threshold retention more precisely.

(4) Microdistribution of tin in organotin(IV)-treated wood should include all type of cells.

(5) Efficacy of these compounds on other white, brown and soft rot fungi should be examined to draw better perspective of the effects of these compounds on the three wood decay types.

(6) Efficacy of these compounds against insects and termites can be evaluated.

(7) Morphological, molecular biology and enzyme kinetic studies on ligninolytic enzymes in relation to organotin(IV) complexes pretreatment on the tested fungus should be carried out to further reveal and understand the mode or mechanism of action and inhibition of the potential preservatives.

REFERENCES

Affan, M.A., Salam, M.A., Ahmad, F.B. and Ismail, J. (2012). Preservation of wood. *BorneoTalk,* 24: 64.

Affan, M.A., Salam, M.A., Ahmad, F.B., Ismail, J., Shamsuddin, M.B. and Ali, H. M. (2011). Synthesis and spectroscopic characterization of organotin(IV) complexes with 2-benzoylpyridine-N(4)-cyclohexylthiosemicarbazone (HBPCT): X-ray crystal structure of [$PhSnCl_2$(BPCT)]. *Inorganica Chimica Acta*, 366: 227-232.

Akhter, K. (2005). Preservative treatment of rubber wood (*Hevea brasiliensis*) to increase it's service life. Document No: IRG/WP 05-40320. The International Research Group on Wood Protection, Stockholm, Sweden.

American Wood-Preservers' Association Standard (AWPA), (1990). "Book of Standard." Stevensville, Maryland.

American Wood-Preservers' Association Standard (AWPA), (1991). Standard method of testing wood preservatives by laboratory soil-block cultures. AWPA standard E10-91. Birmingham, USA. pp. 1-11.

American Wood-Preservers' Association Standard (AWPA), (1997). Standard method of determining the leachability of wood preservatives. AWPA standard E11-97. Birmingham, USA. pp. 1-3.

An, Y., Ruddick, J.N.R. and Morris, P.I. (1998). CCA component distribution in the heartwood of treated lodgepole pine and white spruce. Document No: IRG/WP 98-30173. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-12.

Anagnost, S.E. and Smith, W.B. (1997). Comparative decay of heartwood and sapwood of red maple. *Wood and Fiber Science*, 29(2): 189-194.

Anderson, D.M. (1979). Organotin preservatives for wood structures, David W. Taylor Naval Ship Research and Development Center, Bethesda, MD., Rep. No. DNTSRDC/SME-78141.

Arantes, V., Goodell, B., Milagres, A.M.F. and Qian, Y. (2010). Fungal attack on lignin and cellulose: Elucidation of brown- and white-rot mechanisms comparing biomimetic and in-vivo degradation patterns. Document No: IRG/WP 10-10714. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-20.

Archibald, F. (1992). A new assay for lignin-type peroxidases employing the dye azure B. *Applied and Environmental Microbiology*, 58:3110.

Arora, D.S. and Gill, P.K. (2001). Effects of various media and supplements on laccase production by some white rot fungi. *Bioresource Technology*, 77: 89-91.

Asgher, M., Asad, M.J. and Legge, R.L. (2006). Enhanced lignin peroxidase synthesis by Phanerochaete chrysosporium in solid state bioprocessing of a lignocellulosic substrate. *World Journal of Microbiology and Biotechnology*, 22(5): 445–449.

Asgher, M., Bhatti, H.N., Ashraf, M. and Legge, R.L. (2008). Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation*, 19(6): 771-783.

Asgher, M., Sharif, Y. and Bhatti, H.N. (2010). Enhanced production of ligninolytic enzymes by *Ganoderma lucidum* IBL-06 using lignocellulosic agricultural wastes. *International Journal of Chemical Reactor Engineering*, 8(1): 1-19.

ASTM D-143. (1996). *Standard method of testing small clear specimens of timber*. American society for testing and materials, USA.

Ayer, S.W., Fell, D. and Wan, H. (2003). Hardening of solid wood: Market opportunities and review of existing technologies, Forintek Canada Crop. Project No. 3678.

Baldrian, P. (2003). Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology*, 32: 78-91.

Becker, G. and Buchmann, C. (1966). Comparative chemical tests on the leachability of preservative salts from different wood species. *Holzforschung*, 20: 199–204.

Behbood, M. (2003). *Biological attack of acetylated wood*. PhD thesis. Georg-August University, Goettingen, Germany.

Benetollo, F., Lobbia, G.G., Mancini, M., Pellei, M. and Santini, C. (2005). Synthesis, characterization and hydrolytic behavior of new bis (2-pyridylthio)acetate ligand and related organotin(IV) complexes. *Journal of Organometallic Chemistry*, 690: 1994-2001.

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

Blanchette, R.A. (1995). Degradation of the lignocellulose complex in wood. *Canadian Journal of Botany*, 73: 999-1010.

Blanchette, R.A., Nilsson, T., Daniel, G.E. and Abad, A. (1990). Biological degradation of wood. In: Rowell, R.M. and Barbour, R.J. (eds) *Archaeological wood: properties, chemistry and preservation*, Advances in Chemistry Series 225., Washington, DC: American Chemical Society. pp. 141-174.

Blunden, S.J., Cusack, P.A. and Hill, R. (1985). *The Industrial Uses of Tin Chemicals*. Royal Society of Chemistry, Burlington House, London.

Bodner, J. and Pekny, G. (1991). Distribution of wood preservatives within cell wall. *Holzforschung und Holzverwertung*, 43(2): 46-48.

Bowyer, J.L., Schmulsky, R. and Haygreen, J.G. (2003). *Forest products and wood science: An intoduction.*4th ed. Iowa State Press. Iowa. 554 p.

Brelid, P.L., Simonson, R., Bregman, O. and Nilsson, T. (2000). Resistance of acetylated wood to biological degradation. *HolzalsRoh und Werkstoff,* 58: 331-337.

Brischke, C.R. and Rapp, A.O. (2008). Dose-response relationships between wood moisture content, wood temperature and fungal decay determined for 23 European field test sites. *Wood Science and Technology*, 42: 507-518.

Brooks, K.M. (2000). Environmental impact of preservative-treated wood in a wetland boardwalk. U.S. Department of Agriculture, Forest Service, Forest Product Laboratory Research Paper, FPL-RP-582 (Madison, WI), 136p.

Butcher, J.A. and Nilsson, T.H. (1982). Influence of variable lignin content amongst hardwoods on soft-rot susceptibility and performance of CCA preservatives. Document No: IRG/WP 82-1151. The International Research Group on Wood Preservation, Stockholm, Sweden.

Cai, X., Riedl, B., Zhang, S.Y. and Wan, H. (2007). Effects of nanofillers on water resistance and dimensional stability of solid wood modified by melamine-urea-formaldehyde resin. *Wood and Fiber Science*, 39(2): 307-318.

Campbell, R.N. and Clark, J.W. (1960). Decay resistance of Baldcypress heartwood. *Forest Products Journal*, 10(5): 250-253.

Cao, J. and Kamdem, D.P. (2005). Microdistribution of copper in copper-ethanolamine (Cu-EA) treated southern yellow pine (*pinus* spp.) related to density distribution. *Holzforschung*, 59: 82-89.

Cardwell, R.D. and Sheldon, A.W. (1986). A risk assessment concerning the fate and effects of tributyltins in the aquatic environment. In: Maton, G.L. (Ed.), Proceedings Oceans 86 Conference, Washington DC, September 23-25, 1986, vol. 4, Organotin Symposium, pp. 1117-1129.

Carraher, C.E., Naoshima, Y., Nagao, K., Mori, Y., Zhao, A., Barot, G. and Battin, A. (2008). Bacterial inhibition by organotin-containing polymers. (Ed.) Abd-El-Aziz, A.S. *Inorganic and Organometallic Macromolecules: design and applications*, 421-447.

Chao, W.Y. and Lee, A.W.C. (2003). Properties of southern pine wood impregnated with styrene. *Holzforschung*, 57 (3): 333-336.

Chung, P.A. and Ruddick, J.N.R. (2004). Leaching of copper from ACQ treated wood exposed above ground. Document No: IRG/WP 04-50219. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-11.

Cockcroft, R. and Laidlaw, R.A. (1978). Factors affecting leaching of preservatives in practice. Document No: IRG/WP 78-3113. The International Research Group on Wood Preservation, Stockholm, Sweden.

