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MICRODISTRIBUTION OF CHROMATED COPPER ARSENATE PRESERVATIVE IN RUBBERWOOD (HEVEA BRASILIENSIS MUELL. ARG.)

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MICRODISTRIBUTION OF CHROMATED COPPER ARSENATE PRESERVATIVE IN RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.)

By

Ismail Bin Jusoh

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ABSTRACT

MICRODISTRIBUTION OF COPPER-BASED PRESERVATIVES IN RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.)

By

Ismail Bin Jusoh

Rubberwood is popular for making indoor furniture since rubberwood is relatively abundant and sustainable. Currently more than 60% of the total annual rubberwood produced by rubber plantation is used as fuelwood. Rubberwood has the potential for both indoor and outdoor application. For exterior applications, preservative treatment is needed to extend the service life of rubberwood. The objectives of this study are to (1) assess treatability of rubberwood with chromated copper arsenate (CCA) preservative, (2) evaluate the natural decay resistance and efficacy of CCA on rubberwood, and (3) study the microdistribution of CCA components in rubberwood cells. The treatability of rubberwood was determined by measuring the penetration and retention of CCA type C preservative after a full-cell treatment. Natural decay resistance and efficacy of CCA treatment on rubberwood was estimated using a laboratory soil-block test according to AWPA E10-91. The microdistribution of chromium, copper and arsenic in CCA-treated rubberwood was studied using scanning electron microscope in conjunction with energy dispersive X-ray analyzer (SEM-EDXA). As expected, longitudinal permeability was found to be better than the radial and the tangential permeability. The penetration and retention in the radial direction was about 3 times better than in the tangential direction. Longer pressure period increased penetration and

retention of CCA type C in rubberwood. Complete penetration was achieved after 4 hours of pressure (1240 kPa) treatment. A pre-treatment steaming improved the treatability of rubberwood regardless of the anatomical direction. The average weight loss by white rot and brown rot was about 1.5 times higher than that of soft rot. A linear relationship was found between the weight loss and the incubation period for all the six test fungi. A CCA retention of 4.1 kg/m^3 reduced weight loss to about 10% and retention of 14.5 kg/m^3 reduced the weight loss of all test fungi at less than 2%. Vessels contained high level of chromium, copper, and arsenic compared to fibers. Chromium level was the highest, followed by arsenic and then copper in rubberwood cells. After the full cell treatment, fibers contained about 0.42%, 0.63%, and 1.02% of copper after treatment with 4.1 kg/m^3, 10.5 kg/m^3, and 14.5 kg/m^3 of CCA, respectively. Highest levels of Cr, Cu, and As were recorded in fiber-to-vessel cell corner (FV_{CC}) and Fiber-to-vessel middle lamella (FV_{ML}) and the lowest was recorded in S_2 layer of fiber. Linescan analyses showed that higher count rates of carbon, oxygen, chromium, copper, and arsenic were found in the middle lamella compared to the fiber S_2 layer in CCA-treated rubberwood. The increase of the solution strength in chromium, copper, and arsenic corresponds to an increase in Cr, Cu, and As level in wood cells.

DEDICATION

To my beloved wife, Salviah, and children, Afiqah, Syamimi, Zulhusni, and Khairulhamiz for their patience and sacrifices.

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CHAPTER 1

INTRODUCTION

Rubber plantations are found in more than 30 countries around the world as illustrated in Figure 1. The total plantation surface area world wide is approximately 9 million hectares with almost 90% located in Asia and about 75% of these are in Malaysia, Indonesia and Thailand (Ismariah and Norini 1994).

Rubber trees (*Hevea brasiliensis* Muell. Arg.) reach their prime in 25 years, after which it is no longer economical to for latex production (Hong *et al.* 1994). After 25 years, rubber trees have a clear bole of 3 to 10 meters height depending on the clone. The diameter can reach up to 50 centimeter at breast height.

Old rubber trees are simply burned in the field prior to planting new stock. Some of the old trees are used for firewood for brick manufacturing and for production of charcoal briquettes. In recent years, due to the shortage of forest timbers, rubberwood has become an important source of timber particularly for furniture and wood composites manufacturing (Hong and Sim 1994). Despite the rapid commercial use of the rubberwood, it is estimated that 67% of the total volume of rubberwood obtained from rubber plantations is burned (Lew 1992).

Rubberwood has excellent potential for both interior and exterior wood products. The supply is sustainable and is relatively low cost as it is a by-product of rubber industry Today, by far the largest users of rubberwood are manufacturer of furniture for indoor use. For exterior use, rubberwood is not included because of its susceptibility to biodegradation and dimensional instability.

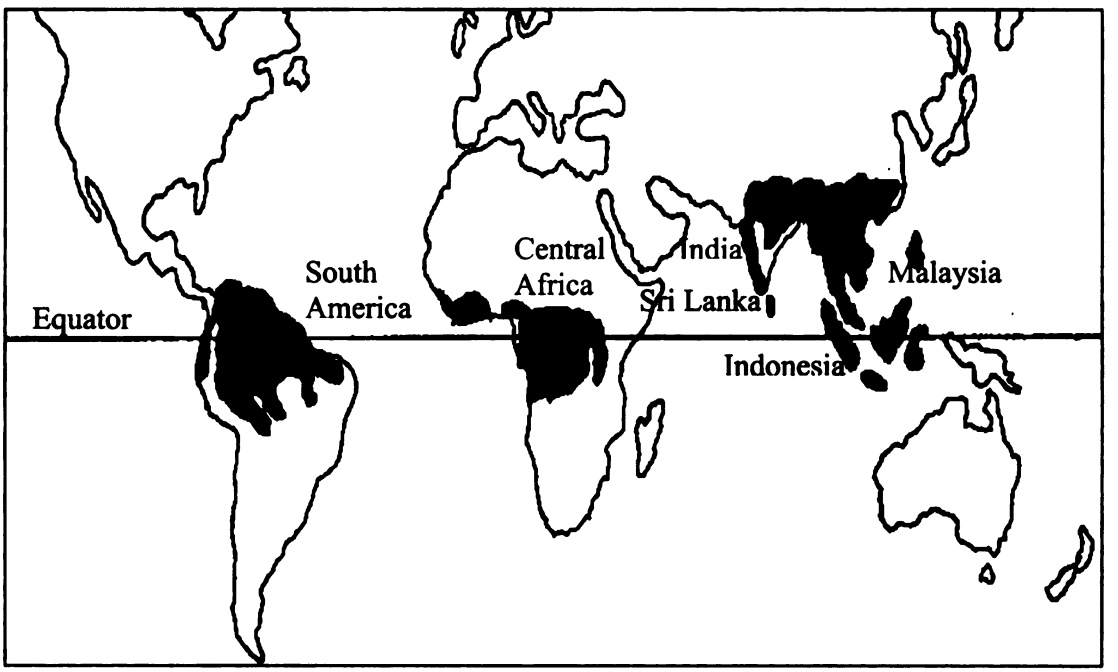


Figure 1. World map of rubberwood distribution.

The protection of hardwood against deterioration is desirable for exterior applications. The service life of wood can greatly be increased by proper treatment. The opportunity for increasing utilization of many hardwoods species, especially for exterior applications, is highly dependent on the development of wood preservation technology. Chemical treatments can make the use of rubberwood in high decay-hazard environment possible. The effectiveness of a wood preservative is dependent not only on the retention, but also on the penetration and distribution of preservative in the wood cells (Janezic *et al.* 1997; Ryan 1986).

Information on the location and distribution of the active ingredients in treated wood is important with regard to the mode of action of the preservative, permeability of wood, and impregnation process. Over the last three decades, the microdistribution of preservative in treated wood has received considerable research. Several studies have investigated the microdistribution of chromated copper arsenate (CCA) in softwood species (Newman and Murphy 1996; Jeihooni *et al.* 1993; Greaves *et al.* 1983; Yata and Nishimoto 1983; Drysdale *et al.* 1980; Chou *et al.* 1973; Petty and Preston 1968). Apart from *Eucalyptus*, little work is reported on the microdistribution of metal elements in tropical hardwoods. The location and amount of preservative elements present in the complex wood microstructure of hardwoods need more study. The amount of preservative in the cell walls may contribute to its efficacy (DeGroot and Kuster 1986). Thus, it is necessary to understand the microdistribution of preservative in rubberwood cells.

The objectives of this study are;

(1) To assess the treatability of rubberwood with chromated copper arsenate (CCA) preservative.

(2) To evaluate the natural decay resistance and the efficacy of CCA on rubberwood.

(3) To examine the microdistribution patterns of CCA components in the wood cell walls and selected morphological regions, at varying treating solution strength.

CHAPTER 2

LITERATURE REVIEW

2.1 Rubberwood - Overview

2.1.1 Rubberwood resource

Rubberwood is the timber produced from rubber trees. The rubber trees of Malaysia, indigenous to Amazon forest of Brazil, were introduced in 1877 for latex production (Hong 1994). Currently, Malaysia has more than 20 clones of rubber trees. In 1992 the total area planted with natural rubber in Malaysia was estimated to be 1.8 million hectares (ha.) (Anonymous 1993a). The rubber plantation contributes the largest man-made forests in the South East Asia region (Ser 1990). Logs with diameter more than 20 cm are used for sawn timber products while smaller diameter are used as fuel in brick factories and processed into charcoal, chipboard and medium density fiberboard plants (Ho 1994).

2.1.2 Properties of rubberwood

Rubberwood is white to pale cream, sometimes with a pinkish tinge, and aged to light straw or light brown. It consists of only sapwood (Lim and Sulaiman 1994; Anonymous 1982; Thomas and Landon 1953). The texture is moderately coarse with an even grain. A characteristic smell of latex is distinct in freshly sawn timbers. The vessels are moderately large and usually filled with tyloses (Lim and Sulaiman 1992). Wood parenchyma cells are narrow and irregularly spaced. Rays are moderately fine and are visible to naked eye. The structural elements of rubberwood consist of about 62% fibers,

9.5% vessels, and 29.0% parenchyma cells (Nagoshi 1970). The fiber length of rubberwood varies from 1.1 to 1.8 mm, fiber width from 26 to 30 microns and cell wall thickness from 5.1 to 7.0 microns (Peel and Peh 1960). Rubberwood has a lignin content of 22 to 29%, holocellulose of 70%, pentosan content of 20%, alpha-cellulose of 40% and extractives of 6 to 10% (Hong and Lim 1985; Peel and Peh 1960).

The green specific gravity of rubberwood is 0.53 (Lee *et al.* 1979). The air-dried modulus of elasticity (MOE) and modulus of rupture (MOR) were 9,240 MPa (1.34 million lb/in^2) and 66 MPa (9,500 lb/in^2), respectively. The strength properties of rubberwood are grouped under the strength group C (Rahman 1978), which is good for light construction. Gnanaharan and Dhamodaran (1993) reported that a 35-year rubberwood plantation from Kerala, India had an average MOE of 15,700 MPa (2.27 million lb/in^2) and MOR of 98.4 MPa (14,200 lb/in^2).

In the tropics rubberwood is classified as non-durable wood (Wong 1988; Jantan and Tam 1987; Hong *et al.* 1987; Scheffer and Cowling 1966). Wood in this class has the natural durability of between 2 to 5 years and should require some protection if used in decay or insect hazardous environment (Findlay 1985). The carbohydrates content of the parenchyma makes rubberwood susceptible to decay attack (Azizol and Sudin 1989). In a laboratory evaluation of susceptibility of rubberwood to the three major types of wood-rot, the severity of attack are in the order of soft rot > white rot > brown rot (Wong 1988).

Freshly sawn rubberwood is susceptible to sapstain and mold fungus. The common fungus reported to be responsible for bluish discoloration is *Botryodiplodia theobromae* (Hong 1976; Kaarik 1980; Tsunoda *et. al.* 1983). Other staining fungi that have been isolated from rubberwood are *Fusarium decemcellulare*, *Aspergillus sydowii*

and *Penicillium citrium* (Balasundram and Gnanaharan 1990). The main surface colonizers or molds are species of *Aspergillus, Curvularia, Fusarium, Gliocladium, Trichoderma, Sphaeronaema and Penicillium* (Hong *et al.* 1987; Tsunoda *et al.* 1983; Hong 1981; Sujan *et al.* 1980).

2.1.3 Utilization and uses of rubberwood

Rubberwood is valuable for furniture making because of its beautiful, light and even colored texture, suitable strength, good machining and processing properties (Tee 1990). The creamish color and figured, ease of machining and high quality surface finish make rubberwood suitable for mouldings and other joinery components, parquet and strip floorings (Chew 1993). Particelboard made from rubberwood is extensively used in furniture and joinery industries for making wardrobes, cabinets, side- boards, desks, tables, room divider, door and kitchen cabinets (Hong and Sim 1994). Currently the main raw material for making MDF in Malaysia is rubberwood (Yusoff 1995). The excellent physical properties of rubberwood MDF (Yusoff 1992; Razali and Diong 1992; Tomimura *et al.* 1990) made it excellent choice for furniture manufacture especially mainly as table tops, cabinets, doors and headboards for beds. The cement-bonded particleboard (CBP) manufactured in Malaysia from rubberwood is marketed under the trade name *Cemboard* and has been widely used as building material in construction of low-cost houses and multi-story buildings (Sudin 1994). Other uses of rubberwood are for pulp and paper (Yusoff 1994), charcoal and briquettes (Hoi 1994) and when properly treated rubberwood can be used as building material (Hong and Sim 1994; Teng and Idrus 1990).

2.1.4 Preservative treatment of rubberwood

Newly felled rubberwood logs are extremely susceptible to staining fungi and insect attacks (Jantan *et al.* 1994). To avoid deterioration the logs are usually converted to sawn timber as soon as possible. However, delay in conversion to sawn timber is unavoidable because of transportation and log yard operations (Ho 1994). To prevent log deterioration during storage, preservative treatment is necessary.

Rubberwood logs can be protected from biodegradation by application of chemical preservatives containing a fungicide and insecticide by means of spraying or end-coating (Jantan *et al.* 1994; Lewis and Spencer 1987; Hong *et al.* 1980). For more effective end-coating, the preservatives could be incorporated into Shellkote-3, a bituminous based emulsion for end coating applied by brushing (Hong *et al.* 1980; Tan *et al.* 1980). The end-coating serves as physical barrier to fungal infection and insect attack and at the same time create an unfavorable condition for fungal development because of moisture content fluctuation at the coated surfaces.

The most effective end coat preservatives are the combinations of 2% Captafol and 1% Fennotox S2 (thiophanates, thiocarbamates and sodium nitrite) incorporated into Shellkote-3 (Hong *et al.* 1980). The performance of Shellkote-3 is enhanced by addition of either 4% sodium pentachlorophenate (NaPCP) or 1.5% of a proprietary fungicide containing methylene-bis-thiocynate (MBT) or 4% Captofol (Lewis and Spence 1987). Storage of rubberwood logs under water is another way of protection. Complete protection of fresh logs can achieved by end coating prior to storing under water. A study by Hong *et al.* (1988) showed that without any prior treatment, fresh logs completely submerged under water for up to seven months had little or no staining, and no

appreciable visual deterioration. Although this method has an added advantage in protecting logs against splitting and insects, large log pond facilities are seldom available. Storing logs under water emit a foul smell after a few days (Jantan *et al.* 1994).

Freshly sawn rubberwood are susceptible to fungal and insects attack. Two types of treatments are employed in Malaysia for temporary and long-term protection (Jantan *et al.* 1994). Temporary treatment is used to protect rubberwood during air-drying and/or prior to kiln drying. Temporary treatment is done by dipping rubberwood in a preservative mixture of fungicide and insecticide for 1 to 2 minutes (Hong 1989). Commonly used preservative mixtures available for dip treatments in Malaysia are listed in Table 1.

Table 1. Preservatives used for rubberwood dip treatment.

Preservative	Recommended amounts per 1000 liters of water
NaPCP + gamma-BHC	15 - 20 kg 7.5 kg
NaPCP + deltametrin/cypermethrin	15 - 20 kg 0.125 L
TCMTB + boron compounds	1 - 5 L 15 - 20 kg
TCMTB + Deltametrin/cypermethrin	1 - 5 L 0.125 L
MBT + boron compounds	4 - 10 L 15 - 20 kg
MBT + deltametrin/cypermethrin	4 - 10 L 0.125

* From Hong, L.T. (1989)
TCMBT - Thiocyanomethylthiobenzothiazole
MBT - Methyl-bis-thiocynate

The growing concern on the use of highly persistent pesticides such as the cost-effective NaPCP has resulted in the search for formulation of alternative fungicides. Tsunoda *et al.* (1983) reported that 2% formulated mixture of 4-chlorophenyl-3-iodopropagylformal and 2-(4-thiazoly)benzimidazole prevents sapstain and mold growth. A study by Lewis and Spence (1987) showed that stain and mold development on rubberwood can be controlled by using 1.5% Antiblu-3739 (MBT and TCMTB) treatment. Wong and Woods (1997) reported that Tuff Brite C^{TM} containing (36% w/w chlorothalonil & 8% carbendazim) with or without borax provided complete control against blue-stain and mold on rubberwood.

Dip treatment is essentially a temporary protective measure as preservative only form a protective layer of about 2-5 mm deep from the surface of treated rubberwood (Jantan *et al.*1994; Hong, *et al.* 1982). During further processing to furniture and other products the superficial preservative layer is removed, exposing fresh untreated surfaces.

For long term protection, preservatives need to penetrate deep into the rubberwood wood microstructure. Two methods employed for long-term protection are dip-diffusion process and pressure impregnation process (Jantan *et al.* 1994). The dip-diffusion process is a simple and economical method to treat fresh sawn rubberwood (Anonymous 1969). The treatment consists of dipping freshly sawn wood in a 20-30% solution mixture of borax and boric acid at 35-45°C for 5 to 40 minutes (Gnanaharan 1982; Tam and Singh 1987). Addition of 0.5-1% NaPCP to the boron compounds prevent staining of wood (Gnanaharan and Mathew 1982).

Pressure impregnation or pressure treatment gave satisfactory results for total protection of rubberwood (Hong *et al.* 1982; Sonti *et al.* 1982; Hong and Liew 1989).

Chemicals that have been tested for pressure treatment of rubberwood are benzylkonium chloride, copper naphthenate, pentachlorophenol, copper-chromium-arsenate (CCA), and cresosote (Martawidjaja 1971; Tan *et al.* 1979; Hong *et al.* 1987). An average uptake of 507.2 l/m^3 (31 lb/ft^3) was achieved when rubberwood was treated with 3% CCA by full cell process for the duration of 2.5 hours (Jantan *et al.*, 1994). In recent study on treatability of rubberwood, Salim (1997) reported retention of 32.58 kg/m^3 and full saturation of 40 x 40 x 750 mm specimen when 5% CCA was used in full cell process for the duration of four hours.

If rubberwood is to be used for furniture making, CCA is seldom used to treat rubberwood because of the undesirable color of treated wood, however if it to be used for construction it is best to treat with CCA (Jantan *et al.* 1994). In a field test, pressure treated rubberwood stakes with 100% and 50% creosote and 4 % copper naphthenate showed no sign of rot after 20 years of ground contact (Martawidjaya 1971).

Boron-based preservatives have been used to treat green rubberwood using pressure treatment (Tan *et al.* 1980, 1983, Hong *et al.*1987; Hong and Liew 1989, Salamah *et al.* 1988). Treatment of rubberwood of varying moisture contents using 3% mixture of borax and boric acid has shown that freshly sawn rubberwood could absorb 187-214 kg/m^3 of preservative solutions giving retention of about 0.36% boric acid equivalent (BAE) and up to a depth of 10 mm (Hong and Liew 1989).

The opportunity for increasing the utilization of rubberwood and other hardwoods in exterior construction is highly dependent on the preservative treatment. However, satisfactory protection of hardwoods vary with species because of the different in

chemical contain, preservatives used, and permeability of various wood tissues (Smith *et al.* 1996; Butcher and Nillson 1982; Butcher 1979).

Fungi decompose wood by secreting enzymes, which in present of moisture, degrade cellulose and other wood constituents (Zabel and Morell 1992). However, the complexity of fungi metabolism and processes provide numerous potential points that can be altered or blocked chemically. For instance, copper acts as a fungicide in which its denatures protein within the fungi and interfere with enzyme reactions (Eaton and Hale 1993). Arsenic is a competitive inhibitor for phosphorus in adenosine triphosphate (ATP) synthesis (Zabel and Morell 1992) while chromium has strong affinity for wood lignin which limits leaching (Hartford 1973).

2.2 Performance of treated hardwoods

Chromated copper arsenate (CCA) is a broad-spectrum wood preservative and its effectiveness in protecting softwoods both in laboratory and field tests is well documented and proven. The excellent performance of CCA-treated softwood, particularly the *Pinus* species, is attributed to deep penetration of preservatives into the cell wall of tracheids (Petty and Preston 1968), preservative loading (Ryan and Drysdale 1988), uniform preservative distribution at cell level (Bodner and Pekny 1991; Dickinson and Sorkhoh 1976; Greaves 1972), and even preservative distribution within the cell wall (Newman and Murphy 1996). However, the performance of CCA-treated hardwoods against soft rot varies. Soft-rot fungi often attacked CCA-treated hardwoods that are used in ground contact (Duncan and Eslyn 1966; Findlay 1984; Smith *et al.* 1996). For

instance, CCA-treated eucalyptus transmission poles in Australia were attacked by soft-rot fungi (Greaves 1977; Butcher and Drysdale 1978).

In laboratory studies (Butcher and Drysdale 1978), specimens of twelve CCA-treated hardwoods were severely attacked by soft-rot fungi. The same study also found that it was possible to control soft-rot attack by increasing the CCA retention from 26 kg/m^3 to 44 kg/m^3, depending on the density of the species. Ryan and Drysdale (1988) noted that it required six times more copper retention in the fibers of *Betula alba* than in tracheids of *Pinus radiata* to prevent soft-rot attack. Witheridge (1983) also suggested that retention of approximately 35 kg/m^3 of CCA can significantly extended the service life of eucalyptus power poles in New South Wales. However, Leightley and Norton (1983) reported that CCA retention of up to 40 kg/m^3 did not protect eucalyptus poles from soft rot in Australia.

Some hypotheses have been put forward to explain the reasons for the poor performance of CCA-treated hardwood. The gross anatomy of hardwoods and softwoods are very different. Hardwoods are composed of mainly fiber (15-60%), vessel elements (20-60%), ray (5-30%), and parenchyma (0-24%) (Haygreen and Bowyer 1996). The vessel elements perform conductive function while fiber is responsible for support, which usually possess a few slit-like simple pits (Greaves 1974). Preservative solution flows principally through the open vessels, which form an easy and continuous pathway (Wilkinson 1979; Siau 1984). It moves into the surrounding cells, through pits into cell lumen, and penetrating the cell wall. Softwoods are compose of 90-95% tracheids, and the remainder, 5-10% is make up of ray cells. In softwood, tracheids are conducting cells, thin walled, with rounded ends and numerous bordered pits, which leads to more

invariable behavior in term of liquid penetration and distribution than in hardwoods. Softwoods have a uniform arrangement of thin-walled tracheids, conversely hardwoods composed of varying proportions of different kinds of cells couple with thick cell-wall fiber, may posses many possibilities as regards to liquid pathways that will influence penetration and distribution of liquids (Nilsson 1982; Greaves 1974). Studies showed that preservatives distribution in softwood were relatively more even and penetrated deeper in the cell wall than hardwood (Levy and Greaves 1978; Greaves 1974).

The differences in chemical make-up of hardwoods and softwoods affect the fixation reactions. The softwoods have generally higher lignin content than hardwoods (Haygreen and Bowyer 1996), and not only the content is different but there are also structurally different. According to Butcher and Nillson (1982), lignin content determines the susceptibility of hardwood to soft-rot, since lignin is encrusting the cellulose microfibril, thus it provides protection from enzymes attack by soft-rot. Softwoods contain a guaiacyl lignin whereas most hardwoods contain syringyl-quaiacyl lignin. Since lignin is one of the reaction sites of CCA thus the amount of CCA in treated wood corresponds to the amount of and distribution of lignin in wood (Buthcer and Nilsson 1982; Daniel and Nilsson 1987). In addition, it has been reported that CCA preferentially react with guaiacyl lignin (Daniel and Nilsson 1987), which may suggests that since hardwood cells are mostly made-up of syringyl-quaiacyl lignin thus its more susceptible to fungi attack.

2.3 Fixation of CCA in wood

The term "fixation" as used in the field of wood preservation refers to the mechanism by which preservative component become stabilized in the wood that resist leaching by water or rain during service. However, there is debate whether the word fixation is appropriate term to use. This is because even CCA, which is fully bound to wood, will leach to some degree. Thus some researchers suggested that CCA fixation refer to the state of the chemical component of the preservative and wood or other substrate when all chemical reactions are complete.

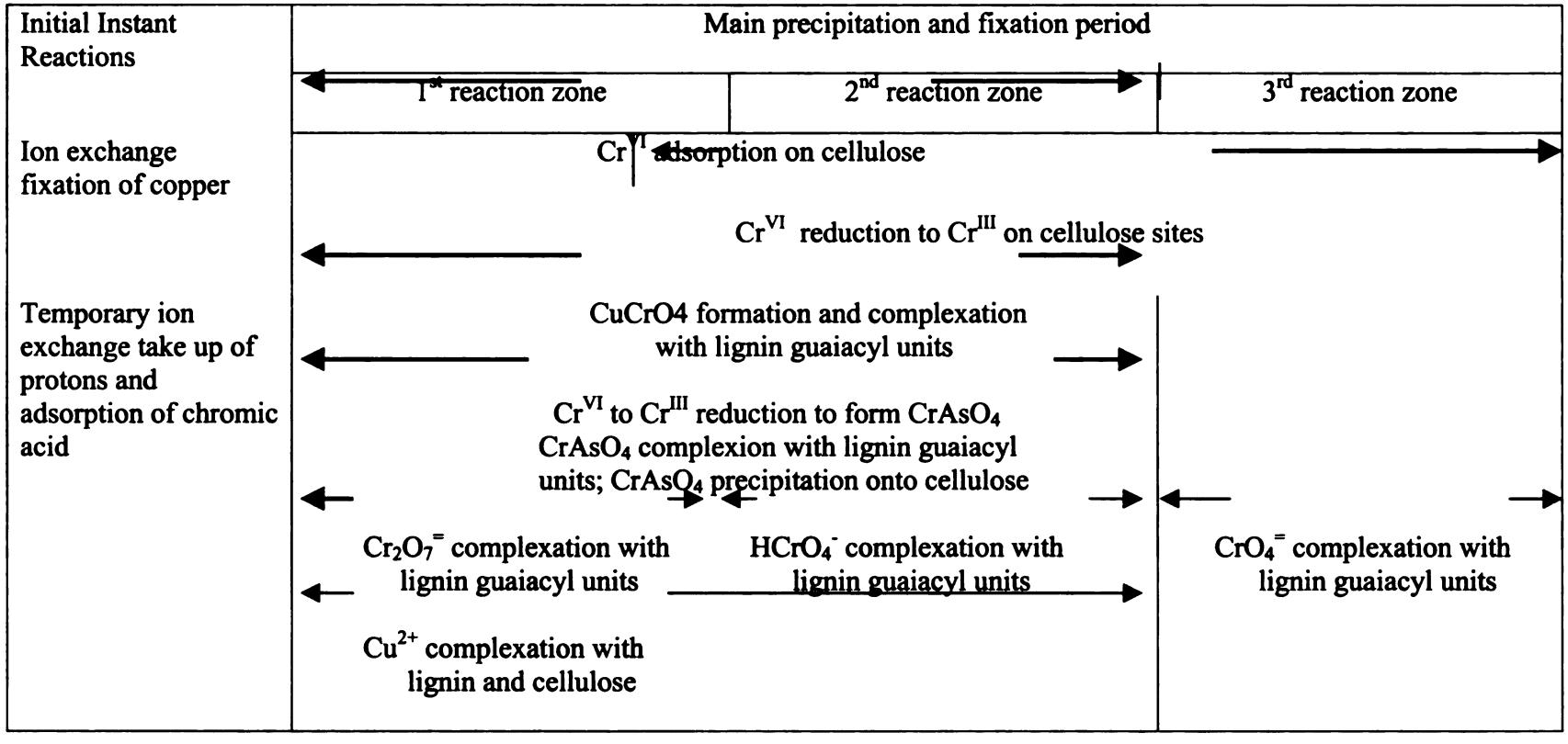
The interactions of CCA preservatives with wood during and after treatment are complex and not completely understood (Eaton and Hale 1993; Lebow 1996). The completion of CCA fixation may take few days, weeks or even months. However, some reactions do occur during and immediately after treatment (Dahlgren and Hartford 1972; Pizzi, 1981, 1982c). In a series of papers published by Dahlgren and Hartford (1972 - 1975) suggested that the process is related to pH changes in wood following treatment. According to their study based on wood flour from sapwood of pine and spruce, the process of fixation undergo three periods namely, momentary initial reactions, primary fixation reactions, and conversion reactions.

In the momentary initial reactions, that is immediately after CCA treatment there is an increase in pH caused by ion exchange and adsorption reactions between copper and chromium and wood components. It appears that some copper and chromium react with wood almost instantly. The next process is the precipitation reactions. During precipitation of the active elements, pH continues to increase but reaches a maximum (ca. pH 5), when all the chromium is consumed. The reactions involve in this period are the reduction of chromic acid to trivalent chromium (Cr^{3+}) that causes a steady increase in

the pH of the solution. Following precipitation reactions, slow fixation reactions may continue within the wood for several months. During the slow process, some of the early reaction products, which are unstable, are slowly converted via dissolution into stable compounds. For instant acid and tertiary copper arsenates are converted into basic copper arsenate. During these conversion reactions the pH of the wood-CCA system increases and decreases due to proton liberating and proton consuming reactions. In this reaction liberated protons are consumed in the reduction of chromates and chromate-wood complexes to trivalent chrome in the form of $Cr(OH)_3$. The final equilibrium products believed to be ion exchange fixation of copper to wood, $CrAsO_4$, $Cu(OH)CuAsO_4$, and $Cr(OH)_3$ (Dahlgren, 1974, 1975).