Coll, P.M., Fernandez-Abalos, J.M., Villanueva, J.R., Santamaria, R. and Perez, P. (1993). Purification and characterization Phenoloxidase (Laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). *Applied and Environmental Microbiology*, 59(8): 2607-2613.

Collins, P.J. and Dobson, A.D.W. (1997). Regulation of laccase gene transcription in *Trametes versicolor*. *Applied Environmental Microbiology*, 63: 3444–3450.

Cooper, P.A. (1990). Leaching of CCA from treated wood. In: Proceedings, Canadian Wood Preservers' Association, 11: 144–169.

Cooper, P.A. (1991). Leaching of CCA from treated wood: pH effects. *Forest Products Journal*, 41(1): 30-32.

Cooper, P.A. and Stokes, D. (1993). Leaching characteristics and fixation of copper dimethyldithiocarbamate treated wood. In: Proceedings, American Wood Preservers' Association, 89:192–203.

Covolan, V.L., Innocentini, M.L.H. and Rossi, C.L. (1997). Chemical modifications on polystyrene latex: Preparation and characterization for use in immunological applications. *Polymers Advanced Technologies*, 8(1): 44-50.

Daniel, G. and Nilsson, T. (1987). Comparative studies on the distribution of lignin and CCA elements in birch using electron microscopic x-ray microanalysis. Document No: IRG/WP 87-1328. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-19.

Darah, I. and Ibrahim, C.O. (1996). Effect of agitation on production of lignin-degrading enzymes by *Phanerochaete chrysosporium* grown in shake-flask cultures. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 4: 174-182.

Dashtban, M., Schraft, H., Syed, T.A. and Qin, W. (2010). Fungal biodegradation and enzymatic modification of lignin. *International Journal of Biochemistry and Molecular Biology*, 1(1): 36-50.

Davies, A.G. and Smith, P.J. (1980). Recent advances in organotin chemistry. *Advances in Inorganic Chemistry and Radiochemistry*, 23: 1-77.

Dawson-Andoh, B.E. and Kamdem, D.P. (1998). Application of environmental scanning electron microscopy to the study of microdistribution of copper in copper naphthenate treated hardwoods. *Holzforschung*, 52(6): 603-606.

de Souza-Cruz, P.B., Freer, J., Siika-Aho, M. and Ferraz, A. (2004). Extraction and determination of enzymes produced by *Ceriporiopsis subvermispora* during biopulping of *Pinus taeda* wood chips. *Enzyme and Microbial Technology*, 34: 228-234.

De Vetter, L., Cnudde, V., Masschaele, B., Jacobs, P.J.S. and Van Acker, J. (2006). Detection and distribution analysis of organosilicon compounds in wood by means of SEM-EDX and micro-CT. *Materials Characterization*, 56: 39-48.

Deka, M. and Saikia, C.N. (2000). Chemical modification of wood with thermosetting resin: Effect on dimensional stability and strength property, *Bioresource Technology*, 73: 179-181.

Deka, M., Saikia, C.N. and Baruah, K.K., (2002). Studies on thermal degradation and termite resistant properties of chemically modified wood. *Bioresource Technology*, 84: 151-157.

Desch, H.E. and Diwoodie, J.M. (1996). *Timber: Structure, Properties, Conversion and Use (7th ed.)*. London: Macmillan Press Limited.

Dhouib, A., Hamza, M., Zouari, H., Mechichi, T. and Hmidi. R., Labat, M., Martinez, M.J. and Sayadi, S. (2005). Screening for ligninolytic enzyme production by diverse fungi from Tunisia. *World Journal of Microbiology and Biotechnology*, 21: 1415-1423.

Dittmer, J.K., Patel, N.J., Dhawale, S.W. and Dhawale, S.S. (1997). Production of multiple laccase isoforms by *Phanerochaete chrysosporium* grown under nutrient sufficiency. *FEMS Microbiology Letters*, 149: 65–70.

Donaldson, L.A. and Ryan, K.G. (1987). A comparison of relative lignin concentration as determined by interference microscopy and bromination/EDXA. *Wood Science and Technology*, 21(4): 303–309.

Doyle, A.K. and Ruddick, J.N.R. (1994). The microdistribution of alkylammonium compounds in ponderosa pine sapwood. *Holzforschung*, 48(2): 106-112.

Drysdale, J.A., Dickson, D.J. and Levy, J.F. (1980). Microdistribution of a CCA preservative in five timbers varying susceptibility to soft rot. *Material und Organismen*, 15(4): 287-303.

Duncan, C.G. and Lombard, F.F. (1965). Fungi associated with principal decays in wood products in the United States, U.S. Forest Service Research paper WO-4, Washington.

Eaton, R.A. and Hale, M.D.C. (1993). *Wood: Decay, Pests and Protection*. Chapman and Hall. Boundary Row, London. 546pp.

Eden, D., Singh, T. and Kreber B. (1999). Effect of mobile fungicides on fungal pre-infection in *radiata* pine. Document No: IRG/WP 99-30191. The International Research Group on Wood Preservation, Stockholm, Sweden.

Edwin, L. and Pillai, A.G.G. (2004). Resistance of preservative-treated rubber wood (*Hevea brasiliensis*) to marine borer attack. *Holz als Roh- und Werkstoff*, 62: 303–306.

Elisashvili, V., Irbe. I., Andersone, I., Andersons, B. and Tsiklauri, N. (2012). Hydrolytic enzyme activity of EN113 standard basidiomycetes in the fermentation of lignocellulosic material and wood colonization. *Holzforschung*, 66: 841-847.

Ellis, W.D. and Rowell, R.M. (1984). Reaction of isocyanates with southern pine wood to improve dimensional stability and decay resistance, *Wood and Fiber Science*, 16(3): 349-356.

Elvy, S.B., Dennis, G.R. and Ng, L.T. (1995). Effects of coupling agent on the physical properties of wood-polymer composites. *Journal of Materials Processing Technology*, 48: 365-371.

EN 350-1. (1993). Durability of Wood Based Products. Natural Durability of Solid Wood Part 1. *Guide to the Principles of Testing and Classification of the Natural Durability of the Wood*.

Enoki, M., Watanabe, T., Nakagame, S., Koller, K., Messner, K., Honda, Y. and Kuwahara, M. (1999). Extracellular lipid peroxidation of selective white rot fungus, *Ceriporiopsis subvermispora*. *FEMS Microbiol Letters*, 180:205–211.

Eriksson, K.E.L., Blanchette, R.A. and Ander, P. (1990). *Microbial and enzymatic degradation of wood and wood components*. Berlin: Springer, Heidelberg, New York.

Eslyn, W.E. (1986). Utility pole decay, Part 4: Growth-temperature relationships and decay capacities of eleven major utility pole decay fungi. *Holzforschung*, 40(2): 69-77.

Evans, C.J. (1998). Industrial uses of tin chemicals. In. Smith, P.J. (ed.). Chemistry of Tin, 2nd edition. Blackie Academic and Professional: London, 442pp.

Evans, F.G. (1987). Leaching from CCA-impregnated wood to food, drinking water and silage. Document No: IRG/WP 87-3433. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-17.

Evans, P.D. (2003). Emerging technologies in wood protection. *Forest Products Journal*, 53(1): 14-22.

Exley, R.R., B.G. Butterfield and B.A. Meylan. (1974). Preparation of wood specimens for the scanning electron microscope. *Journal of Microscopy*, 101(1): 21-30.

Fearnside, P.M. (1997). Wood density for estimating forest biomass in Brazilian Amazonia. *Forest Ecology and Management*, 90(1): 59–87.

Feldhaar, H., Fiala, B., Rosli, H. and Maschwitz, U. (2000). Maintaining an ant-plant symbiosis: Secondary polygyny in the *Macaranga triloba* - *Crematogaster sp*. association. *Naturwissenschaften*, 87: 408–411.

Fengel, D. and Wegener, G. (1983). *Wood: Chemistry, Ultrastructure and Reactions*. Walter de Gruyter. New York.

Ferraz, A., Cordova, A.M. and Machuca, A. (2003). Wood biodegradation and enzyme production by *Ceriporiopsis subvermispora* during solid-state fermentation of *Eucalyptus grandis*. *Enzyme and Microbial Technology*, 32(1): 59–65.

Fiala, B. (1996). Ants benefit pioneer trees: the genus *Macaranga* as an example of ant-plant associations in dipterocarp forest ecosystems. In Schulte, A. and Schoene, D. (eds). Dipterocarp Forest Ecosystems: Towards Sustainable Management, World Scientific, Singapore, pp. 102-123.