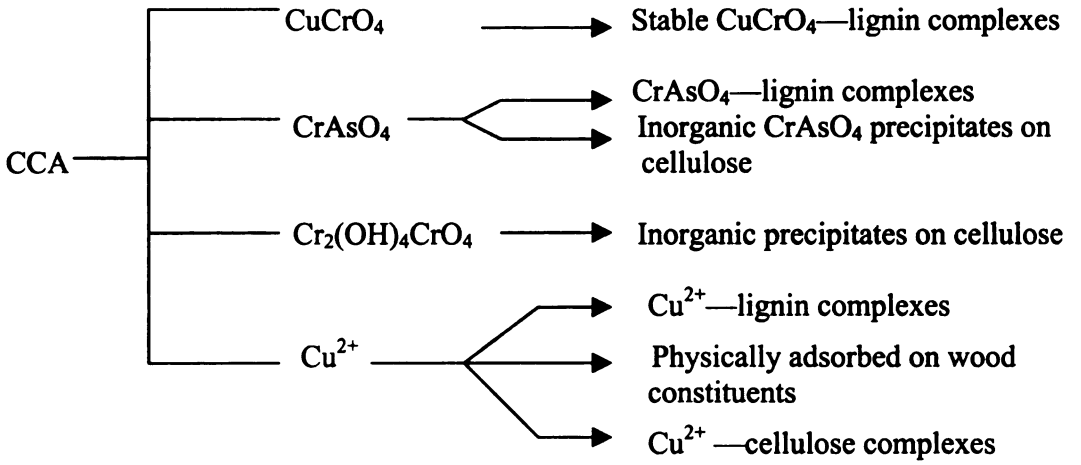
Studies by Pizzi (1981, 1982a, 1982b, 1982c) on the mechanisms of CCA fixation using cellulose and lignin model compounds and also wood flour showed that a series of reactions and fixation products occur in wood cell appear to have some similarity to those described by Dahlgren and Hartford (1972). However, Pizzi (1982) suggested that during the initial reactions, the initial fixation of copper was only physical absorption not adsorption reactions as suggested by Dahlgren and Hartford (1972). He also concluded that copper might also be involved in much slower reactions with cellulose and lignin. According to Pizzi (1982c), the main precipitation and fixation reactions, which follow the initial reactions, are divided into three zones that occur within the first 2 hours. The details of reactions in the three zones are described in Table 2.

Table 2. Schematic diagram of fixation of CCA wood preservative according to Pizzi (1982c).



Bold line arrows indicate predominant reactions in reaction zone.

According to Pizzi (1982c) the products formed when CCA is reacted with wood are composed of mainly the $CuCrO_4^-$—lignin complexes, $CrAsO_4^-$—lignin complexes, and $CrAsO_4$ precipitates on cellulose. The actual proportions of various chemical species are dependent on relative proportion of Cu, Cr, and As present in the formulation. About 10% of the copper in the system is bound to chromium. The rest of the copper is bound to the cellulose and lignin. Copper is present in the treated wood in four different forms: (1) $CuCrO_4$ bound to guaiacyl units of lignin by Cr^{6+} (10 - 15%), (2) Cu^{2+} directly bound to carbohydrates and lignin guaiacyl units (10 - 22%), (3) Cu^{2+} directly bound to lignin functional groups other than guaiacyl units (40 – 70%), and (4) $CuSO_4$ physically adsorbed by the various wood constituents (5 – 20%). It can be seen from the different forms of copper, that the majority of copper is associated with lignin in wood. The fixation reactions with wood and the products formed when CCA reacts with wood can be summarized as follows (Pizzi 1982c):



Earlier work by Smith and Williams (1973) had shown that chromium was crucial to the fixation process and was responsible for fixation of most of the arsenic and copper. When chromium is reduced from Cr^{6+} to Cr^{3+} it react readily with arsenic to form

CrAsO4, which in turn, has the ability to complex with lignin and cellulose. In treated wood, approximately 85% of the arsenic reacts with chromium, the remaining arsenic forms fairly soluble complexes with lignin and cellulose (Pizzi 1982b).

In term of leach resistance, Pizzi (1981) postulated that the formation of polymeric complex of guaiacyl—CrO_3 provides a significant amount of water repellency, which contributes to the efficacy of CCA-treated wood against leaching.

In a study by Yata *et al.*(1982) on the distribution of chromium in cell wall showed that chromium concentration was high in the primary wall and S_1 layer, which are areas of high lignin content, suggests that preferential association of chromium with lignin.

The current trend of research on CCA fixation is to study the effect of fixation reactions on wood. Ostmeyer *et al.* (1988, 1989) using x-ray photoelectron spectroscopy (XPS) and diffuse reflectance Fourier transform infrared spectroscopy (DRIFT) on CCA-treated southern pine, found that carbon-hydrogen bonds of the aromatic ring are being oxidized, with the possibility that hexavalent chromium chromate esters being formed. According to them, this suggest that the preservative components undergo substitution reactions with aromatic portion of guaiacyl lignin only, because the second methoxyl group on syringyl units would limit formation of stable wood-metal complexes. In a XPS study by Yamamoto and Inoue (1990) on CCA-treated coniferous wood, detected an increase in the proportion of carbon-carbon-hydrogen bonds, and a decrease in hydroxyl concentration.

A study using infrared, nuclear magnetic resonance (NMR), and ultraviolet spectroscopic analysis by Hon and Chang (1985) on model lignin compounds of quaicol

and catechol demonstrated that phenolic hydroxyl compunds were altered by reaction of chromium trioxide.

2.4 Microdistribution of CCA in hardwoods

The microdistribution of wood preservatives in hardwoods has been studied in various ways. Most of the research was done with waterborne chromated copper arsenate (CCA) preservative using scanning electron microscopy (SEM) in conjunction with X-ray microanalysis (Greaves 1974; Dickinson and Sorkhoh 1976; Greaves and Levy 1978; Greaves *et al.* 1982; Daniel and Nilsson 1987). Another method employed to study the microdistribution of CCA was by electron probe microanalyser (EPMA) (Salamah and Ani 1995).

Studies on the microdistribution of CCA components in wood using SEM and EPMA employed bulk sections or specimen. For more detailed X-ray microanalysis the transmission electron microscope (TEM) in combination of X-ray microanalysis has been used to determine the microdistribution of CCA elements in wood cell walls (Dickinson 1974; Drysdale *et al.* 1980; Newman and Murphy 1996). The use of TEM-X-ray microanalysis provides higher spatial resolution and eliminates the complication encounters with the specimen electron interactions as in normal bulk specimen. However, SEM fitted with X-ray microanalysis has been used to determine CCA microdistribution in the cell wall of wood by employing semi-thin sections (Daniel and Nilsson 1987).

2.4.1 Microdistribution study using bulk samples of hardwoods

In some early works using SEM in conjunction with energy dispersive X-ray analysis (EDXA), Greaves (1974) reported that CCA components were unevenly distributed through different anatomical structures in which preservative was accumulated in vessel of *Eulyptus regnans*, *E. maculata* and *Fagus sylvatica*. A similar instrument was used in the analysis of CCA-treated birch (*Betula* sp.) by Dickinson and Sorkhoh (1976) and found that high levels of chromium (Cr) in vessels and rays together with high levels of copper (Cu) and arsenic (As) in vessels and overall lower distribution of the three elements in fibers. Similarly, in SEM-EDXA analysis of several hardwoods species, Levy and Greaves (1978) noted that individual CCA component was unevenly distributed with the exception of *Alstonia scholaris*. In a more recent study using EPMA, Salamah and Ani (1995) detected high concentration of copper in vessel and ray cells in CCA-treated dark red meranti (*Shorea leprosula*).

It has been shown that X-ray generated from bulk sample is affected by three known factors namely atomic number (Z), absorption (A), and fluorescence (F), or in short ZAF factors. The atomic number correction Z, is concerned with the efficiency with which an element generates X-ray. When an electron enters a specimen it may undergo elastic scattering, in which case it may re-emerge (backscattered) before it has excited any X-rays. Similarly, X-ray may not be generated because the electron may lose energy so rapidly to excite further X-rays as it penetrates deeper into the specimen. Both of these effects are dependent on atomic number, and if left uncorrected will cause error in due to overestimation of X-rays. The absorption effect correction A, adjust for X-rays generated at the surface of the specimen are emitted without loss, whereas X-rays produced from

deeper portion of the specimen have to travel through the specimen and are likely to be absorbed before they can escape to the detector. Absorption corrections take into account numerous factors such as accelerating voltage, take-off angle, mean atomic number and mean atomic mass. However, in thin samples the effect is almost negligible. If correction on absorption is not done it will underestimate the X-rays. The factor fluorescence F, corrects for 'secondary' X-rays. The X-rays produced within the specimen may be of high energy X-rays that are capable of fluorescing lower energy X-ray of another element. The effect causes abnormally high counts in the lower energies. Although generally considered the least important of the three corrections secondary fluorescence can cause errors as large as 15% when analyzing elements in adjacent atomic numbers (Goodhew and Hunphreys 1988).

2.4.2 Microdistribution study using thin samples of hardwoods

Study on the distribution of metal element in wood using SEM-EDXA employed bulk sections. This means that the specimens are always thicker than the depth of electron during irradiation (Daniel and Nilsson 1987). As a consequence, the results will always be affected by electron backscattering, absorption and fluorescence (Williams and Carter 1996; Flegler *et al.* 1993; Russ 1984). Furthermore, SEM-EDXA bulk analysis would not reflect real loading in the S_2 cell wall layer (Ryan 1986) due to the low spatial resolution. However, the SEM-EDXA of bulk analysis is useful in evaluating the penetration pathways of preservative in treated wood.

To overcome the low spatial resolution due to bulk sections, TEM-EDXA is the method use for more detail X-ray microanalytical studies on wood cell wall layer (Daniel

and Nilsson 1987). A single cell wall layer could be analyzed in a thin section with confidence that no X-ray had been generated from outside the region (Ryan 1986). However, SEM-EDXA of semi-thin sections (0.5 μm) have shown to be useful in analyzing the microdistribution of Cr, Cu, and As in wood cell walls (Daniel and Nilsson 1987). Although the spatial resolution with conventional SEM may not approach that of TEM, sufficient resolution can be obtained to analyze many cell wall regions without risk of overlapping 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 provide a convenient alternative to TEM-X-ray microanalysis (Daniel and Nilsson 1987).

In an early study of CCA distribution in cell walls of several hardwoods by TEM-EDXA, Hulme and Butcher (1977) reported that CCA component is well distributed with the exceptions of three *Eucalyptus* species. However, Drysdale *et al.* (1980) using electron microscope micro-analyser (EMMA) fitted with EDXA, reported that CCA levels within the S_2 cell wall layer varies and often low in *Betula alba*, *Fagus sylvatica*, *Alstonia scholaris*, and *Acer psedoplatanus*. Similarly, SEM-EDXA of thin section of CCA-treated birch (*Betula verrucosa*), Daniel and Nilsson (1987) found that fiber and ray S_2 cell wall layers have the least Cr, Cu, and As. It appeared that results from the analyses of thin section for microdisribution of CCA components varies. This is attributed to the differences in the preparation of treated wood, sample preparation for X-ray microanalysis, different species used and the use and capabilities of the electron microscope and X-ray detector itself.

Studies on the microdistribution of CCA components in hardwood cell walls after pressure treatment showed that the middle primary wall region between cell walls and cell corners tends to be better treated than the adjacent S_2 layers (Daniel and Nilsson 1987; Drysdale *et al.* 1980). Drysdale *et al.* (1980) reported that increasing preservative retention did not resulted in proportional increase of preservative in the S_2 layers. Daniel and Nilsson (1987) showed that relative microdistribution of CCA follow closely that of lignin distribution.

An examination of the methods used in X-ray microanalysis to determine microdistribution of Cr, Cu, and As in woods, both in bulk and thin samples, indicate two approaches; X-ray counts in certain time interval and peak:background (P:B) ratios. There are relatively few data that gave microdistribution of CCA components in terms of elemental composition. It is important to quantified the present of Cr, Cu, and As in wood in order to determine preservative threshold and to assist preservative formulation. High or low X-ray counts would not reveal element quantities in treated wood. Similarly, P:B ratios cannot be used for direct comparison between Cr, Cu, and As in different region of cells and cell walls.

CHAPTER 3

SOME CHEMICAL AND ANATOMICAL PROPERTIES OF RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.)

3.1 Introduction

3.1.1 Chemical properties

There are three major chemical components in wood namely lignin (18-35%) and cellulose (40-55%), and hemicellulose (25-40%). There are complex and polymeric materials. Minor amounts of extraneous materials, mostly in the form of organic extractives and inorganic minerals (ash), are also present in wood usually 4-10%. Overall, wood has an elemental composition of 50% carbon, 6% hydrogen, 44% oxygen, and trace amount several metal ions (Fengel and Wegener 1984). The chemical composition of cellulose is similar in all woods but the hemicellulose and the lignin components differ between hardwoods and softwood. The approximate composition of chemical components in both hardwoods and softwood are shown in Table 3.

Table 3. The chemical composition of hardwood and softwood (Haygreen and Bowyer 1996).

Type	Dry weight percent (%)				
	Cellulose	Hemicellulose	Lignin	Extractives	Ash
Hardwood	40-44	15-35	18-25	4-10	2%
Softwood	40-44	20-32	25-35	4-10	<1%

Cellulose is the main structural components in wood cells consisting of D-anhydroglucopyranose units linked by β 1-O-4 glycosidic bonds (Eaton and Hale 1993). The average degree of polymerization (DP) is about 8000-10000 arranged in linear chain.

It exists in the form of microfibrils which are reportedly more or less square in cross-section (ca. 10 x 5 nm) of indeterminate length (Eaton and Hale 1993). The function of cellulose microfibrils is to impart strength to the cell wall. Cellulose is insoluble in most solvents including strong alkali. It is difficult to isolate because it is intimately associated with the lignin and hemicelluloses.

Hemicelluloses are relatively short, branched, and of much lower molecular weight. There are made up of glucose and other hexose and pentose sugar and having DP seldom exceeds 200 (Haygreen and Bowyer 1996; Eaton and Hale 1993). Hemicelluloses exist as a matrix material and appear to contribute as structural components in plant. They are soluble in alkali and easily hydrolyzed by acids.

Lignin is a complex three-dimensional polymer of phenylpropane units, which is completely amorphous and serves as encrusting material surrounding microfibrils. Lignin gives considerable rigidity to the cell wall and because of its hydrophilic properties; its also influences the swelling and shrinkage properties of wood. Lignin can be isolated by several methods. Acid hydrolysis of wood isolates Klason lignin, which can be quantified, but is too severely degraded for used in structural studies. Milled wood lignin procedure (Bjorkman) yields a lignin that is much less degraded and is more useful for structural studies. Lignin for structural studies can also be obtained by enzymatic hydrolysis of carbohydrates.

Extraneous components (extractives and ash) in wood are substances other than cellulose, hemicelluloses, and lignin. They do not contribute to the cell wall structure and most are soluble in neutral solvents. Extractive constitute 4-10% of the dry weight of

wood species that grow in temperate climates and as much as 20% of the wood in tropical species (Eaton and Hale 1993; Pettersen 1984).

Ash is the inorganic residue remaining after ignition at a high temperature. It is usually less than 1% of wood from temperate zones. It is slightly higher in wood from tropical climates (Haygreen and Bowyer 1996).

3.1.2 Anatomical properties

The water-conducting elements in hardwoods are the vessel. Vessels make up of about 20-60% of the total volume in hardwoods, composed of wide, cylindrical cells arranged in vertical columns. Vessels only found in hardwoods and are often called pores. They have relatively thin walls with overall diameter ranging from 25-500 μm (Eaton and Hale 1993). Majority of tropical and many temperate hardwood species, including birch, beech and maple are described as diffuse-porous and exhibit little difference in the size and distribution of the vessels across each growth ring. Other temperate hardwood species are classifies as ring-porous such as ash, elm and oak. There produce prominent large-diameter vessels in the earlywood and small vessels in the latewood of successive growth rings. Some species are known as semi-ring porous, showing large-diameter pores in the earlywood but a more gradual decrease in size through to the latewood region of the growth ring.