Florence, M.E.J., Gnanaharan, R., Singh, P.A. and Sharma, J.K. (2005). Weight loss and cell wall degradation in rubberwood caused by sapstain fungus *Botryodiplodia theobramae*. *Holzforschung*, 56: 225-228.

Frankland, E. (1849). Ueber die Isolirung der organischen Radicale. *Liebigs Ann. Chem.* 71: 171-213.

Gezer, E.D., Yalinkilic, M.K., Kizilkaya, K. and Michael, J.H. (1999). Estimation of preservative toxic threshold retention from laboratory decay tests: a new method. *Wood Science and Technology*, 33: 63 -71.

Girasolo, M.A., Schillaci, D., Salvo, C.D., Barone, G., Silvestri, A. and Ruisi, G. (2006). Synthesis, spectroscopic characterization and in vitro antimicrobial activity of diorganotin(IV) dichloride adducts with [1,2,4]triazolo-[1,5- *a*]pyrimidine and 5,7-dimethyl-[1,2,4] triazolo-[1,5- *a*] pyrimidine. *Journal of Organometallic Chemistry*, 691(4): 693-701.

Glenn, J.K. and Gold, M.H. (1985). Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics*, 242(2): 329-341.

Glenn, J.K., Akileswaran, L., and Gold, M.H. (1986). Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics*, 251(2): 688–696.

Gnanaharan, R. and Dhamodaran, T.K. (1993). Mechanical properties of rubber wood from a 35 year-old plantation in Central Kerala, India. *Journal of Tropical Forest Science*, 6: 136–140.

Gold, M.H. and Alic, M. (1993). Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Microbiological Reviews*, 57**:** 605-622.

Gold, M.H., Kuwahara, M., Chin, A.A. and Glen, J.K. (1984). Purification and characterization of an extracellular H_2O_2-requiring diaryl propane oxygenase from the white-rot basidiomycete, *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics*, 234(2): 353-362.

Gold, M.H., Warushi, W. and Valii, K. (1989). Extracellular peroxidases involved in lignin degradation by the white-rot basidiomycete *Phanerochaete chrysosporium*. American Chemical Society Symposium Series, 389: 127-140.

Goldstein, S. D. (1988). Biological control in alkaline paper machine systems; An update, Proc. 1988 TAPPI papermakers Conference, Chicago, p. 81.

Goni, M.A., Nelson, B., Blanchette, R.A. and Hedges, J.I. (1993). Fungal degradation of wood lignins: Geochemical perspectives from CuO-derived phenolic dimmers and monomers. *Geochimica et Cosmochimica Acta*, 57(16): 3985-4002.

Gray, S.M. (1993). Chromated copper preservative systems-The performance of treated wood. In: Winandy, J.; Barnes, M., eds. *Chromium-containing waterborne wood preservatives: fixation and environmental issues*. Madison WI: Forest Products Society; 14-22.

Greaves, H. and Nilsson, T. (1982). Soft rot and microdistribution of waterborne preservatives in three hardwoods following the field test exposure. *Holzforschung*, 36(4): 207-213.

Greaves, H., Tighe, M.A. and McCarthy, D.F. (1982). Laboratory tests on light organic solvent preservatives for use in Australia. 1. Evaluation of candidate fungicides, including some commercial formulations. *The International Journal of Wood Preservation*, 2: 21-27.

Green, D.W., Winandy, J.E. and Kretschmann, D.E. (2007). Chemical treatment. In: Mechanical properties of wood, The encyclopedia of wood, U.S. Department of Agriculture, Skyhorse Publishing, Inc. New York, 496 p.

Gruzdyev, G.S., Zinchenko, V.A., Kalinin, V.A. and Slovtsov, R.I. (1980). Means for controlling plant pests. In G.S. Gruzdyev (Ed), *The chemical protection of plants*, (pp. 236-239). Mir Publishers, Moscow.

Haddadin, M., Al-Natour, R., Al-Qsous, S. and Robinson, R. (2002). Biodegradation of lignin in olive pomace by freshly-isolated species of Basidiomycete. *Bioresource Technology*, 82: 131–138.

Hadi, S., Irawan, B. and Efri. (2008). Antifungal activity test of some organotin (IV) carbohydrates. *Journal of Applied Sciences Research*, 4(11): 1521-1525.

Haglund, C. (1999). Biodegradation of xenobiotic compounds by the white-rot fungus *Trametes trogii*. *Molecular Biotechnology Programme*, Uppsala University School of Engineering, 30pp.

Hammel, K. E. (1997). Fungal degradation of lignin. In: Cadisch G, Giller KE (eds) *Driven by nature: plant litter quality and decomposition*. CAB International, Wallingford, pp. 33-46.

Hanif, M., Hussain, M., Ali, S., Bhatti, M.H., Ahmed, M.S., Mirza B. and Stoeckli-Evans, H. (2010). *Invitro* biological studies and structural elucidation of organotin(IV) derivatives of 6-nitropiperonylic acid: Crystal structure of $\{[(CH_2O_2C_6H_2(o\text{-}NO_2)COO)SnBu_2]_2O\}_2$. *Polyhedron*, 29(1): 613-619.

Haque, M.M., Hasan, M., Islam, M.S. and Ali, M.E. (2009). Physico-mechanical properties of chemically treated palm and coir fiber reinforced polypropylene composites. *Bioresource Technology*, 100: 4903-4906.

Harrison, J.G., Lowe, R. and Williams, N.A. (1994). Humidity and fungal diseases of plants – problems. In: *Ecology of plant pathogens*. J.P. Blakeman and B. Williamson (eds.). Wallingford: CAB International, pp. 79-97.

Hastrup, A.Ch.S., Green, III.F., Clausen, C. and Jensen, B. (2005). *Serpula lacrymans* – the dry-rot fungus tolerance towards copper-based wood preservatives. Document No: IRG/WP 05-10555. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-7.

Hatakka, A. (2001). Biodegradation of lignin. In: Biopolymer. *Biology, Chemistry, Biotecnology, Applications*. Vol 1. Lignin, Humic substances and coal, Hofrichter, M. and Steinbuchel, A. (eds.), Wiley-WCH, 129-180.

Haygreen, J.G. and Bowyer, J.L. (2003). *Forest products and wood science an introduction*. Iowa State University Press, United States of America, 500pp.

Hedley, M.E., Nasheri, K. and van der Wales, J. (1990). Effect of treating process on efficacy of CCA in a laboratory decay test. Document No: IRG/WP 90-3628. The International Research Group on Wood Preservation, Stockholm, Sweden.

Heiserman, D.L. (1992). Exploring Chemical Elements and their Compounds. Blue Ridge Summit, PA: Tab Books, TAB Books. 376p.

Helmer, T., Trockenbrodt, M. and Fruhwald, A. (2000). Wood density and elastomechanical properties of *Macaranga gigantifolia* and *M. hypoleuca* from Sabah, Malaysia. *Journal of Tropical Forest Products*, 6(1): 62-67.

Hilditch, E.A. (1991). Organic solvent preservatives: application and composition, in The Chemistry of Wood Preservation, (ed. R. Thompson), The Royal Soc. Chem., pp. 69-87.

Hill, R., Smith, P.J., Ruddick, J.N.R. and Sweatman, K.W. (1983). A laboratory evaluation of tributyltin ethanesulphonate as an aqueous fungicide in wood preservation. Document No: IRG/WP 83-3229. The International Research Group on Wood Preservation, Stockholm, Sweden.

Hillis, W.E. (1984). High temperature and chemical effects on wood stability. *Wood Science and Technology*, 18(4): 281-293.

Hiziroglu, S. (1997). Treatment of rubberwood (*Hevea brasiliensis*). Document No: IRG/WP 97-40081. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-7.

Hoch, M. (2001). Organic compounds in the environment - an overview. *Applied Geochemistry*, 16: 719-743.

Hofrichter, M., Vares, T., Kalsi, M., Galkin, S., Scheibner, K., Fritsche, W. and Hatakka, A. (1999). Production of manganese peroxidase and organic acids and mineralization of ^{14}C-labelled lignin (^{14}C-DHP) during solid-state fermentation of wheat straw with the white rot fungus *Nematoloma frowardii*. *Applied and Environmental Microbiology*, 65(5): 1864-1870.

Hong, L.T. and Liew, C.C.K. (1989). Protection of rubber wood timber 1. Impregnation with boron preservatives. Document No: IRG/WP 89-3551. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-11.