In hardwoods, the main strength of the timber is provided by fibers. Fibers make up 15-60% of hardwoods volume and are with narrow cells with finely tapering end. Fiber cells can be more than 1.5 mm with thick cell wall surrounding the narrow central

cell lumen. The overall diameters of fiber cells are normally between 20 and 40 μm (Haygreen and Bowyer 1996; Eaton and Hall 1993).

Parenchyma is often seen in the transverse sections as lines or bands running more or less at right angles to the rays. They make up 0-24% of the hardwood volume. In some hardwood species parenchyma form a sheath surrounding the vessels and is known as paratracheal parenchyma. Parenchyma that is not associated with vessels is known as apotracheal.

Hardwood rays are much more variable in form than softwood rays and occur as uniseriate and also multiseriate aggregation of cells. Rays consists of 5-30% of the total hardwood volume. They show considerable variation in height. Rays in hardwoods divided into two types. The upright cells are found at the upper and lower margin of the ray; procumbent cells make up the remainder of the ray and more or less brick-shaped with longest diameter oriented radially (Haygren and Bowyer 1999; Hoadley 1993). Hardwood rays are described as homocellular when only procumbent cells are present or heterocellular when both cell types occur in the ray.

In softwood 90-95% of the total wood volume is make up of tracheids. Tracheids can be up to 5 mm long or even longer in some species. The tangential diameter of tracheids ranges from 20 and to 70 μm but the radial diameter are variable depending on their position in the annual ring and are quite narrow in the latewood zone (Hoadley 1990). In softwoods, the strength of the wood is derived largely from latewood tracheids. The latewood tracheids are composed of thick-walled cells with small lumina. The wide diameter and thin-walled tracheids in the adjacent earlywood zone in softwoods function as water conducting and nutrient transfer in living trees. Rays make up the rest of the 5%

wood volume in softwood. Generally they are one cell wide or occasionally biseriate extending from 1-15 and sometimes up to 50 cells in height (Hoadley 1990)

The objectives of this study are to (1) determine the chemical component of rubberwood and (2) investigate the anatomical structures that are important to liquid transport in rubberwood.

3.2 Materials and Methods

3.2.1 Chemical analysis

Rubberwood samples were cut into small pieces and milled (Wiley) to pass 20-mesh. The sample was chemically analyzed for ash, extractives, Klason lignin, cellulose and hemicellulose content. Ash content was determined using TAPPI T 211 om-85 (1992) procedure. Extractives content were determined based on TAPPI T 264 om-88 (1992) 'Preparation of wood for chemical analysis' procedure. To determine the amounts and types of extractives approximately 2 g of oven dried-milled rubberwood was placed in a thimble and successively extracted with ethanol-toluene (1:2 by volume), 95% ethanol, and water in a soxhlet extractor. Each extraction time was about 10 hours. Total extractives were determined on the weight residue remaining after the final extraction with water. The residue was placed into a crucible and oven dried to constant weight and placed into a desiccator prior to final weighing.

Klason lignin was determined using TAPPI T 222 om-88 (1992) procedure. One gram of dried extractive free rubberwood was treated with 72% sulfuric acid, for Klason lignin determination. Holocellulose content was determined by Ona's *et al.* (1995) procedure. Three grams of extractive free rubberwood samples were treated with 20%

sodium chlorite. The residues was filtered and washed prior to drying. To determine the amount of α-cellulose, 1 g of hollocellulose was treated with 12% potassium hydroxide. Then the residues was filtered, washed, and dried prior to weighing. Hemicellulose was determined by subtracting hollocellulose content by α-cellulose obtained from the 12% potassium hydroxide treatment.

3.2.2 Light and confocal laser scanning microscopy (CLSM)

Rubberwood samples were cut into 1 cm cubes. Prior to sectioning, the cubes were softened by boiling in water for 48 hours. The transverse, radial, and tangential sections were cut approximately to 20 μm thick using a sliding microtome. The sections were stained with 1% Safranin-O and mounted permanently in resin. All three sections were examined and photographed with both brightfield and confocal laser fluorescence using Carl Zeiss confocal laser scanning microscope LSM 210. Fluorescence of the sections was observed using a 488 nanometers (nm) argon ion laser line for excitation and 520 nm long phase emission filters. Three transverse sections were used to measure vessel diameter and frequency. Ten vessels were measured for diameters from each transverse section. The number of vessels per square millimeter was determine by counting individual vessels present in a field of 1 x 1 mm. From each section five fields were measured.

3.2.3 Scanning electron microscopy (SEM)

Rubberwood were cut to 2 x 2 x 10 mm specimen with 10-mm dimension in the radial direction for SEM examination. The transverse, radial, and tangential surfaces of

the specimens were prepared by a razor blade (Exley *et al.* 1974). The prepared surface specimens were dried in an oven of 60°C for 24 hrs. Dried specimen were mounted on aluminum stubs and coated with approximately 28 nanometers (nm) of gold in Emscope SC500 sputter coater.

Small matchstick samples of 0.5 x 0.5 x 10.0 mm were cut. The samples were infiltrated and embedded in Spurr's resin. Selected embedded samples were sectioned using ultramicrotome to obtain semi-thin section of 0.5 μm thick. The sections were mounted on copper grid. The semi-thin sections were used to examine the size of middle lamella, fiber pits, cell corners and the cell wall thickness vessels, fibers, parenchyma, and rays. The samples were examined in a JEOL 6400V scanning electron microscope using a accelerating voltage of 20 kV.

3.3 Results and Discussion

3.3.1 Chemical constituents of rubberwood

The results of chemical studies are summarized in Table 4. The average hollocellulose yield, corrected for extractives and ash content, was 77.5%. The yield obtained for hollocellulose is comparable to those of other hardwoods ranging from 69 to 80% (Ona *et al.* 1995). Potassium hydroxide extraction for α-cellulose yield about 40.3% and by subtraction, hemicellulose is approximately 37.2%. Comparison of hollocellulose data available for rubberwood is limited. In a chemical analysis of rubberwood pulp, Peel and Peh (1960) reported a hollocellulose yields of 70%, α-cellulose 40%, and pentosan content of 20%. In wood cellulose exist as in the form of microfibrils and it is the main structural component of wood cell walls.

The Klason lignin yields was about 18.5%. This compares well with result showing 15.9 to 22.9% of Klason lignin in various hardwoods (Ona *et al.* 1995).

Total extractives content is about 6%. Ethanol-toluene extractives yield about 4.7% and water-soluble extractives were about 1.3%. Extractives are a variety of organic compounds including fats, waxes, alkaloids, proteins, simple and complex phenolic, simple sugars, pectins, mucilages, gums, resins, terpenes, starches, glycosides, saponins, and essential oil (Pettersen 1984). Many of these functions as intermediates in tree metabolism, as energy reserves, or as part of the tree's defense mechanism against microbial attack (Haygreen and Bowyer 1996). They contribute to wood properties such as color, odor, and decay resistance (Eaton and Hale 1993; Hoadley 1990). Ash content is about 1%.

Table 4. Chemical composition of rubberwood.

Chemical constituents	Content (%)
Hollocellulose	77.5 ±0.7
Hemicellulose	37.2 ±0.6
α-cellulose	40.3 ±0.9
Lignin	18.5 ±0.8
Extractives	6.0 ±0.5
Ethanol-toulene	4.7 ±0.2
Hot-water	1.3 ±0.2
Ash	1.0 ±0.01

In general, cellulose microfibrils make up the backbone structure of the wall and that hemicellulose and lignin exist as matrix materials around the microfibrils (Eaton and hale 1993; Haygreen and Bowyer 1996). The individual chemical components vary widely across cell walls. The distribution of wood chemical components can be generalized as follows (Fengel and Wegener 1984); cellulose is concentrated in the

secondary walls and is least abundant in the middle lamella and cell corner. Hemicellulose concentrations are highest in the S_1 and lowest in the S_2 of secondary wall of tracheids and fibers. The hemicellulose types and distribution vary greatly among species. High levels of lignin present in the cell corner, middle lamella, and primary wall and lower but relatively uniform distribution in the secondary wall. However, because of the thickness of the secondary wall 70 to 80% of lignin occurs in the secondary wall (Eaton and Hale 1993).

3.3.2 Microscopic features of rubberwood

The shape of vessels or pores was round to oval, mostly in a group of 2 to 4 and with tangential diameter (widest point) ranging from 150 to 300 μm (Figure 2). Vessels are diffuse, with average frequency of 3-4 vessels/mm^2, and sometimes filled with tyloses. The pores have simple perforation plates (Figure 3). Intervessel pitting arrangement is scalariform and sometime opposite and is about 20-30 μm in diameter. The cell wall of the vessel varies from 2.5 to 8 μm thick. The thickest part of the cell wall is usually when the vessel cell walls are adjacent to each other while the thinnest are when the vessel cell walls are adjacent to other cell. Vessel consists of about 9.5% of the total rubberwood structural volume (Nagoshi 1970).

Fibers have thick cell wall ranging from 4 to 7 μm and lumen width from 10 to 20 μm (Figure 4). Fiber pits are confined to radial walls and approximately 0.3 to 0.5 μm in diameter (Figure 5). Fiber makes up the bulk of rubberwood and it was estimated that 61.5% of rubberwood volume is made up of fibers (Nagoshi 1970).

Parenchyma consists of both apotracheal and paratracheal (Figure 2). Apotracheal parenchyma appears as irregularly spaced bands, joining ray to ray to form a net-like pattern (Figure 2). Close examination of apotracheal parenchyma show that they are diffuse-in-aggregates and discontinuous lines up to 3 cells wide. Paratracheal parenchyma is sparse and adjacent to the vessels. Cell wall of parenchyma is about 2.5 – 3.5 μm thick and is the thinnest cell. In rubberwood parenchyma is about 29% of the total rubberwood volume (Nagoshi 1970).

Rays are moderately fine and visible to the naked eye. They are heterogeneous, with 1 to 4 rows of square upright cells and 8 to 12 procumbent cells. Rays are mostly about 0.5 mm in height but can reach up to 0.8 mm. The cell wall of ray ranged from 2.5 to 4 μm thick. Close examination of parenchyma and ray cells shows that they contain considerable amount of starch grains (Figures 6 and 7). Azizol and Sudin (1989) reported that rubberwood contains high starch reserves deposited in the parenchyma cells. According to Esau (1977) starch are products of cell metabolism and deposition occurs commonly in the parenchyma of stem tissue.

The middle lamella and cell corners of rubberwood are measured based on semi-thin sections as examined by SEM (Figure 8). The examination of semi-thin sections by SEM gave a high resolution that enables the cell walls, middle lamella, and cell corners to be measured, but not high enough to resolve the S_1 and S_3 cell wall layers. Measurements on middle lamella showed that it ranges from 1 to 1.8 μm while cell corners is between 2 and 3.5 μm wide.

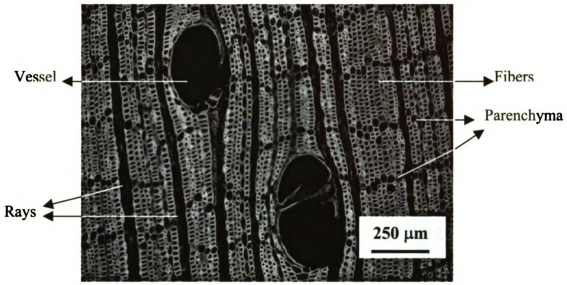


Figure 2. CLSM image of transverse section of rubberwood.

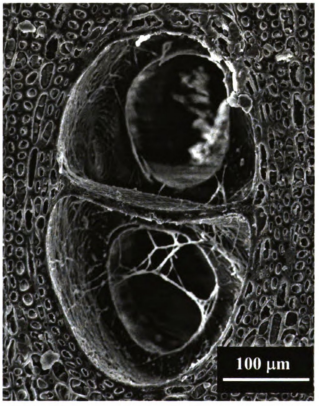


Figure 3. SEM micrograph showing simple perforation plate in vessels.

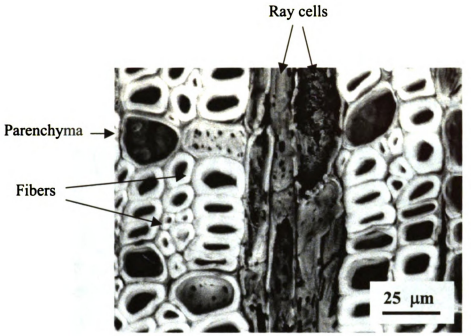


Figure 4. CLSM image of transverse section of rubberwood showing relative cell wall thickness of fiber, parenchyma, and ray cells. The dark spots in ray and parenchyma cell represent the pits.

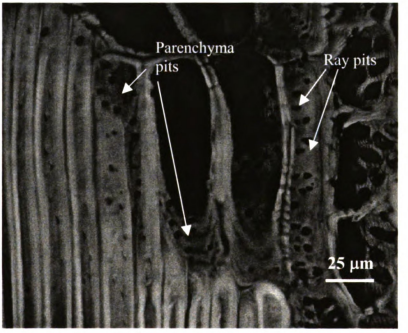


Figure 5. CLSM image of radial section of rubberwood showing pits of fiber, parenchyma, and ray cells.

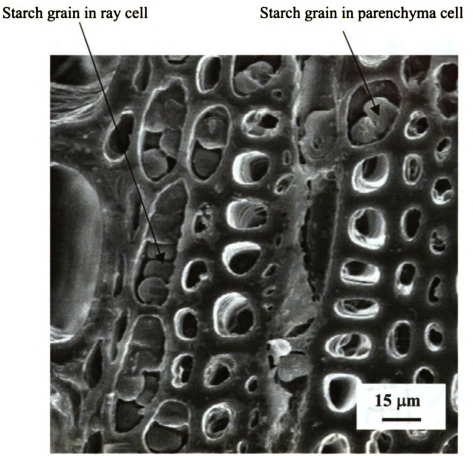


Figure 6. SEM micrograph showing starch grains in ray and parenchyma cells.

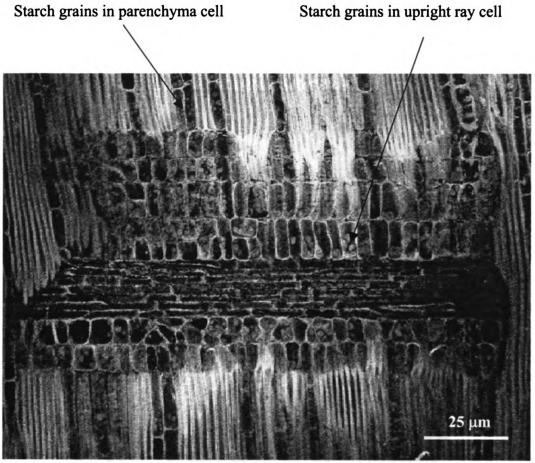


Figure 7. CLSM image showing starch grains in parenchyma and upright ray cell.

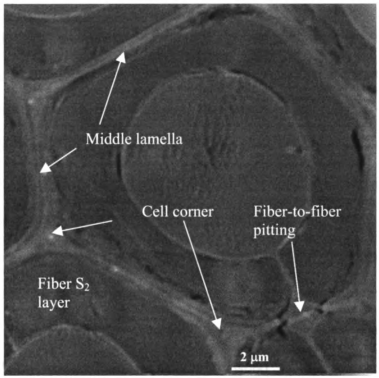


Figure 8. SEM micrograph of transverse section of rubberwood showing S_2 layer of fiber, middle lamella, cell corner, and fiber-to-fiber pitting.

CHAPTER 4

PENETRATION AND RETENTION OF CCA IN RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.)

4.1 Introduction

The importance of Rubberwood in Malaysia was realized only two decades ago when the supply of traditional forest timbers declined and pushed the price high make it unaffordable for the furniture manufacturers. The properties and utilization of rubberwood have been studied since the 1950's, but the commercial utilization of wood only began in the late 1970's (Hong *et al.* 1994). Rubberwood is primarily used for furniture making due to its, light and even texture, suitable strength, good machining and processing properties (Hong and Sim 1994, Tee 1990). Rubberwood is also extensively used for manufacturing particleboard, blockboard, medium density fiberboard and cement-bonded particleboard (Hong and Sim 1994). Despite the rapid expansion of the rubberwood industry, about 67% of the total rubberwood obtained from rubber plantations are still used as fuelwood (Lew 1992).

Rubberwood is classified as non-durable and does not produce visible heartwood (Wong 1988, Jantan and Tam 1987, Thomas and Landon 1953). Rubberwood is highly susceptible to sapstain, mold and decay fungi (Tsunoda *et al.* 1983, Wong 1988). The current use of rubberwood for making furniture and related products does not warrant protection against decay fungi.

Forest land area in Southeast Asia has been in continual decline due to agricultural conversion and urbanization. Thus, the future wood supply will increasingly rely on forest plantations. The cost of establishing forest plantations is high, but some 9

million hectares of existing rubber plantations in Southeast Asia could produce 19 million m^3 of wood annually (Ser 1990). The supply is sustainable and is relatively low cost as it is a by-product of rubber industry (Ismariah and Norini 1994). The utilization of rubberwood can supplement the wood supply.

Using rubberwood for general outdoor application will depend on the ability to effectively protect against decay and insect attack. Copper-chromium-arsenic (CCA) type C wood preservative is a cheap and broad-spectrum pesticide that leaves wood paintable after treatment. Treatability of wood with CCA type C is influenced by its permeability, which is a function of anatomical structure (Nicholas and Siau 1973).

Permeability in the longitudinal direction is usually several thousand times greater than in the transverse direction due to the vessels and fibers, and the pits arrangement on the cell walls (Nicholas and Siau 1973). Hardwood permeability is generally higher in the radial than in tangential direction although there are some exceptions (Hunt and Garrat 1953, Rudman 1965, 66). Variability in transverse penetration may be attributed to the proportion of ray parenchyma (Siau 1984) and the presence or absence of aspirated pits. A pre-treatment steaming may help in dissolving water soluble deposits in ray parenchyma or weakening the adhesion between the pit membrane and overarching border in the aspirated pits. The objective of this study was to determine the influence of anatomical orientation, pre-treatment steaming and pressure duration on the treatability of rubberwood.

4.2 Materials and Methods

4.2.1 Wood samples

Samples of sodium pentachlorophenol (NaPcP)-treated and kiln-dried rubberwood (50 x 50 x 800 mm) from a 30-year-old Malaysian plantation were quarter-sawn to 35 x 35 x 125 mm defect-free and uniform grain wood blocks. A set of 75 end-matched blocks was prepared for modified full-cell treatment and another set of 25 end-matched blocks was used for pre-treatment steaming. The blocks were labeled and conditioned to constant weight at 20°C and 70% relative humidity. The average moisture content (MC) before treatment was 10 ± 2% and average air-dry density was 670 kg/m^3 (41.8 lb/ft^3).

4.2.2 Pre-treatment steaming

A set of 25 end-matched blocks was autoclaved at 250°F (120°C) for 4 hours and then conditioned for 10 to 14 days until the MC was about 10 ± 2%.

4.2.3 Anatomical direction

Selected block surfaces of pre-treatment steamed and unsteamed blocks were sealed with SCS 1200 GE Silicon Rubber Adhesive Sealant to permit chemical penetration only in one or two anatomical directions. Unseal blocks allows preservative penetration from all directions. End-seal blocks allow both tangential and radial penetration while end- and radially-seal blocks expose only the tangential surface. End- and tangential-seal blocks expose the radial surface permitting penetration in the

tangential direction. Sealing all four edges and leaving the end or the transverse surface expose allows longitudinal penetration. Five replicates were used for each surface.

4.2.4 Pressure treatment

Pressure treatment of blocks used a modified full-cell process. The treating schedule included an initial vacuum of 25 in Hg for 30 minutes followed by 1240 kPa (180psi) pressure for 1, 2, or 4 hours, and a final vacuum of 85 kPa (25 in Hg) for 10 minutes. A 50% stock solution of CCA-type C from Hickson Corporation was diluted to 2% CCA. The actual concentration of CCA was determined by Atomic Absorption Spectroscopy (AAS) and the oxides was calculated according to AWPA Standard A2-96 (1998). The concentration of CrO_3, CuO, and As_2O_5 used were 0.90, 0.41, and 0.66%, respectively. For pre-treatment steamed blocks, the duration of pressure applied was 1 hour.

4.2.5 Determination of CCA retention

Each block was weighed before and after treatment to determine preservative uptake. The retentions were determined on the basis of net weight gain after treatment, using the actual solution concentration, the volume of the block according to the following expression

$$R = \frac{GC}{V} \times 10$$

Where R is the retention in kg/m^3, G the net weight gain after the treatment in grams, and C the concentration of CCA solution in percent, and V the volume of block in cm^3.

The CCA retention was determined in the assay zone of the outer 13 mm-thick portion of the blocks. The chemical analysis was performed according to AWPA Standard A7-93 (1998). The retentions of chromium, copper and arsenic were expressed on oxides basis then converted from a weight percent to kg of preservative per m^3 wood based on the measured density.

4.2.6 Determination of CCA penetration

CCA-treated blocks were air-dried for 14 days before been then cut in two halves. The freshly cut surfaces were sprayed with 0.5% chrome azurol-S to reveal the presence of copper at ≥ 25 ppm concentration (AWPA Standard A3-97, 1998). The penetration of depth of copper in mm was obtained by measuring the depth of the colored area with a digital caliper.

The penetration of CCA was assessed according to its penetration direction. The radial and tangential penetrations were determined by measuring preservative penetration at right angle to the block edge or face. The maximum penetration for both directions was set as the half of the width or length of the treated blocks.

The percentages of preservative penetration in each block were measured by tracing the penetration pattern on tracing paper and redrawing the pattern on graph paper. The areas covered by the penetration pattern over the total cross-sectional area of each block were expressed as percentage.

4.3 Results and Discussion

4.3.1 Retention and penetration

Average values of retention, maximum penetration and percentage rating of cross section penetrated for rubberwood are listed in Table 5. Observations of penetration patterns of unsealed blocks due to pressure show that some sections were penetrated completely. Others were penetrated only in the outer shell, and some had patchy penetration. Full penetration of unsealed wood blocks was achieved by applying 4 hours of pressure and the retention (13.3 ± 0.6 kg/m^3) was well above that recommended for most applications as specified in the Malaysia Standard MS 360 (1991).

Hong *et al.* (1982) reported that complete penetration of CCA was achieved in 5 cm thick of rubberwood board with an average retention of 16 kg/m^3 after 2 hours of full-cell treatment. The concentration of the CCA treating solution used was not reported. Hiziroglu (1997) reported CCA retention of 32.6 kg/m^3 for CCA in rubberwood using a 5% CCA treating solution in a 4-hour treatment schedule at room temperature with 2 hours of pressure. Higher CCA retention may be achieved with increase concentrations although special care should be given to the viscosity and the fixation rate of chromium in such treatment. Treatment parameters such as duration and level of initial and final vacuum, concentration of CCA, and pressure level and duration may significantly affect the retention and penetration of preservatives.

Increased in retention and penetration values with increase pressure duration suggests that pressure duration is an important parameter in the treatment of rubberwood. Acidic solutions like CCA with pH varying from 1.5 to 3.0 can hydrolyze hemicellulose

Table 5. The effect of pressure duration on the retention and penetration in rubberwood treated with 2% CCA-C solution.

	Pressure	Fluid pathway				
		All direction	Radial and tangential	Radial	Tangential	Longitudinal
Average retention (kg/m^3)	1 hr.	9.9	9.3	7.0	2.2	9.5
	Pre-steam/1hr.	11.1	10.1	9.3	3.8	11.4
	2 hr.	13.0	10.9	10.1	4.2	10.4
	4 hr.	13.3	11.0	10.7	5.1	11.6
Average maximum penetration (mm)	1 hr.	5.8	4.4	4.2	2.0	23.3
	Pre-steam/1hr.	15.5*	15.5*	12.2	5.8	58.5
	2 hr.	10.4	9.2	9.4	3.8	42.5
	4 hr.	15.5*	13.2	15.0	6.8	60.0
Average percentage of cross section penetrated (%)	1 hr.	68.0	44.4	15.0	6.0	24.7
	Pre-steam/1hr.	91.6	90.8	43.2	28.0	91.7
	2 hr.	69.0	63.8	40.2	15.6	57.3
	4 hr.	100.0	79.0	63.6	25.2	97.0

* Indicates full penetration

(Winandy *et al.* 1983). Kamdem and Chow (1999) reported a 33% strength reduction in CCA-treated hardwoods, which was attributed to the hydrolysis of hemicellulose.

The effect of anatomical direction on penetration and retention was obvious (Table 5). Copper penetrated deeper in longitudinal direction than in the radial and maximum longitudinal penetration was achieved after 4 hours of pressure. The cross-sectional coverage was about 97%. Complete penetration and cross-sectional coverage was observed in longitudinally penetrated blocks treated for 4 hours.

Penetration in longitudinal direction was almost identical to that of unsealed blocks indicating that the longitudinal penetration was the dominant flow path in rubberwood. In hardwood species, without tyloses, penetration in the longitudinal direction is usually greater than in the radial and tangential direction because vessel elements provide a flow path (Nicholas and Siau 1973; Greaves 1974; Siau 1984).

Transverse direction penetration results showed that radial and tangential permeability were not similar. Retention and penetration in the radial direction was about 2 times greater than in the tangential direction (Table 5). Retention and penetration values in the radially penetrated blocks were similar to those of end-sealed blocks, indicating that radial penetration is more important than tangential penetration in rubberwood.

Radial permeability is generally attributed to the ray cells (Nicholas and Siau 1973; Siau 1984). The role of rays as penetration pathways in hardwoods varies. Hunt and Garratt (1953) stated that ray cells in hardwoods do not assist penetration and suggested that lateral movements are carried out by parenchyma. Rudman (1965, 1966) observed that liquids of different polarities did not penetrate ray parenchyma of Eucalyptus. Other studies have shown that hardwood rays are penetration pathways.

Hayashi *et al.* (1967) mentioned that rays of three Japanese hardwoods achieve good penetration. Greaves (1974) found that ray tissues play a significant role in the distribution of CCA in *Fagus sylvatica* , *Eucalyptus regnans* and *E. maculata.* A more recent study but Maturbongs and Schneider (1996) showed rays of 10 non-durable Indonesian hardwoods are well penetrated by CCA after pressure treatment.

CCA retention in the 0 to 13 mm assay zone of end-sealed blocks and function of the pressure duration during the treatment are listed in Table 6. End-sealed samples were

Table 6. The effect of pressure duration on the average chemical retention in the assay zone (0 – 13 mm) of end sealed rubberwood samples.

Pressure duration	Chemical retention (kg/m^3, oxides basis)*
1 hr.	8.4
Pre-steam/1hr.	10.2
2 hr.	11.8
4 hr.	15.8

* The chemical retention was obtained by multiplying the measured density by the percent oxide determined by using AAS (AWPA Standard A7-93, 1998).

used in assaying the CCA in the outer 0 to 13 mm in order to minimize the longitudinal penetration and exhibit a transverse penetration. The Malaysia Standard MS 360 (1991) on pressure treatment of hardwood lumber with CCA specifies retention of 8 kg/m^3 and 12 kg/m^3 in the 0 to 12 mm assay zone for C5 and C4 commodity for above ground and soil contact, respectively. The retention value of 15.8 kg/m^3 CCA oxides for end-sealed blocks after 4 hours pressure treatment satisfies the retention requirements for C4 commodity (MS 360 1991). C4 commodity is for ground contact application such as railway sleepers, bridge decking, fence and gardening posts and stakes.

One-hour pressure duration was sufficient to satisfy the C5 commodity requirement. Thus a 4 hours pressure treatment may be unnecessary. Two hours of pressure resulted in 11.8 kg/m^3 CCA retention in the 0 to 13 mm assay zone, satisfying the C5 and C4 requirements. Subjecting rubberwood to pressure treatment for more than 2 hours and less than 4 hours would result in satisfying C4 and C5 requirement (MS 360, 1991).

4.3.2 Effect of pre-treatment steaming of wood blocks

Full penetration was observed in unsealed and end-sealed blocks. The penetration was improved by about 2.9 times for radial and tangential direction. The CCA retention was improved from 9 to 73% depending on the direction of penetration. The chemical retention was improved by 21% in end-sealed blocks (Table 6).

Pre-treatment steaming improved longitudinal permeability by 150%. It was reported earlier that longitudinal permeability was increased in black walnut after steaming at 210°F (100°C) prior to treatment (Chen 1975). In pre-treatment steamed blocks, CCA penetrated deeper in both radial and tangential direction. The pre-treatment steaming appears to improve lateral penetration. Retention was increased by 33% in radially and 73% in tangentially penetrated samples. Increase in lateral penetration after pre-steaming of wood was also observed in red oak (Simpson 1976). The chemical retention was also increased by pre-treatment steaming (Table 6).

The mechanism responsible for increase permeability after steaming is not well known. Simpson (1976) suggested that pre-treatment steaming of the wood caused the cell walls and/or pit membrane to crack or check, and help in removing the encrusted

material in the pit membrane. Chen and Workman (1980) also mentioned the increase in permeability caused by the removal of the encrusted material during pre-treatment steaming. Their study showed that during steaming some of the water-soluble extractives were removed and resulted in improved permeability of black walnut.

Nicholas and Thomas (1968) suggested that steaming affected pit aspiration. The aspirated pits after a pre-treatment steaming formed weaker bond between torus and cell wall, resulting in improved treatability. Further study is underway to assess the detail effect of pre-steaming parameters such as different pre-steaming duration, pre-steaming temperature, and pressure duration. The assessment on the effect of pre-steaming on the wood mechanical and physical properties is necessary since pre-steaming can impact the mechanical properties of wood.

4.3.3 Gradient retention of Cr, Cu and As

Average elemental retention values for outer and core sections of end-sealed block are presented in Table 7. The amount of Cr, Cu, and As retained in the core section was less than outer section. However, result of pre-treatment steamed and unsteamed blocks did not show much differences in the retention of Cr, Cu, and As between the outer and core section. The average Cr, Cu, and As retention for outer pre-steamed blocks are 2.3 kg/m^3, 1.8 kg/m^3, and 2.3 kg/m^3, respectively. Comparable values were obtained for pre-steamed core samples.