Hong, L.T. and Sim, H.C. (1994). Products from rubberwood – an overview. *In*: *Rubberwood Processing and Utilization*. Hong, L.T. and Sim, H.C. (eds.), Forest Research Institute of Malaysia, Kuala Lumpur, mlaysia. pp. 173-184.

Hong, L.T. and Wong, A.H.H. (1994). Biodeterioration and preservation of rubberwood (*Hevea brasiliensis*). Document No: IRG/WP 94-10084. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-12.

Hong, L.T. and Wong, A.H.H. (1994 a). In Wong, A.H.H. and Sabri, A. (2000). Variation in infection rates of blue-stain, mould and white-rot tropical fungi on mixed light hardwoods. Document No: IRG/WP 00-10334. The International Research Group on Wood Preservation, Stockholm, Sweden.

Hong, L.T., Surian, M.A., Goh, T.A. and Daljeet Singh, K.D. (1982). Preservation and protection of rubber wood against biodeteriorating organisms for more efficient utilization. *The Malaysian Forester*, 45: 299–315.

Hong, L.T., Wong, A.H.H. and Ho, Y.F. (1994). Durability of rubberwood. *In*: Rubberwood Processing and Utilization. Hong, L.T. and Sim, H.C. (eds.), Forest Research Institute of Malaysia, Kuala Lumpur, mlaysia. pp. 37-50.

Hortling, B., Tarja, T. and Kentta, E. (1997). Determination of carboxyl and non-conjugated carbonyl groups in dissolved and residual lignins by IR spectroscopy. New York. *Holzforschung*, 51(5): 405-410.

Huang, R.T., Wang, M.C., Yan, J.Y., Wu, T.W., Kai, J.J. and Chen, F.R. (2005). Synthesis and microstructural study of Fe-doped $Zn_{1-x}Cu_xO$ diluted magnetic semiconductor nanowires. *Microscopy and Microanalysis*, 11: 1924-1925.

Humar, M., Petric, M. and Pohleven, F. (2001). Changes of the pH value of impregnated wood during exposure to wood-rotting fungi. *HolzalsRoh-und Werkstoff*, 59: 288-293.

Humphrey, C.J. and Siggers, P.V. (1933). Temperature relations of wood destroying fungi. *Journal of Agricultural Research*, 47: 997-1008.

Husaini, A., Fisol, F.A., Liew, C.Y., Hussain, M.H., Muid, S. and Roslan, H.A. (2011). Lignicellulolytic enzymes produced by tropical white rot fungi during biopulping of *Acacia mangium* wood chips. *Journal of Biochemical Technology*, 3(2): 245-250.

Ikehata, K., Pickard, M.A., Buchanan, I.D. and Smith, D.W. (2004). Optimization of extracellular fungal peroxidase production by 2 *Coprinus* species. *Canadian Journal of Microbiology*, 50(12): 1033-1040.

Iqbal, H.M.N., Asgher, M. and Bhatti, H.N. (2011). Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by novel strain of *Trametes versicolor*. *Bioresources*, 6(2): 1273-1287.

Ismail, H., Edyhan, M. and Wirjosentono, B. (2002). Bamboo fiber filled natural rubber composites: the effects of filler loading and bonding agent. *Polymer Testing*, 21(2): 139-144.

Izekor, D.N., Fuwape, J.A. and Oluyege, A.O. (2010). Effects of density on variations in the mechanical properties of plantation grown *Tectona grandis* wood. *Archives of Applied Science Research*, 2(6): 113-120.

Josue, J. (2004). Some wood properties of *Xylia xylocarpa* planted in Sabah. *Sepilok Bulletin*, 1: 1-15.

Jusoh, I. and Kamdem, D.P. (2000). Penetration and retention of CCA in rubberwood (*Hevea brasiliensis*). *Journal of Tropical Forest Products*, 6(1): 77-84.

Jusoh, I. and Kamdem, D.P. (2001). Laboratory evaluation of natural decay resistance and efficacy of CCA-treated rubberwood (*Hevea brasiliensis* Muell. Arg.). *Holzforschung*, 55(3): 250-254.

Jusoh, I. and Kamdem, P. (2009). SEM-EDXA of CCA-treated rubberwood (*Hevea brasiliensis*). Document No: IRG/WP 09-20399. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-18.

Kadir, A.A. and Sudin, R. (1989). Carbohydrates in rubberwood (*Hevea brasiliensis* Muell.). New York. *Holzforschung*, 43: 173-178.

Kaldas, M.L. and Cooper, P.A. (1996). Effect of wood moisture content on rate of fixation and leach ability of CCA-treated red pine. *Forest Products Journal*, 46(10): 67-71.

Kalita, D.K., Das, D.K. and Gogoi, P.K. (2002). Synthesis, characterization and antibacterial activity of 2- methylpiperazine dithiocarbamates of Ru(III), Rh(III), Pd(II),Os((III) and Pt(II). *Proceedings of National Academy of Sciences, India*, 72(A): 7-13.

Kamdem, P.D. and Chow, P. (1999). The effect of pressure treatment on the mechanical properties of CCA-treated hardwoods. *Wood and Fiber Science*, 41(2): 128-135.

Kamdem, P.D. and Mcintyre, C.R. (1998). Chemical investigation of 23-years-old CDDC-treated southern pine. *Wood and Fiber Science*, 30(1): 64-71.

Kazemi, S.M. (2007). An investigation on use and durability of some industrial and domestic woods of Iran against destructive factors in Caspian sea. *Journal of Biological Sciences*, 7(7): 1187-1193.

Kennedy, M.J. and Palmer, G. (1994). Leaching of copper, chromium, and arsenic from CCA-treated slash pine heartwood. Document No: IRG/WP 94-50020. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-7.

Kennedy, M.J. and Woods, T.L. (1996). Selective adsorption of antisapstain actives from two aqueous suspensions, and movement of actives into wood. Document No: IRG/WP 96-30103. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-7.

Kerem, Z., Jensen, K.A. and Hammel, K.E. (1999). Biodegradative mechanism of the brown-rot basidiomycete *Gloeophyllum trabeum*: evidence for an extra cellular hydroquinone-driven Fenton reaction. *FEBS Letter*, 446: 49-54.

Khan, M.I., Baloch, M.K. and Ashfaq, M. (2004). Biological aspects of new organotin(IV) compounds of 3-maleimidopropionic acid. *Journal of Organometallic Chemistry*, 689(21): 3370-3378.

Kidwai, M., Dave, V., Misra, P., Saxena, R.K. and Singh, M. (2000). Novel synthetic approach for antifungal and antibacterial organotin compounds. *Inorganic Chemistry Communications*, 3(9): 465-468.

Kim, G.H., Song, Y.S. and Lee, D.H. (2008). Fixation and leaching characteristics of Douglas-fir treated with CCA-C. *Forest Products Journal*, 58(7): 73-76.

Kim, Y.S., Singh, A.P., Wong, A.H.H., Eom, T-J. and Lee, K.H. (2006). Micromorphological and chemical characteristics of cengal (Neobalanocarpus heimii) heartwood decayed by soft rot fungi. *Journal of the Wood Science and Technology*, 34(2): 68-77.

Kirk, T.K. (1988). Lignin degradation by *Phanerochaete chrysosporium* ISI Atlas Sci. Biochem., 1: 71-76.

Kirk, T.K. and Cowling, F.B. (1984). Biological decomposition of wood. In: Rowell, R.M. (ed) *The chemistry of solid wood*. Adv. Chem. Series 207. American Chemical Society, Washington DC, pp. 455-487.

Kirk, T.K. and Cullen, D. (2005). Enzymology and molecular genetics of wood degradation by white-rot fungi. In: Environmentally friendly technologies for pulp and paper industry. Edited by Young, R.A. and Akhter, M., Madison, Wisconsin. pp. 273-307.

Kirk, T.K. and Farrell, R.L. (1987). Enzymatic "combustion": the microbial degradation of lignin. *Annual Review of Microbiology*, 41: 465–505.

Kirk, T.K. and Shimada, M. (1985). Lignin biodegradation: The microorganisms involved and the physiology and biochemistry of degradation by white-rot fungi. In "Biosynthesis and Biodegradation of Wood Components" (T. Higuchi, eds.), Academic Press, Orlando, Florida, pp.579-605.

Kreber, B., Kimberley, M., Eden, D., Chittenden, C., Van der Waals, J., Wakeling, R. and Dorset, I. (2001). Arrest of fungal preinfection in raw logs and freshly sawn lumber of radiate pine using Sentry. *Forest Products Journal*, 51: 66–72.

Kuivila, H.G., Menapace, L.W., Warner, C.R. (1962). Reduction of alkyl halides by organotin hydrides. Evidence for a free radical mechanism. *Journal of the American Chemical Society*, 84(18): 3584-3586.

Lee, J.S., Furukawa, I. and Sakuno, T. (1992). Microdistribution of elements in wood after pre-treatment with chitosan and impregnation with chrome-copper-arsenic preservative. *Mokuzai Gakkaishi*, 38: 186-192.

Lemmens, R.H.M.J., Soerianegara, I. and Wong, W.C. (eds.). (1995). Plant Resources of South-east Asia. No 5(2). Timber trees: minor commercial timbers. Backhuys Publishers, Leiden.

LeVan, S. and Winandy, J.E. (1990). Effects of fire-retardant treatments on wood strength: a review. *Wood and Fiber Science*, 22(1): 113–131.

Lever, A.B.P., Mc Cleverty, J.A. and Meyer, T.J. (2004). *Comprehensive coordination chemistry II. Vol 1: Fundamentals: Ligands, complexes, synthesis, purification and structure*. Elsevier Ltd., United Kingdom, pp.507.

Li, M.X., Zhang, D., Zhang, L.Z., Niu, J.Y. and Ji, B.S. (2011). Diorganotin(IV) complexes with 2-benzoylpyridine and 2-acetylpyrazine N(4)-phenylthiosemicarbazones: synthesis, crystal structures and biological activities. *Journal of Organometallic Chemistry*, 696(4): 852-858.

Liew, C.Y., Husaini, A., Hussain, H., Muid, S., Liew, K.C. and Roslan, H.A. (2011). Lignin biodegradation and ligninolytic enzyme studies during biopulping of *Acacia mangium* wood chips by tropical white rot fungi. *World Journal of Microbiology and Biotechnology*, 27(6): 1457-1468.

Lowich, C. (1852). Ueber Zinnathyle. *Justus Liebigs Annalen Chemmie*, 84(3): 308-333.

Luo, J., Chen, H. and Morrell, J.J. (2005). Effect of borate on uptake and efficacy of an anti-sapstain treatment. Document No: IRG/WP 05-30380. The International Research Group on wood Protection, Stockholm, Sweden. pp. 1-11.

Lyon, F., Thevenon, M.F., Pizzi, A. and Gril, J. (2009). Resistance to decay fungi of ammonium borate oleate treated wood. Document No: IRG/WP 09-30505. The International Research Group on wood Protection, Stockholm, Sweden. pp. 1-10.

Malaysian Standard. MS 360 (1991). Specification for treatment of timber with copper/chrome/arsenic wood preservatives.

Malaysian Timber Industry Board. MTIB (2010). 100 Malaysia Timbers, Malaysian Timber Industry Board. P. 283.

Marienfeld, S., Schmohl, N., Klein, M., Schroder, W.H., Kuhn, A.J. and Horst, W.J. (2000). Localization of aluminium in root tips of *Zea mays* and *Vicia faba*. *Journal of Plant Physiology*, 156: 666-671.

Matan, N. and Kyokong, B. (2003). Effect of moisture content on some physical and mechanical properties of rubber wood (*Hevea brasiliensis* (Willd.) Muell.-Arg.). *Songklanakarin Journal of Science and Technology*, 25(3): 327–340.

Matsunaga, H., Kiguchi, M. and Evans, P.D. (2007). Micro-distribution of metals in wood treated with a nano-copper wood preservative. Document No: IRG/WP 07-40360. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-10.

Matsunaga, H., Kiguchi, M., Roth, B. and Evans, P.D. (2008). Visualization of metals in pine treated with preservative containing copper and iron nanoparticles. *IAWA Journal*, 29(4): 387-396.

Matsunaga, H., Matsumura, J. and Oda, K. (2004). X-ray microanalysis using thin sections of preservative-treated wood: Relationship of wood anatomy features to the distribution of copper. *IAWA Journal*, 25(1): 79-90.

Mayer, A.M. (1987). Polyphenol oxidases in plants: recent progress. *Phytochemistry*, 26: 11-20.

Mendes, I.C., Moreira, J.P., Speziali, L.N., Mangrich, A.S., Takahashi, J.A. and Beraldo, H. (2006). N(4)-tolyl-2-benzoylpyridine thiosemicarbazones and their copper(II) complexes with significant antifungal activity. Crystal structure of N(4)-tolyl-2-benzoylpyridine thiosemicarbazone. *Journal of the Brazilian Chemical Society*, 17(8): 1571-1577.

Mester, T. and Field, J.A. (1998). Characterization of a novel manganese peroxidase-lignin peroxidase hybrid isozyme produced by Bjerkandera species strain BOS55 in the absence of manganese. *The Journal of Biological Chemistry*, 273(25): 15412-15417.

Miller, G. (1972). Tributyltin oxide: some factors influencing its development and application as a preservatives, in *Biodeterioration of Materials*, vol. 2 (eds A.H. Walters and E.H. Hueck-van der plas), Applied science, London, pp. 279-285.

Mohd. Dahlan, J., Hong, L.T., Azlan, M. and Wong, A.H.H. (1994). Preservation of rubberwood. In L.T. Hong & H.C., Sim (eds.), Rubberwood processing and utilization. Malayan Forest Records No. 39. Forest Research Institute of Malaysia.

Momeni, B.Z., Rominger, F. and Hosseini, S.S. (2009). Tetrachlorido (1,10-phenanthroline-κ^2N,N')tin(IV) 1,2-dichloroethane hemisolvate. *Acta Crystallogr. E65: m690.*

Mtui, G. and Masalu, R. (2008). Extracellular enzymes from brown-rot fungus *Laetiporus sulphureus* isolated from mangrove forests of coastal Tanzania. *Scientific Research and Essay*, 3(4): 154-161.

Myneni, R.B., Dong, J., Tucker, C.J., Kaufmann, R.K., Kauppi, P.E., Liski, J., Zhou, L., Alexeyev, V. and Hughes, M.K. (2001). A large carbon sink in the woody biomass of northern forests. *PNAS*, 98:14784–14789.

Nan, Z.B., Skipp, R.A. and Long, P.G. (1991). Fungal invasion of red clover roots in a soil naturally infested with a complex of pathogens: Effects of soil temperature and moisture content. *Soil Biology and Biochemistry*, 23(5)**:**415-421.

Nath, M., Goyal, S., Sharma, C.L., Eng, G. and Whalen, D. (1995). Spectral, thermal and biological studies of thiosemicarbazones of organotin(IV) chlorides. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*. 25(5): 821-844.

Nath, M., Pokharia, S., Song, X., Eng, G., Gielen, M., Kemmer, M., Biesemans, M., Willem, R. and Vos, D.D. (2003). New organotin(IV) derivatives of dipeptides as models for metal-protein interactions: in vitro anti-tumour activity. *Applied Organometallic Chemistry*, 17(5): 305-314.

Nath, M., Yadav, R., Eng, G., Nguyen, T.T. and Kumar, A. (1999). Characteristic spectral studies, and antimicrobial and anti-inflammatory activities of diorganotin(IV) derivatives of dipeptides. *Journal of Organometallic Chemistry*, 577(1): 1-8.

Newman, P.R. and Murphy, R.J. (1996). Variation in biological performance of CCA caused by preservative application method. Document No: IRG/WP 96-40072. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-16.

Nilsson, T. (1997). Natural durability of larch heartwood against decay. Document No: IRG/WP 97-10201. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-6.

Nilsson, T., Obst, J.R. and Daniel, G. (1988). The possible significance of the lignin content and lignin type on the performance of CCA-treated timber in ground contact. Document No: IRG/WP 88-1357. The International Research Group on Wood Preservation, Stockholm, Sweden.

Norimoto, M. and Gril, J. (1993). Structure and properties of chemically treated woods. pp. 135–154 in Shiraishi, N., Kajita, H. and Norimoto, M. (Eds.) *Recent Research on Wood and Wood-based Materials.* Elsevier, Barking, UK.

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H. and Sederoff, R. (2010). Lignin and biomass: a negative correlation for wood formation and lignin content in trees. *Plant Physiology*, 154: 555-561.

Ohkuma, M., Maeda, Y., Johjima, T. and Kudo, T. (2001). Lignin degradation and roles of white rot fungi: Study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation. *RIKEN Review*, 42: 39-42.