The retention trend of Cr, Cu, and As between outer and core sections are illustrated in Figure 9. The retention of Cr, Cu, and As of pre-treatment steamed blocks are higher than and unsteamed blocks. In the core zone, an apparent improvement in the

retention of Cr, Cu, and As in pre-treatment steamed blocks can be observed. Retention of Cr, Cu, and As in pre-steamed blocks of treated with 1 hour pressure surpasses the retention values in unsteamed blocks treated with 2 hour pressure. This further emphasized the improvement of CCA penetration and retention in pre-treatment steamed rubberwood blocks.

Table 7. Average retention of Cr, Cu, and As in the outer (0-13) zone and core of end-sealed 2% CCA-treated rubberwood blocks.

Pressure duration	Outer (kg/m^3)			Core (kg/m^3)		
	Cr	Cu	As	Cr	Cu	As
1 hr.	1.9	1.4	1.9	0.7	0.5	0.7
Pre-steam/1hr.	2.3	1.8	2.3	2.1	1.7	2.1
2 hr.	2.8	1.8	2.7	1.8	1.2	1.8
4 hr.	3.8	2.5	3.4	2.8	1.8	2.5

CCA retention and penetration increase as the pressure duration increased. Full penetration of unsealed wood blocks was achieved by applying 4 hours of pressure. It was reported that using two hours of pressure full penetration of CCA was achieved in 5 cm thick rubberwood samples. However, this study showed that two hours of pressure resulted in only about 63% of the cross section penetrated with CCA in the end sealed samples of 3.5 cm thick samples.

Retentions in the unsteamed rubberwood blocks treated with different pressure duration were usually lower than pre-treatment steamed blocks. Pre-treatment steaming resulted in the improvements of Cr, Cu, and As retentions in the outer and core zone of rubberwood blocks.

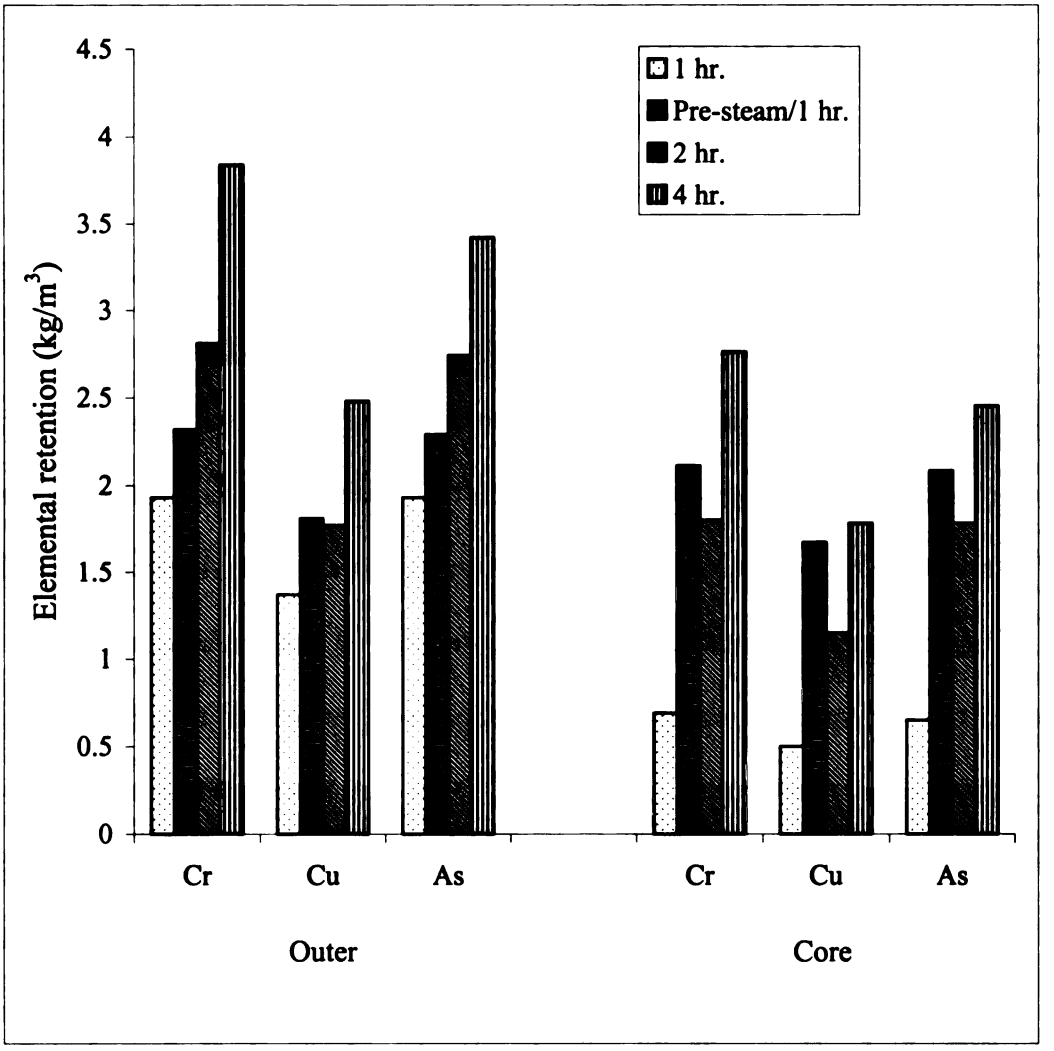


Figure 9. Average Cr, Cu, and As retention by outer and core zones of 2% CCA-treated rubberwood.

CHAPTER 5

LABORATORY EVALUATION OF THE NATURAL DECAY RESISTANCE AND THE EFFICACY OF CCA-TREATED RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG).

5.1 Introduction

The opportunity for increasing the utilization of rubberwood is highly dependent on the efficacy of the preservatives that will protect against biodegradation. Currently CCA preservative has been shown to be effective in protecting some Malaysian hardwoods species such as *Koompassia malaccensis*, *Dipterocarpus* spp., *Shorea leprosula*, *Shorea talura*, and *Drypbalanops* spp. for exterior use (Jantan *et al.* 1994; Aston 1985). Few studies were done on the natural decay resistance and the performance of CCA-treated rubberwood.

The retention and penetration of CCA in rubberwood was established (Chapter 4). The retention and penetration are established as function of protection. CCA-treated cubes were tested against pure monoculture of *Irpex lacteus*, *Trametes versicolor*, *Gloeophyllum trabeum*, *Postia placenta*, *Chaetomium globosum* and *Phialophora* sp. after 12 weeks of incubation, using laboratory soil block test.

5.2 Materials and Methods

5.2.1 Sample preparation

Six 30-year-old rubber trees from a Malaysian plantation located at Kota Samarahan, Sarawak were felled. The logs were sprayed with sodium pentachlorophenol (NaPCP) to protect against mould and insect attacked. The logs were quarter-sawn to 50

x 50 x 800 mm boards and kiln-dried prior to shipment to East Lansing, Michigan, USA. No attempt was made to separate the wood into sapwood and heartwood since the volume of heartwood in rubber trees is negligible. The boards were further planed, ripped and cut into 19 mm cubes. The cubes were conditioned at 20°C and 70% relative humidity until they reached a constant weight. The average moisture content (MC) and the air-dried density of the cubes before treatment were averaged at 6 ± 2 % and 670 kg/m^3, respectively.

5.2.2 Sample treatment

A 50% stock solution of CCA type C (CCA-C) obtained from Hickson Corporation, Conley, Georgia was diluted with water to 1%, 2% and 3% total oxides and used for treatment. The actual strength of CCA solution (total oxides) was determined by using Atomic Absorption Spectroscopy (AAS). The cubes were treated with the three different CCA solution strengths via a modified full-cell process. The control cubes were pressure-treated with distilled water. The treatment schedule involved an initial vacuum of 85 kPa for 30 minutes followed by 1240 kPa of pressure for 1 hour and a final vacuum of 85 kPa for 10 minutes. Treated samples were blotted with paper towel and weighed to determine the weight of the treating solution absorbed. Treated samples were stored in conditioning room kept at 20°C and 68% relative humidity for 4 weeks.

After 4 weeks of drying in the conditioning room, the cubes were then conditioned at 40°C in an oven to a constant weight. The treated and conditioned cubes were weighed prior to the sterilization of the cubes. Cubes were sterilized by using gamma irradiation according to the procedures describe in AWPA E10-91 (1999).

5.2.3 Chemical analysis

Chromium, copper, and arsenic concentrations in treated cubes were determined with atomic absorption spectrometer (AAS). Wood samples were digested in perchloric acid according to the AWPA 97-93 (1999) standard. Chromium, copper, and arsenic concentrations in the digested sample solution were determined by AAS. The retentions of chromium, copper, and arsenic, expressed as oxides, were calculated based on measured density of the treated samples in kg/m^3. Table 8 contains the CCA retention determined by weight difference and chemical analysis of CCA-treated rubberwood blocks.

Table 8. Average CCA and copper retention from CCA-treated rubberwood blocks.

CCA solution strength determined by AAS (%)	CCA retention by weight gain (kg/m^3)	CCA retention in wood by AAS (kg/m^3)	Copper retention in wood by chemical analysis and AAS (kg/m^3)
Control (untreated)	0	0	0
0	0	0	0
0.93	5.18	4.12	0.97
2.12	13.24	10.54	2.68
3.29	20.90	14.48	3.57

5.2.4 Fungal exposure

The cubes were evaluated for resistance to attack by the white-rot decay fungi *Irpex lacteus* Fries (FP-105915) and *Trametes versicolor* (L. ex Fr.) Pilat (R-105), the brown-rot fungi *Gloeophyllum trabeum* (Pers. Ex Fr.) Murr (Madison 617) and *Postia placenta* (Fries) M. Larsen et Lomb.(Madison 698), and the soft-rot fungi *Chaetomium globosum* Kunze (ATTC 32237) and *Phialophora* sp. (ATTC 66725) by a slightly

modified soil-block test as described in AWPA standard method E10-91. Polycarbonate boxes of 400 ml volume from Sigma Inc. were filled with 95 g of forest soil of about 70 percent MC. The forest soil was first screened for roots and other wood debris. Then the soil clumps were broken into particles and passed through No. 6 sieve and stored in plastics bag.

An aspen feeder strip measuring 3 x 25 x 30 mm was placed above the soil in each box and then the boxes were autoclaved for one hour at 121°C. The boxes were then inoculated with an agar plug cut from the edge of actively growing colony of the appropriate test fungus. Culture boxes were incubated until the fungus covered the feeder strips. Boxes with vigorous fungus growth without contamination were selected for the soil-block test. Treated and conditioned wood cubes were weighed prior to sterilization using gamma irradiation. Sterilized cubes were aseptically placed on the feeder strip with end exposed to the feeder strips. Twelve sterilized cubes from each treatment were used for each decay fungus. Untreated and distilled water-treated cubes were included for reference. Sterilized reference and treated cubes were placed in uninnoculated culture boxes to determine the operational weight loss of the samples. The cubes were incubated at 25°C and 75% relative humidity for 12 weeks.

5.2.5 Determination of weight loss

The degree of fungal attack was estimated by determining the weight loss after 2, 4, 6, 8, 10, and 12 weeks. Test cubes were removed from the boxes and the mycelium was wiped with soft foam from the cube surfaces. Every two weeks five replicates per sample treatment and per decay fungus were removed and their MC and weight loss were

determined. The clean cubes were dried in an oven set at 40°C to a constant weight. The weight loss due to decay was calculated and expressed in percentage as follow:

$$\text{Weight loss (WL), \%} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Where;

Initial weight = Weight of conditioned cube prior to fungal exposure.
Final weight = Weight of conditioned cube after fungal exposure.

To determine the cube MC after decay, the cubes exposed for 2, 4, 6, 8, 10, and 12 weeks were weighed immediately after removal from culture boxes. The MC of each cube was calculated as:

$$\text{Moisture content (MC), \%} = \frac{\text{Weight of decay cube from box - Final weight}}{\text{Final weight}} \times 100$$

Where;

Weight of decay cube from box = Weight test cube immediately after removal from box and after mycelium has been wiped.
Final weight = Weight of conditioned cube after fungal exposure.

All data were subjected to statistical analysis.

5.2.6 Statistical analysis

Simple linear regression analysis was conducted to determine the relationship between the weight loss and the incubation duration for each fungus. Covariance analysis was used to determine whether the difference was statistically significant among the

coefficients. The incubation duration was used as covariate for the analysis of covariance of the weight loss.

One-way ANOVA was used to compare and determine any significant difference of weight loss and the CCA retention of treated samples exposed to a laboratory soil block test. Tukey multiple comparison tests were used to further analyze the variation of weight loss between treatments of different fungi using SYSTAT (SYSTAT 1996).

5.3 Results and Discussion

5.3.1 Relation between moisture content and weight loss

Fungus colonization of wood is reported to occur at moisture content around 20% (Zabel and Morell 1992; Eaton and Hale 1993). The optimal moisture levels for growth of most decay fungi in laboratory evaluation lie between 40 and 80% (Scheffer 1973). The moisture content in untreated test cubes exposed to different fungi for 12 weeks show different trend for different decay type as shown in Table 9.

The moisture content in cubes exposed to white and brown rot fungi are in the range of 44 to 87%, in agreement with Scheffer (1973). The MC of cubes exposed to white rot, particularly *Irpex lacteus*, increases with weight loss. However, the MC of cubes exposed to brown rot showed a decreasing trend with weight loss. According to Zabel and Morell (1992), the reduction in MC associated with brown-rot attack is due to the destruction of the cellulose. White rot decay fungi decompose lignin with little cellulose modifications. Lignin is more hydrophobic than cellulose. This explain why after white rot attack sample exhibit a higher moisture content compared to brown rot.

Table 9. Average biweekly moisture content (%) of untreated rubberwood cubes exposed to different decay fungi for 12 weeks.

	White rot		Brown rot		Soft rot	
Week	*Irpex lacteus*	*Trametes versicolor*	*Gloeophyllum trabeum*	*Postia placenta*	*Chaetomium globosum*	*Phialophora sp.*
2	49.7 (5.7)[1]	43.9 (17.1)	86.9 (6.3)	70.7 (9.1)	39.0 (9.0)	27.9 (5.4)
4	50.3 (5.7)	52.5 (24.7)	71.2 (11.6)	76.7 (15.2)	44.3 (8.5)	27.1 (2.4)
6	54.4 (8.9)	65.2 (17.2)	75.9 (6.6)	64.2 (12.3)	46.2 (3.3)	41 (16.7)
8	57.0 (14.8)	63.2 (7.69)	66.7 (10.4)	65.6 (10.5)	52.4 (5.9)	40.0 (11.1)
10	57.0 (12.0)	63.2 (11.0)	66.5 (10.7)	54.9 (10.5)	48.4 (6.4)	41.6 (7.9)
12	67.0 (15.5)	65.3 (3.3)	47.3 (5.5)	49.8 (4.0)	53.3 (7.6)	41.4 (5.5)

[1]Values in parenthesis is the standard deviation of the mean.

For cubes exposed to soft-rot, the MC appears to increase as decay progresses. Eaton and Hale (1993) stated that wood permeability increases as of soft rot cavity in wood developed. Thus this explains the increasing MC in cubes exposed soft-rot. High level of MC not only supports the growth of soft-rot fungi, but also it increases the availability of nutrients particularly nitrogen (Eaton and Hale 1993).