Oluwafemi, O.A. and Adegbenga, S.O. (2007). Preliminary report on utilization potential of *Gliricidia sepium* (Jacq.) steud for timber. *Research Journal of Forestry*, 1: 80-85.

Onuorah, E.O. (2000). The wood preservative potential of heartwood extracts of Miliciaexcelsa and Erythrophleumsuaveolens. *Bioresource Technology*, 75: 171–173.

Orth, A.B., Royse, D.J. and Tien, M. (1993). Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi. *Applied and Environmental Microbiology*, 59(12): 4017-4023.

Otjen, L., Blanchette, R.A., Effland, M. and Leatham G.F. (1987). Assessment of 30 white rot basidiomycetes for selective lignin degradation. *Holzforschung*, 41:343.

Pallaske, M. 2004. Chemical wood protection: Improvement of biocides in use. COST Action E22 – Environmental Optimization of Wood Protection, 22-23.3.2004, Lisboa-Portugal, 11 p.

Palmieri.G., Giardina, P., Bianco, C., Fontanella, B. and Sannia, G. (2000). Copper induction of laccaseisoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. *Applied and Environmental Microbioogyl*, 66(3): 920-924.

Pankras, S., Cooper, P., Ung, T. and Awoyemi, L. (2009). Copper to quat ratio in alkaline copper quat (ACQ) wood preservative: Effects on fixation and leaching of preservative components. Document No: IRG/WP 09-30496. The International Research Group on wood Protection, Stockholm, Sweden. pp. 1-12.

Panshin, A.J. and Zeeuw, C.D. (1980). Textbook of Wood Technology. New York, McGraw-Hill.

Paszczynski, A., Crawford, R. and Huynh, V. (1988). Manganese peroxidase of Phanerochaete chrysosporium: purification. *Methods in Enzymology*, 161:264–270.

Patrick, F., Mtui, G., Mshandete, A.M. and Kivaisi, A. (2011). Optimization of laccase and manganese peroxidase production in submerged culture of *Pleurotus sajor-caju*. *African Journal of Biotechnology*, 10(50): 10166-10177.

Pellerito, C., Nagy, L., Pellerito, L. and Szorcsik, A. (2006). Biological activity studies on organotin(IV) complexes and parent compounds. *Journal of Organometallic Chemistry*, 691(8): 1733-1747.

Perez, J., Martinez, J. and Rubia, T. (1996). Purification and characterization of laccase from white rot fungus *Phanerochaete flavido-alba*. *Applied and Environmental Microbiology*, 62(11): 4263-4267.

Perie, F.H. and Gold, M.H. (1991). Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. *Applied and Environmental Microbiology*, 57(8): 2240-2245.

Petric, M., Murphy, R.J. and Morris, I. (2000). Microdistribution of some copper and zinc containing waterborne and organic solvent wood preservative in spruce wood cell walls. *Holzforschung*, 54(1): 23-26.

Piver, W.T. (1973). Organotin compounds: industrial applications and biological investigation, *Environmental Health Perspectives*, 4: 61–79.

Pohleven, F., Humar, M., Amartey, S. and Benedik, J. (2002). Tolerance of wood decay fungi to commercial copper based wood preservatives. Document No: IRG/WP 02-30291. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-12.

Popp, J.L., Kalyanaraman, B. and Kirk, T.K. (1990). Lignin peroxidase oxidation of Mn2+ in the presence of veratryl alcohol, malonic or oxalic acid, and oxygen. *Biochemistry*, 29(46): 10475-10480.

Preston, A.F. (2000). Wood preservation – Trends of today that will influence the way tomorrow. *Forest Products Journal*, 50(9): 12-19.

Rao, K.S., Santhakumaran, L.N., Balaji, M. and Srinivasan, V.V. (1993). Natural resistance of rubber wood to marine borers and a preliminary note on the effects of the preservative treatment. *Indian Journal of Natural Rubber Research*, 6: 71–74.

Ray, M., Dickinson, D.J. and Archer, K. (2010). A comparison of the performance of related copper based preservatives against soft-rot. Document No: IRG/WP 10-30540. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-16.

Rayner, A.D.M. and Boddy, L. (1988). Fungal Decomposition of Wood: Its Biology and Ecology. John Wiley & Sons Ltd. New York. United Kingdom. 587p.

Rebolledo, A.P., Vieites, M., Gambino, D., Piro, O.E., Castellano, E.E., Zani, C.L., Souza-Fagundes, E.M., Teixeira, L.R., Batista, A.A. and Beraldo, H. (2005). Palladium(II) complexes of 2-benzoylpyridine-derived thiosemicarbazones:spectral characterization, structural studies and cytotoxic activity. *Journal of Inorganic Biochemistry*, 99(3): 698-706.

Reinprecht, L. (2007). Selected laboratory tests of boron efficacy against wood damaging fungi. *ProLigno*, 3(3): 27-37.

Richardson, B.A. (1993). *Wood Preservation Second Edition*. Spon Press, London. 226p.

Rieble, S., Joshi, D.K. and Gold, M.H. (1994). Purification and characterization of a 1,2,4-trihydroxybenzene 1,2-dioxygenase from the basidiomycete *Phanerochaete chrysosporium*. *Journal of Bacteriology*, 176(16):4838-4844.

Robinson, J.M. (1990). Lignin, land plants, and fungi: Biological evolution affecting Phanerozoic oxygen balance. *Geology*, 18(7): 607-610.

Ross, P. (2002). Appraisal and repair of timber structures. Great Britain; Thomas Telford.

Rouhi, A.M. (1998). The squeeze of tributyltin. *Chemical Engineering News*, 27: 41-42.

Rowan, N.J., Johnstone, C.M., McLean, R.C., Anderson, J.G. and Clarke, J.A. (1999). Prediction of toxigenic fungal growth in buildings by using a novel modelling system. *Applied and Environmental Microbiology*, 65(11)**:** 4814-4821.

Rowell, R.M. (1984). Bonding of toxic chemicals to wood. *Applied Biochemistry and Biotechnology*, 9: 447-453.

Rowell, R.M. (2005). Chemical modification of wood. In: Handbook of Wood Chemistry, Wood Composites, Rowell R.M. (Ed.). CRC Press, Boco Raton, pp. 381-420.

Rowell, R.M., Esenther, G.R., Younguist, J.A., Nicholas, D.D., Nilsson, T., Imamura, Y., Kerner-Gang, W., Trong, L. and Deon, G. (1988). Wood modification in the protection of wood composites, Proceedings of the IUFRO wood protection subject group, Honey Harbor, Ontario, Canada, Canadian Forestry Service, pp.238-266.

Rubber Board, Ministry of Commerce and Industry, Government of India. (2005). Online at http://www.rubberboard.org.in/RubberWood.asp

Ruddick, J.N.R. (1993). The roles of standard setting organizationsand regulatory agencies inwood preservation. In: Winandy, J.; Barnes, M., ed. Chromium-containing waterbornewood preservatives: Fixation and environmental issues. Madison, WI: Forest Products Society: 26–31.

Rudjiman, Gintings, N., Martawijaya, A. and Ilic, J. (1994). Alstonia R.Br. In: Soerianegara, I. and Lemmens, R.H.M.J. (Eds.): Plant Resources of South-East Asia 5(1). Timber Trees: major commercial timbers. Prosea Foundation, Bogor, Indonesia, pp. 88-89.

Ryan, K.G. (1986). Preparation techniques for X-ray analysis in the transmission electron microscope of wood treated with copper-chrome-arsenate. *Material und Organismen*, 21(3): 223-234.

Ryan, K.G. and Drysdale, J.A. (1988). X-ray analysis of copper chromium and arsenic within the cell walls of treated hardwood - new evidence against the microdistribution theory. *Journal of the Institute of Wood Science*, 11(3): 108-113.

Saka, S. (2000). Chemical composition and distribution. *Wood and Cellulosic Chemistry*. New York, Marcel Dekker. p. 51-81.

Salam, M.A. (2012). Synthesis, structural characterization and biological activities of new organotin(IV) complexes with *N*(4)-substituted thiosemicarbazone/hydrazone ligands containing NNS/ONS/ON donor atoms. PhD Thesis. Faculty of Resource Science and Technology. Universiti Malaysia Sarawak.

Salam, M.A., Affan, M.A., Arafat, M.A., Saha, R. and Nasrin, R. (2013). Synthesis, characterization, and antibacterial activities of organotin(IV) complexes with 2-

acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone (HAPCT). *Heteroatom Chemistry*, 24(1): 43-52.

Salamah, S., Zaitun, S. and Fauzidah, A. (1993). Effectiveness of copper-chrome-boron as wood preservatives. *Journal of Tropical Forest Science*, 6(2): 98-115.

Salim, S., Shahomlail, S., Ashaari, Z., Choi, Y. and Kim, G. (2012). Fixation and leaching characteristics of Malaysian tropical hardwoods treated with CCA-C. Document No: IRG/WP 12-50289. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-7.

Salmiah, U., Shaharudin, H., Roazaini, K. and Salamah, S. (2005). Performance of Treated Sentang in Aboveground Exposure. Forest Research Institute of Malaysia (FRIM). CFFPR.

Salmiah, U., Wong, A.H.H. and Jones, E.B.G. (2007). Wood degrading fungi. In, E.B.G. Jones, K.D. Hyde and S. Vikineswary (eds.), Malaysian Fungal Diversity, Mushroom Research Centre, University of Malaya and Ministry of natural resources and environment, Malaysia, pp. 163-183.

Sasaki, T., Kajino, T., Li, B., Sugiyama, H. and Takahashi, H. (2001). New pulp biobleaching system involving manganese peroxidase immobilized in a silica support with controlled pore sizes. *Applied and Environmental Microbiology*, 67(50): 2208-2212.

Saxena, A. and Tandon, J.P. (1984). Structural features of some organotin(IV) complexes of semi- and thio-semicarbazones. *Polyhedron*, 3(6): 681-688.

Scheffer, T.C. (1973). Microbiological degradation and the causal organisms. In: Wood deterioration and its prevention by preservative treatment, vol.1: Degradation and protection of wood. Ed. D. D. Nicholas, Syracuse University Press, Syracuse, New York, pp. 31-106.

Schilling, S.J. and Inda, J.J. (2010). Towards an assessment of copper bioavailability in treated wood. Document No: IRG/WP 10-20445. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-10.

Schmidt, O. (2006). *Wood and tree fungi – Biology, damage, protection and use.* Springer-Verlag, Berlin Heidelberg, 334 p.

Schneider, M.H. (1994). Wood polymer composites. *Wood and Fiber Science*, 26(1): 142-151.

Schoemaker, H.E. and Piontek, K. (1996). On the interaction of lignin peroxidase with lignin. *Pure and Applied Chemistry*, 88(11): 2089-2096.

Schultz, T.P. and Nicholas, D.D. (2000). Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. *Phytochemistry*, 54(1): 47-52.

Schwarze, F.W.M.R., Engels, J. and Mattheck, C. (2000). Fungal strategies of wood decay in trees. Springer, Berlin Heidelberg, New York. 185p.

Schweinfurth, H., Ventur, D. and Lange, R. (1991). TBTO and TBTN: Safe and effective biocides for wood preservation, in The Chemistry of Wood Preservation, (ed. R. Thompson), *The Royal Society of Chemistry*, pp. 192-223.

Sharma, N., Sharma, S., Kumar, V., Sharma, R. and Chaudhry, S.C. (2011). Synthesis, characterization and acceptor behavior of *n*-butyltin(IV)-2,4- dimethylphenoxides. *Journal of Coordination Chemistry*, 64(2): 351-368.

Singh, A.P., Nillson, T. and Daniel, G.F. (1992). Resistance of *Alstonia scholaris* vestures to degradation by tunneling bacteria. Document No: IRG/WP 92-1547. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-19.

Singh, R. and Kaushik, N.K. (2008). Spectral and thermal studies with anti-fungal aspects of some organotin(IV) complexes with nitrogen and sulphur donor ligands derived from 2-phenylethylamine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 71(2): 669-675.

Singh, T., Kreber, B., Chittenden, C., Wakeling, R. and Stewart, A. (2001). Minimum inhibitory concentration of methylene bisthiocyanate towards selected wood inhabiting fungi in liquid culture and on *radiata* pine. Document No: IRG/WP 01-30261. The International Research Group on Wood Preservation, Stockholm, Sweden.

Singh, T., Kreber, B., Wakeling, R., Stewart, A. and Jaspers, M. (2004). Effect of methylene bis thiocyanate on propagules and established mycelium of two sapstain fungi. *Forest Products Journal*, 56(1): 40-45.

Sjostrom, E. (1981). *Wood Chemistry - Fundamentals and Applications*. New York, The Academic Press, Inc.

Soerianegara, I. and Lemmens, R.H.M.J. (eds.). (1993). *Plant Resources of South-East Asia. No. 5(1): Timber trees: major commercial timbers*. Backhuys Publishers, Leiden.

Srinivasan, U., Ung, Y.T., Taylor, A. and Cooper, P.A. (1999). Natural durability and water-borne preservative treatability of tamarack. *Forest Products Journal*, 49(1): 82-87.

Stevanovic-Janezic, T., Cooper, P.A. and Ung, Y.T. (2000). Chromated copper arsenate preservative treatment of North American Hardwoods. Part 1.CCA fixation performance. *Holzforschung*, 54(6), 577-584.

Stirling, R. (2009). Natural durability classification systems used around the world. Document No: IRG/WP 09-10694. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-10.

Stirling, R., Drummond, J., Zhang, J. and Ziobro, R. (2008). Micro-distribution of micronized copper in southern pine. Document No: IRG/WP 08-30479. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-16.

Sundaramoorthy, M., Kishi, K., Gold, M.H. and Poulos, T.L. (1994). The crystal structure of manganese peroxidase from Phanerochaete chrysosporium at 2.06-angstrom resolution. *The Journal of Biological Chemistry*, 269(52): 32759-32767.

Talley, S.M., Coley, P.D and Kursar, T.A. (2002). The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecology*, 2(7).

Tang, J.D., Sonstegard, T., Burgess, S. and Diehl, S.V. (2010). A genomic sequencing approach to study wood decay and copper tolerance in the brown-rot fungus *Antrodia radiculosa*. Document No: IRG/WP 10-10720. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-14.

Taylor, A.M., Gartner, B.L. and Morrell, J.J. (2002). Heartwood formation and natural durability- a review. *Wood and Fiber Science*, 34(4): 587-611.

Tekere, M., Mswaka, A.Y., Zvauya, R. and Read, J.S. (2001). Growth, dye degradation and ligninolytic activity studies on Zimbabwean white rot fungi. *Enzyme and Microbial Technology*, 28:420–426.

ten Have, R. and Teunissen, P.J. (2001). Oxidative mechanisms involved in lignin degradation by white rot fungi. *Chemical Reviews*, 101(11): 3397-3413.

Teoh, S.G., Ang, S.H., Fun, H.K. and Ong, C.W. (1999). Synthesis, crystal structure and biological activity of thiophene-2-carboxaldehyde thiosemicarbazone and its tin complexes. *Journal of Organometallic Chemistry*, 580: 17-21.

Teunissen, P.J. and Field, J.A. (1998). 2-Chloro-1,4-dimethoxybenzene as a mediator of lignin peroxidase catalyzed oxidations. *FEBS Letters*, 439(3): 219 – 223.

Thurston, C.F. (1994). The structure and function of fungal laccases. *Microbiology*, 140: 19-26.

Tian, L., Sun, Y., Li, H., Zheng, X., Cheng, Y., Liu, X. and Qian, B. (2005). Synthesis, characterization and biological activity of triorganotin 2-phenyl-1,2,3-triazole-4-carboxylates. *Journal of Inorganic Biochemistry*, 99(8): 1646–1652.

Tien, M. and Kirk, T.K. (1988). Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods in Enzymology*, 161: 238–249.

Tien, M. and Kirk, T.K. (1983). Lignin-degrading enzyme from the Hymenomycete *Phanerochaete chrysosporium* burds. *Science*, 221(4611): 661-663.

Tien, M. and Kirk, T.K. (1984). Lignin-degrading enzyme from the *Phanerochaete chrysosporium*: Purification, characterization and catalytic properties of a unique H_2O_2-requiring oxygenase. Proceedings of the National Academy of Science, USA, 81: 2280-2284.

Tillott, R.J. and Coggins, C.R. (1981). Non-arsenical water-borne preservatives - a review of performance and properties. Rec. Brit. Wood Pres. Assoc. Ann. Conv., pp.32-46.

Timell, T.E. (1986). Compression wood in gymnosperms. Springer, Berlin.