5.3.2 Natural decay resistance

The average WL of untreated rubberwood blocks due to decay after 12 weeks exposure are given in Table 10. Average weight losses of about 61 % were obtained after 12 weeks exposure to white (*Irpex lacteus* and *Trametes versicolor*) and brown rot

(*Gloeophyllum trabeum* and *Postia placenta*). According to ASTM standard D 2017-81 "Standard Method of Accelerated Laboratory Test of Natural Decay Resistance of Woods" an average weight loss of 25 to 44% is classified as moderately resistant.

Table 10. Average weight loss (%) of untreated rubberwood cubes exposed to different decay fungi for 12 weeks.

Week	White rot		Brown rot		Soft rot	
	Irpex Lacteus	*Trametes versicolor*	*Gloeophyllum trabeum*	*Postia placenta*	*Chaetomium globosum*	*Phialophora* sp.
2	3.2	2.1	3.9	0.9	1.0	1.4
4	8.4	12.3	16.9	5.5	2.3	4.9
6	19.5	23.4	24.1	13.0	6.7	9.7
8	27.4	33.6	33.8	31.6	20.1	19.2
10	44.3	40.4	42.2	39.4	27.6	26.6
12	63.2	61.8	61.4	59.1	35.9	39.5

Weight loss higher than 45% is classified as slightly resistant or nonresistant. Average weight loss of 63.2% with *Irpex lacteus* and 61.8% with *Trametes versicolor* are obtained after 12 weeks of incubation (Table 10). As for brown rot average weight losses of 61.4% and 59.1% were obtained with *Gloeophyllum trabeum* and *Postia placenta*, respectively. According to ASTM criteria, rubberwood is nonresistant.

Findlay (1985) classified laboratory weight loss of 30% and higher as perishable, which corresponds to the service life less than 2 years in tropical conditions and maximum 5 years under temperate conditions. In some tropical countries the term perishable is not used and non-durable or not durable is used to describe this class (Findlay 1985, Eaton and Hale 1993). In this case moderately durable is used to describe service life of 2 to 5 years and that correspond to weight loss between 10 and 30% in laboratory evaluation.

In this study the susceptibility of rubberwood to white rot and brown rot is identical. It has been reported that hardwood is relatively susceptible to white rot in field test (Schultz and Nicholas 1997). In laboratory testing the samples are exposed to pure fungus culture, with no or negligible competition and under artificial microbial ecosystem. In rubber plantations, white rot fungi (*Rigidoporous lignosus, Phellinus noxius, Lenzites palisotii, Ganoderma applanatum*) were reported to cause major damage to rubber trees (Geiger *et al.* 1986; Sujan *et al.* 1980).

The highest weight losses after 12 weeks of incubation were observed with white rot fungi, *Irpex lacteus* (63%) and *Trametes versicolor* (62%) followed by brown rot, *Gloephyllum trabeum* (61%) and *Postia placenta* (59%) and the lowest weight losses were achieve with soft rot *Phialophora* sp. (40%) and *Chaetomium globosum* (36%). White and brown rot weight losses were about 1.5 times higher than soft rot after 12 weeks of incubation. The slower decay rate of soft rot fungi has been explained by the lack of nutrients particularly nitrogen (Butcher and Drysdale 1974). Anagnost and Smith (1997) reported a significant increased in weight loss due to *Chaetomium globosum* upon addition of nutrient solution consisted of 1.5g/L NH_4NO_3, 2.5 g/L KH_2PO_4, 2.0 g/L K_2HPO_4, 1.0 g/L $MgSO_4 \cdot 7H_2O$, 0.1 mg/g thiamine and 2.5 g/L glucose at 8^{th} week of incubation period.

5.3.3 Relationship between weight loss and incubation period

Simple linear regression equations for all decay fungi are summarize in Table 10. Linear relationship exists between weight loss and incubation period. The coefficients of determination (r^2) range from 0.87 to 0.96 for all test fungi. The rate of decay of

rubberwood exposed to decay fungi used in this study significantly increased with incubation period. The equations (Table 11) is only valid for the incubation period of 2 to 12 weeks and with the conditions used in this study.

The regression equations for brown rot and white rot were almost identical (Table 10). The slopes ranged from 5.3 to 5.9 and intercept from – 6.7 to –13.0. The highest slopes were observed with weight loss due to *Irpex lacteus* (5.9). *Postia placenta* had the next highest slope (5.87) followed by *Trametes versicolor* (5.6) and *Gloeophyllum trabeum* (5.32). The soft rot fungi appeared to have lower regression slope than white and brown rot fungi, which indicates that the their rate of decay was much slower than white and brown rot.

Covariance analysis was carried out to determine the differences between the regression equations in Table 11. The analysis revealed that differences for slope and intercepts are significant at 1% level.

Table 11. Regression equation of WL (%)[1] vs duration (t)[2] of rubberwood exposed to decay fungi.

Fungi	Regression equation	Coefficient of determination (r^2)	P[3]
White rot			
Irpex lacteus	WL (%) = 5.94*t – 13.00	0.90	**
Trametes versicolor	WL (%) = 5.60*t – 10.31	0.96	**
Brown rot			
Gloeophyllum trabeum	WL (%) = 5.32*t – 6.67	0.96	**
Postia placenta	WL (%) = 5.87*t –16.12	0.94	**
Soft rot			
Chaetomium globosum	WL (%) = 3.77*t – 10.8	0.87	**
Phialophora sp.	WL (%) = 3.79*t – 9.62	0.90	**

[1] Weight loss in percent
[2] Incubation periods in week
[3] Significant at 1% level

5.3.4 CCA efficacy

Weight losses of CCA-treated rubberwood exposed to *Irpex lacteus*, *Trametes versicolor*, *Gloeophyllum trabeum*, *Postia placenta*, *Chaetomium globosum*, *Phialophora* sp. and no fungus (or operational weight loss) are summarized in Table 12. Operational weight loss is defined as the weight loss associated with variation of equilibrium moisture content of treated samples before exposure (AWPA 1999). The operational weight loss in this study varies from 0.5 to 1.8%.

Treatment of rubberwood at CCA retention of 4.1 kg/m^3 resulted in significant reduction in weight loss (Table 12). The increase in CCA retention from 4.1 to 10.5 kg/m^3 has reduce the weight loss due to decay by *Irpex lacteus* (2.8%), *Trametes versicolor* (2.0%), *Postia placenta* (3.3%) and *Phialophora* sp. (3.1%). At retention of 14.5 kg/m^3, the weight loss was similar to the operational weight loss, which indicates that no significant fungal attack occurred at 14.5 kg/m^3 retention. The threshold values (Table 13) shows that higher CCA retention is needed to control brown rot, followed by white rot and then soft-rot. A study by Kamdem *et al.* (1996) also found that brown rot fungi needed higher copper content, followed by white rot and soft rot for the protection of northern white red oak (*Quercus rubra*) and soft maple (*Acer rubrum*) with water-borne copper naphthenate.

This laboratory study showed that CCA-C could be used as preservative to prevent rubberwood from decay fungi. Minimum retention of 14.7 kg/m^3 is required rubberwood against all test fungi. Field test is necessary to validate this result.

Table12. Weight loss (%) of CCA type C treated-rubberwood exposed to decay fungi.

CCA-C retention (kg/m^3)	Operational weight loss (%)	White rot		Brown rot		Soft rot	
		I. lacteus	*T. versicolor*	*G. trabeum*	*P. placenta*	*C. globosum*	*Phialophora* sp.
Untreated	0.7 (0.2)[1]	78.6 (8.2) a	55.9 (7.6) a	60.0 (7.7) a	56.2 (3.6) a	35.2 (8.5) a	35.5 (5.2) a
0 (water)	0.7 (0.2)	66.9 (14.9) b	57.1 (3.7) a	58.7 (0.5) a	56.6 (4.9) a	38.6 (6.8) a	41.3 (1.8) a
4.1	0.5 (0.3)	9.6 (1.4) c	9.7 (2.0) b	8.9 (1.8) b	10.2 (2.7) b	9.4 (2.0) b	9.4 (2.4) b
10.5	1.0 (0.2)	2.8 (0.9) d	2.0 (1.0) c	6.5 (2.0) b	3.3 (2.1) c	6.9 (2.4) b	3.1 (1.6) c
14.5	1.8 (0.2)	0.8 (0.7) e	0.9 (1.0) d	1.6 (1.5) c	1.6 (3.6) c	1.8 (0.6) c	1.3 (1.5) c

[1]Means followed by a different letter within a column are statistically different at $P \geq 0.05$ using Tukey Multiple Comparison test.

Table 13. Estimated threshold retention values of CCA type C for rubberwood using intercept method (AWPA E10-91).

Fungus	CCA-C retention (kg/m^3)
Irpex lacteus	13.1
Trametes versicolor	12.8
Gloeophyllum trabeum	14.7
Postia placenta	13.8
Chaetomium globosum	13.1
Philophora sp.	12.3

CHAPTER 6

MICRODISTRIBUTION OF COPPER-CHROME-ARSENIC PRESERVATIVE IN THE CELLS OF RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.)

6.1 Introduction

It is well known that preservative effectiveness depends not only on the amount of uptake or retention, but also upon the preservative distribution in the wood cells. The efficacy of preservatives is also the function of its permanence in wood. The performance of CCA in protecting softwoods both in laboratory evaluation and field tests is well established and it is attributed to the deep penetration into the cell wall of tracheids of *Pinus* species (Petty and Preston 1968), the preservative loading (Ryan and Drysdale 1988), the uniform preservative distribution in wood cells (Bodner and Pekny 1991; Dickinson and Sorkhoh 1976; Greaves 1972), and within the cell wall (Newman and Murphy 1996). In hardwoods, the poor performance of CCA has been associated with uneven distribution of CCA components in hardwoods cells and within the cell wall structure (Greaves 1972, 1974; Dickinson 1974; Butcher 1979).

Some hypotheses have been put forward to explain the poor performance of CCA-treated hardwood. The gross anatomy of hardwoods and softwoods are different. Hardwood species are composed of mainly fiber (15-60%), vessel elements (20-60%), rays (5-30%), and parenchyma (0-24%) (Haygreen and Bowyer 1996). The vessel elements function as conduction of liquids while fibers are responsible for mechanical support. Hardwood fibers possess a few slit-like simple pits (Greaves 1974). Liquids are reported to flow through open vessels, which form continuous pathway (Wilkinson 1979; Siau 1984). Liquids move in the surrounding cells, through the pits into cell lumen.

Softwoods are composed of 90-95% tracheids, and the remaining 5-10% is make up of ray cells. Tracheids are thin wall conducting cell with rounded ends and numerous bordered pits. Hardwoods are composed of cells with thick cell-wall fiber, which influence the penetration of preservative solutions into cell wall (Greaves 1974; Wilkinson 1979; Siau 1984;).

The differences in chemical make-up of hardwoods and softwoods may affect the permanence of preservatives in wood. Generally, softwoods have generally higher lignin content that hardwood (Haygreen and Bowyer 1996). Softwoods lignin contain mostly guaiacyl group whereas most hardwoods lignin contain syringyl and guaiacyl group. It has been reported that CCA preferentially react with guaiacyl lignin (Daniel and Nilsson 1987). Softwood species with higher lignin content will be more reactive with CCA than hardwood species.

The location and distribution of metal elements in copper-based treated wood is reported to influence performance (Chou *et al.* 1973; Greaves 1978; Bodner and Pekny 1991). The uneven distribution of copper is suggested to be the cause of poor performance of CCA-treated hardwoods against soft-rot. Soft rot fungi are known to attack CCA-treated refractory hardwoods species. Uneven distribution of copper in S_2 layers of fibers may explain soft rot decay in CCA-treated wood (Greaves and Levy 1978; Greaves and Nilsson 1982; Greaves *et al.* 1982). Some studies have pointed out that an even distribution of copper in wood cell of hardwoods will still remain susceptible to soft-rot decay (Butcher 1979; Drysdale *et al.* 1980; Ryan and Drysdale 1988). High copper loading in CCA-treated hardwoods is required to protect against soft-rot decay. Ryan and Drysdale (1988) reported that increasing CCA retention could control soft-rot

attack. Their study showed that it required six fold copper retention in the fibers of *Betula alba* than in the tracheids *of Pinus radiata*. CCA retention of about 32 to 35 kg/m^3 was required to significantly extend the service life of hardwood poles in New South Wales, Australia (Witheridge 1983). Field test data of CCA-treated *Koompassia malaccensis* and *Shorea leprosula* indicate that service life of these timbers can be extended to 30 years with a CCA retention of 15 kg/m^3.

Numerous studies have been conducted to evaluate the microdistribution of CCA in hardwoods (Greaves 1974; Dickinson and Sorkhoh 1976; Greaves and Levy 1978; Greaves *et al.* 1982; Daniel and Nilsson 1987). Limited information is available on the factors that may influence the performance of CCA-treated rubberwood. This chapter reports on the distribution of CCA components in the rubberwood cells, at varying treating solution strength using scanning electron microscope fitted with X-ray microanalysis.

6.2 Materials and Methods

6.2.1 Wood treatment

Wood treatment was previously described in chapter 5.

6.2.2 Samples preparation for X-ray microanalysis

Three cubes from each treatment concentration were used. From each cube, two small strips of 1 x 3 x 9 mm were cut perpendicular to each other from the transverse section. The surface of the strips was cut with razor blade (Exley *et al.* 1974). The samples were mounted on aluminum stub using double-sided adhesive tape and then

coated with a thin layer of carbon using a carbon string evaporator in Emitech K450 sputter coater.

6.2.3 Scanning Electron Microscope – Energy Dispersive X-ray Analysis (SEM-EDXA)

The SEM-EDXA were performed using JEOL 6400V scanning electron microscope fitted with Noran Vantage Energy dispersive X-ray system equipped with a light element detector using a Moxtek window with detection capabilities down to beryllium. A 20 kV accelerating voltage, 200 μamps, working distance 15 mm, and take off angle 30° were used to examine the samples. The samples were analyzed for Cr - K_α 5.41 keV, Cu - K_α 8.04 keV, and As - K_α 10.54 keV. Each X-ray analysis was done at 500X magnification. Spot analyses of fibers, vessels, parenchyma, and rays (Figure 10 and 11) were made at 0, 3, 6, and 9 mm depth from the outer surface. Each analysis was repeated at 10 different spots of a specific cell for 100 second. For each CCA concentration, three cubes were examined and a total of 30 examination spots on each wood cell were performed. The data from matched depth from each strip were pooled giving a total of 120 examination spots on each wood cell.

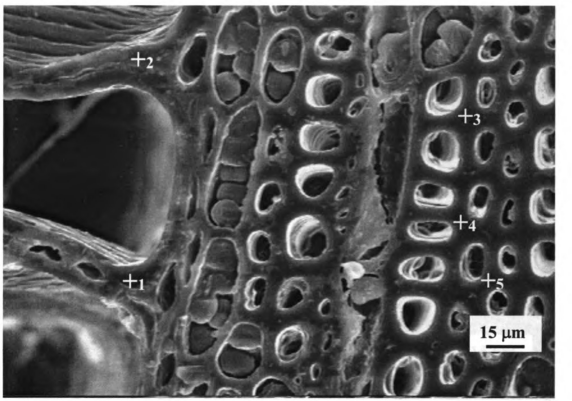


Figure 10. SEM micrograph of transverse section showing cell walls of vessel ($+_1$ and $+_2$) and fiber ($+_3$, $+_4$, $+_5$) used for X-ray spot analyses.