Tolunay, A., Akyol, A. and Ozcan, M. (2008). Usage of trees and forest resources at household level: a case study of Asagi Yumrutas village from the west Mediterranean region of Turkey. *Research Journal of Forestry*, 2(1): 1-14.

Tolvaj, L. and Faix, O. (1995). Artificial ageing of wood monitored by DRIFT spectroscopy and CIE color measurements. 1. Effect of UV light. *Holzforschung*, 49(5): 397-404.

van der Kerk, G.J.M. (1976). Organotin chemistry: past, present and future, in *Organotin Compounds: New Chemistry and Applications*, J. J. Zuckerman, Ed., vol. 1, pp. 204–226, American Chemical Society, Washington, DC, USA.

Vrenon, P. (2000). Analysis of Wood Extractives. Online at www.ftns.wau.nl/oc/research/phytochemistry/extractives%20from%20wood/poster/poster.htm

Waldron, L., Ung, Y.T. and Cooper, P.A. (2005). Prediction of long term leaching potential of preservative treated wood by diffusion modeling. *Holzforschung*, 59: 581-588.

Wang, Y., Minato, L. and Iida, I. (2007). Mechanical properties of wood in an unstable state due to temperature changes and analysis of the relevant mechanism III: Effect of quenching on stress relaxation of chemically modified wood. *Journal of Wood Science*, 53(2): 94-99.

Wiedenhoeft, A. and Miller, R.B. (2005). Structure and function of wood. *Handbook of Wood Chemistry and Wood Composites*. New York, Taylor and Francis. p. 9-33.

Williams, G.R., Eaton, R.A. and Lewis, D.A. (1985). Observation on the penetration of preservative into green timber. Document No: IRG/WP 85-3335. The International Research Group on Wood Preservation, Stockholm, Sweden.

Wilson, A. (1971). The effects of temperature, solution strength and timber species on the rate of fixation of a copper-chrome-arsenate wood preservative. *Institute of Wood Science*, 5(6): 36-40.

Wong, A.H.H. (1989). Detecting Soft Rot Decay in Wood. FRIM. Technical Information. 1(13), Forest Research Institute Malaysia.

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 prospects. Document No: IRG/WP 05-10568. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-33.

Wong, A.H.H., Pearce, R.B., Grime, G.W. and Watt, F. (1996). Macrodistribution of copper-chrome-arsenic preservative in Malaysian hardwoods determined by PIXE imaging. In: Donaldson, L.A., Singh, A.P., Butterfield, B.G. and Whitehouse, L.J. (eds.), *Recent advances in wood anatomy*, New Zealand For. Res. Inst., Ltd., pp.255-257.

Wong, A.H.H., Pearce, R.B., Grime, G.W. and Watt, F. (1999). Preliminary PIXE micro-analysis of copper-chrome-arsenic preservative treated Malaysian hardwoods. Document No: IRG/WP 99-20169. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-17.

Wong, P.T.S., Chau, Y.K., Kramar, O. and Bengert, G.A. (1982). Structure-toxicity relationship of tin compounds on algae. *Canadian Journal of Fisheries and Aquatic Sciences*, 39(3): 483-488.

Woo, Ch.S. and Morris, P.I. (2010). Sensitivity of copper of basidiospores from copper tolerant fungi *Fomitopsis pulastris* and *Oligoporus placentas*. Document No: IRG/WP 10-10707. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-9.

Yalinkilic, M.K., Imamura, T., Takahashi, M., Demirci, Z. and Yalinkilic, A.C. (1999). Biological, mechanical, and thermal properties of compressed-wood polymer composite (CWPC) pretreated with boric acid. *Wood and Fiber Science*, 31(2): 151-163.

Yamamoto, K. and Rokova, M. (1991). Differences and their causes of CCA and CCB efficacy among softwoods and hardwoods. Document No: IRG/WP 91-3656. The International Research Group on Wood Preservation, Stockholm, Sweden. pp. 1-7.

Yap, M.G.S., Chia, L.H.L. and Teoh, S.H. (1990). Wood polymer composites from some tropical hardwood. *Journal of Wood Chemistry and Technology*, 10(1): 1-19.

Yildiz, S., Gezer, E.D. and Yilditz, U.C. (2006). Mechanical and chemical behaviour of spruce wood modified by heat. *Building and Environment*, 41: 1762-1766.

Yildiz, U.C., Temiz, A., Gezer, E.D. and Yildiz, S. (2004). Effects of the wood preservatives on mechanical properties of yellow pine (*Pinussylvestris* L.) wood. *Building and Environment*, 39: 1071 – 1075.

Yin, H.D. and Chen, S.W. (2006). Synthesis and characterization of di- and tri- organotin(IV) complexes with Schiff base ligand pyruvic acid 3-hydroxy-2-naphthoyl hydrazone. *Inorganica Chimica Acta*, 359(10): 3330-3338.

Yin, H.D., Chen, S.W., Li, L.W. and Wang, D.Q. (2007). Synthesis, characterization and crystal structures of the organotin(IV) compounds with the Schiff base legands of pyruvic acid thiophene-2-carboxylic hydrazone and salicylaldehyde thiophene-2-carboxylic hydrazone. *Inorganica Chimica Acta,* 360 (7): 2215-2223.

Yoshida, H. (1883). Chemistry of lacquer (urushi). *Journal of the Chemical Society***,** 43: 472-486.

Zabel, R.A. and Morrell, J.J. (1992). *Wood microbiology: Decay and its prevention*. Academic Press, San Diego.

Zaidon, A., NorhairulNizam, A.M., Faizah, A., Paridah, M.T., Jalaluddin, H., Mohd Nor, M.Y. and Nor Yuziah, M.Y. (2008). Efficacy of pyrethroid and boron preservatives in protecting particleboards against fungus and termite. *Journal of Tropical Forest Science*, 20(1): 57-65.

Zhang, J. and Kamdem, P.D. (2000). FTIR characterization of copper ethanolamine - wood interaction for wood preservation. *Holzforschung*, 54(2): 119-122.

Zhang, J. and Ziobro, R. (2009). Micronized copper preservative system: Observations on the release of cupric ion (Cu2+) from treated wood and performance against wood decay fungi. Document No: IRG/WP 09-30519. The International Research Group on Wood Protection, Stockholm, Sweden. pp. 1-17.

Zhang, Y., Zhang, S., Yang, D. and Wan, H. (2006). Dimensional stability of wood-polymer composites. *Journal of Applied Polymer Science*, 102(6): 5085-5094.

List of Relevant Publications

Rahman, M.M., Jusoh, I., Affan, M.A., Husaini, A. and Hamdan, S. (2013). Efficacy of novel organotin(IV) complexes on non-durable tropical wood against decay fungi. European Journal of Wood and Wood Products, 71(4): 463-471.

Rahman, M.M., Jusoh, I., Husaini, A., Seman, I.A. and Sing, N.N. (2013). Biodegradation and ligninolytic enzymes profiles of the newly synthesized organotin(IV)-treated non durable tropical wood species. *Journal of Biochemical Technology*, (In review).

Jusoh, I., **Rahman, M.M**., Affan, M.A., Hamdan, S. and Salam, M.A. (2012). Treatability of tropical wood using newly synthesized organotin(IV) complexes. Journal of Applied Sciences, 12(6): 535-543.

Rahman, M.M., Jusoh, I., Affan, M.A., Husaini, A., Hamdan, S. and Islam, M.S. (2012). Physico-mechanical properties of tropical wood treated with novel organotin(IV) complexes. European Journal of Scientific Research, 89(4): 512-522.

Rahman, M.M., Jusoh, I., Affan, M.A. and Husaini, A. (2012). Evaluation of five novel organotin(IV) complexes as wood preservatives. Document No: IRG/WP 12-30590. The International Research group on Wood Preservation, Stockholm, Sweden. pp. 1-14.

Rahman, M.M., Jusoh, I. and Seman, I.A. (2013). Retention and toxicity of synthesized organotin(IV)-treated tropical wood. 26th Regional Symposium of Malaysia Analytical Sciences, Hilton Kuching, Malaysia.

Rahman, M.M., Jusoh, I., Affan, M.A. and Husaini, A. (2013). Preservative treatment of non-durable wood using newly synthesized organotin(IV) complexes. In: Assim *et al*. 2013, Current research trends in resource science and technology. FRST, 53-60.

Rahman, M.M. and Jusoh, I. (2012). Treatability and efficacy of novel organotin(IV) compounds as wood preservatives. UNIMAS Graduate Research Poster competition 2012.