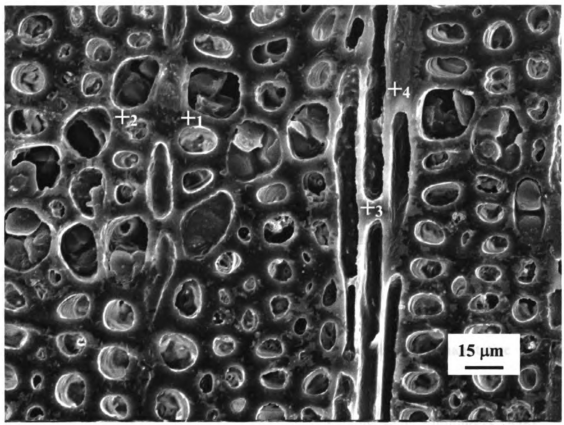


Figure 11. SEM micrograph of transverse section showing cells of parenchyma ($+_1$ and $+_2$) and ray ($+_3$, $+_4$) used for X-ray spot analyses.

6.2.4 Data handling

The first step in the estimation of the concentration of an element in a specimen is to measure the specific X-ray counts in a fixed time interval and to compare the specific X-ray count to the total number of X-ray emitted from the sample in a similar time. The ratio obtained is called k-ratio and represents approximately the elemental concentration of the sample. It is an approximation because of some interference due to the matrix as illustrated in Figure 12. The volume produced by specimen/electron interaction is dependent on several factors: the accelerating voltage, the beam current, the specimen density, the specimen atomic number, and the surface angle orientation. Since the operating conditions are identical, correction is therefore needed for three factors: the atomic number effect (Z), the absorption (A) and the fluorescence (F). The X-rays data are expressed as elemental weight percent using the built-in computerized software PROZOA, which uses ZAF and Phi Rho Z corrections as described in Flegler *et al.* (1993) and Russ (1984). The ZAF corrections are frequently used for quantitative analysis of bulk sample. The following equation is used to adjust the k-ratio to get the concentration (C) of an element (Flegler *et al.* 1993; Goodhew and Humphreys 1988):

$$C_{specimen} = \text{k-ratio} \times Z \times A \times F$$

The atomic number correction Z, refers to the adjustment for X-rays differences due to the Z of samples. The Z correction is combination of stopping power and backscattering effects.

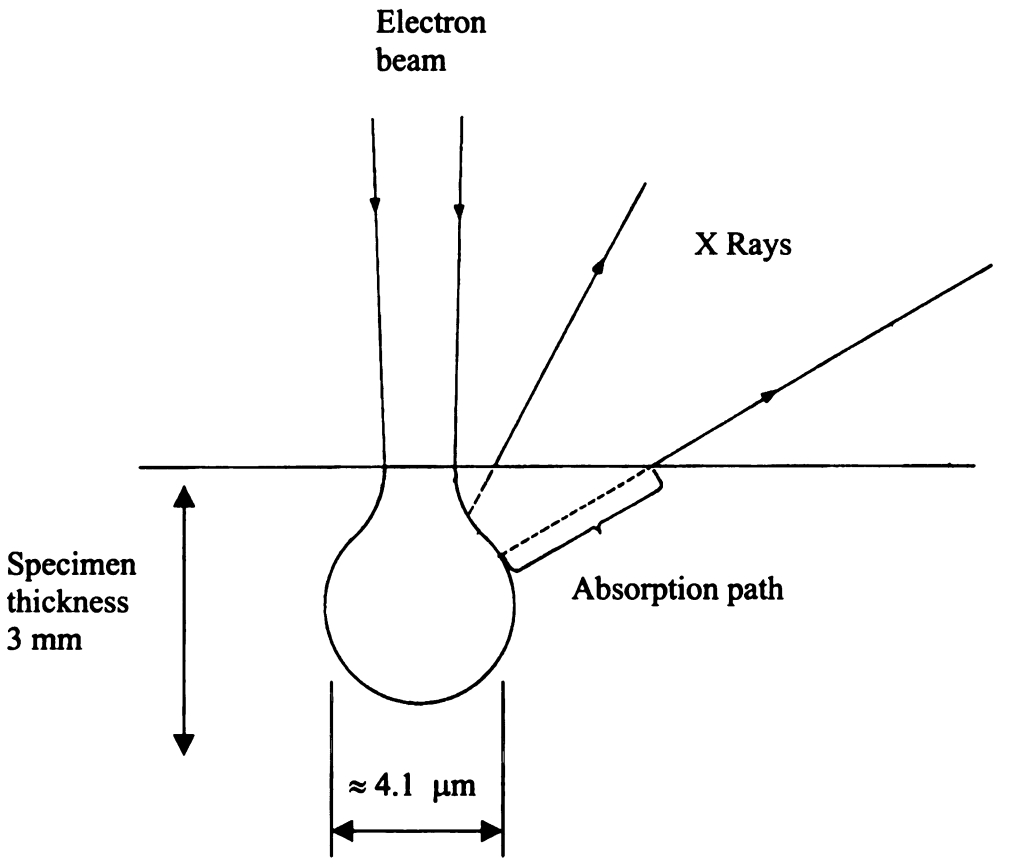


Figure 12. A schematic diagram of electron beam/specimen interaction within a bulk specimen.

The absorption effect correction A, is used to adjust for X-rays differences due to absorption. X-rays produced from deeper portion of the specimen travel through the specimen thickness and likely will be absorbed before they can escape and be detected (Figure. 11). The absorption corrections take into account the accelerating voltage, the take-off angle, the mean atomic number and the mean atomic mass. With thin samples of less than 0.15 μm the effect is almost negligible. If correction on absorption is not done, it will underestimate the X-rays produced.

The fluorescence F factor, corrects for secondary X-rays. The X-rays produced within the specimen may be of high energy capable of fluorescing lower energy X-ray of another element. This may cause abnormally high counts for the lower energies element. Although generally considered the least important of the three corrections, secondary fluorescence could cause errors as large as 15% during analysis of elements of adjacent atomic numbers (Goodhew and Humphreys 1988).

The Phi Rho Z corrects for the differences of areas of X-ray production. As incident electron penetrates deeper into the specimen it loses energy. As a result X-rays are generated from elements with a high K_{ab} and elements with a low K_{ab} due to differences in electron energy as it penetrates deeper into the specimen.

6.3 Results and Discussion

6.3.1 X-ray microanalysis of wood

The volume of wood tissue analyzed in bulk specimen is large. Based on the operating conditions employed in this study, and using Electron Flight Simulator

software version 3.1 (1998) to estimate the volume of specimen-electron beam interactions, the diameter of the analyzed volume in CCA-treated rubberwood is approximately 3.6 – 4.1 μm, corresponding to a volume up to 36 μm^3 (Figure 12). In a SEM-EDXA study of treated Hinoki (*Chamaecyparis obtusa*), Yata and Nishimoto (1983) reported that 20 kV accelerating voltage produced an analyzed volume with a diameter of 3.6 – 4.5 μm.

The cell wall thickness 4 to 7 μm for fibers, 4.3 to 8 μm for vessels, 2.5 to 4 μm for rays, and 2.5 to 3.5 μm for parenchyma (Chapter 3), indicate that examination of fibers and vessels lies well within the cell regions analyzed. For ray, x-ray microanalyses were within the cell region provided that the widest points are used for analyses. For parenchyma, X-rays analyzed may include X-rays generated from adjacent regions. Since the thickness of parenchyma cells and the diameter of specimen-electrons beam interactions are comparable, it is expected that effect of X-rays generated from adjacent cells is minimal.

Variability in the X-ray microanalysis of wood in bulk samples is expected. It is explained by the heterogeneous nature of the biological tissues. Although the operating and analyses conditions were kept constant, the different types and the density of wood cells influence the x-ray analyses. Therefore, X-ray microanalysis of biological material can only be semi-quantitative (Doyle and Ruddick 1994; Greaves *et al.* 1982; Drysdale *et al.* 1980).

The elemental composition of CCA-treated rubberwood from a spot analysis by SEM-EDXA is listed in Table 14. Hydrogen content is not represented, because hydrogen does not generate X-rays.

Table 14. Elemental composition in percent (%) of CCA-treated rubberwood as obtained by SEM-EDXA after ZAF corrections.

Element	CCA solution strength by AAS		
	0.93%	2.12%	3.29%
C	50.46	54.67	55.23
O	41.91	38.68	34.83
N	5.87	3.98	6.12
Na	0.02	0.00	0.00
Mg	0.11	0.15	0.25
S	0.00	0.02	0.03
K	0.01	0.04	0.20
Ca	0.00	0.04	0.17
Cr	0.69	0.81	1.06
Cu	0.24	0.54	0.99
As	0.69	1.07	1.12
Total	100.00	100.00	100.00

6.3.2 Chromium, copper, and arsenic in wood cells

The average composition of Cr, Cu, and As from 60 spots of each wood cells is shown in Table 15. Chromium appeared to be concentrated in the vessels. The composition of chromium in vessels increased from 1.85% to 4.66% after treatment with 1% and 3% CCA. High levels of chromium were also present in ray cells. The concentration of chromium in rays was about 1.72% in 1% CCA-treated samples and increased to 3.32% in 3% treated samples. Fibers have the lowest concentration of chromium (Table 15).

Copper appeared to be distributed in lower concentration in all wood cells compare to Cr and As. Among wood cell, vessels recorded the highest concentration of copper followed by rays, parenchyma, and fibers (Table 15). The concentration of copper in the vessel was about 1.67% in 1% treated samples and increased to 3.26% in 3%

treated samples. Fibers, which constitute the major portion of wood cells in rubberwood contain the lowest Cu concentration. The concentration of Cu in fibers was only about 0.42%, 0.63%, and 1.02% in 1%, 2% and 3% treated samples, respectively.

Table 15. Comparison of chromium, copper, and arsenic elemental composition in percent in various rubberwood cells after treatment with different treating solution strength.

CCA-C solution strength (%)	Cell type	Elemental composition (wt%)		
		Cr	Cu	As
0.93	Vessel	1.85* (0.34)**	1.67 (0.25)	1.85 (0.50)
	Ray	1.72 (0.85)	1.01 (0.50)	1.55 (0.80)
	Parenchyma	1.31 (0.28)	0.77 (0.34)	1.12 (0.60)
	Fiber	0.67 (0.21)	0.42 (0.25)	0.58 (0.21)
2.12	Vessel	3.55 (0.55)	2.42 (0.37)	3.45 (0.45)
	Ray	1.89 (0.95)	1.39 (0.65)	2.47 (0.67)
	Parenchyma	1.40 (0.35)	0.88 (0.40)	1.23 (0.54)
	Fiber	1.12 (0.35)	0.63 (0.22)	0.94 (0.57)
3.29	Vessel	4.66 (1.08)	3.26 (0.91)	3.73 (1.05)
	Ray	3.32 (0.97)	2.47 (0.88)	2.76 (0.99)
	Parenchyma	2.39 (0.45)	1.55 (0.66)	2.03 (0.93)
	Fiber	1.71 (0.67)	1.02 (0.52)	1.45 (0.52)

* Mean of 120 readings
** Standard deviation

Arsenic concentration in wood cells follows closely that of Cr. Concentration of As was highest in the vessels. Composition of As in vessel was 1.85% in 1% CCA and increased to 3.73% in 3% CCA. Fibers have the lowest concentration of As, about 0.58%, 0.94%, and 1.45% in 1%, 2%, and 3% CCA, respectively.

The uneven distribution of Cr, Cu, and As in different cells occurs regardless of treating solution strength. Chromium and arsenic appeared to be concentrated in the vessel (Table 15). High level of chromium was found in rays. Among the three elements, copper concentration was found to be the lowest in all type of cells. This indicates that CCA treatment did not result in a homogenous distribution of all three elements throughout rubberwood structure. Fiber cells have the lowest concentration of Cr, Cu, and As.

The result showed that microdistribution of CCA components differed according to cells types. Previous work by Levy and Greaves (1976) and Greaves *et al.* (1982) reported that the uneven distribution of Cr, Cu, and As in sapwoods of *E. regnans*, *E. maculata*, *Fagus sylvatica,* and *E. obliqua* occurred in different types of cells. They noted that each timber has its own specific characteristics penetration and distribution of Cr, Cu, and As within the wood structure. This study confirms that penetration and distribution of Cr, Cu, and As occurs even between cells within one species.

6.3.3 Relative composition of Cr, Cu, and As in wood cells

The relative composition of Cr, Cu, and As in vessel, rays, parenchyma, and fibers are given in Table 16. The relative compositions of individual CCA components in the wood cells were expressed as a percentage of the total concentration of chromium, copper, and arsenic as shown below;

$$\text{Composition ratio (\%)} = \frac{\text{Element(\%)}}{\text{Cr\%} + \text{Cu\%} + \text{As\%}} \times 100$$

For CCA solution and CCA retention in wood, the relative composition of Cr, Cu, and As is expressed as a percentage of the total amount in part per million (ppm) of chromium, copper, and arsenic as shown below;

$$\text{Composition ratio (\%)} = \frac{\text{Element(ppm)}}{\text{Cr(ppm)} + \text{Cu(ppm)} + \text{As(ppm)}} \times 100$$

Table 16. Relative ratios of element concentration in various rubberwood cells at different treating solution concentration.

CCA-C solution strength (%)	Cell type	Composition ratio (wt%)		
		Cr	Cu	As
0.93	Vessel	38	24	38
	Ray	40	24	36
	Parenchyma	41	24	35
	Fiber	40	25	35
	Cr, Cu, and As in treating solution	39	27	34
2.12	Vessel	39	22	38
	Ray	43	21	37
	Parenchyma	40	25	35
	Fiber	42	24	35
	Cr, Cu, and As in treating solution	39	25	36
3.29	Vessel	41	27	33
	Ray	41	25	34
	Parenchyma	40	26	34
	Fiber	41	24	35
	Cr, Cu, and As in treating solution	39	25	37

There is a small variation in the proportion of Cr, Cu, and As in different cells and treating solution strength (Table 16). The mean magnitude of CCA composition in

rubberwood is in the order of Cr > As > Cu and the order of magnitude is similar to that of the Cr, Cu, and As in treating solution (Table 16).

The distribution of individual CCA components among all cell types in treated rubberwood was uneven. It has been reported that the distribution of Cr, Cu, and As in hardwood cells and tissues were even or uneven depending on species (Greaves and Levy 1978). They noted that species with uneven distribution tend to show a low proportion of Cr, Cu and As in fibers. Among hardwood species that showed even distribution are *Alstonia scholaris*, *Antiaris toxicaria*, and *Elaeocarpus sphaericus* (Greaves and Levy 1978). Hardwoods species showed more uneven than even distribution. Low proportion of CCA components was observed in *Betula* sp. (Dickinson and Sorkhoh 1976), *Eucalyptus regnans*, *Flindersia pimenteliana*, *Magnifera minor*, *Pterygota horsfieldii*, and *Sterculia conwenysii* fibers (Greaves and Levy 1978).

Previous studies have showed that variation in distribution reflects the performance of timber in field exposure (Greaves and Levy 1978, Greaves and Nilsson 1982, Greaves *et al.* 1982). After 9 years field exposure *A. scholaris*, *A. toxicaria*, and *E. sphaericus* were rated as 100 (sound), while *S. conwentzii* scored 82, *P. horsfieldii* 55, *M. minor* 35 and *F. pimenteliana* 17 (Greaves and Levy 1978).

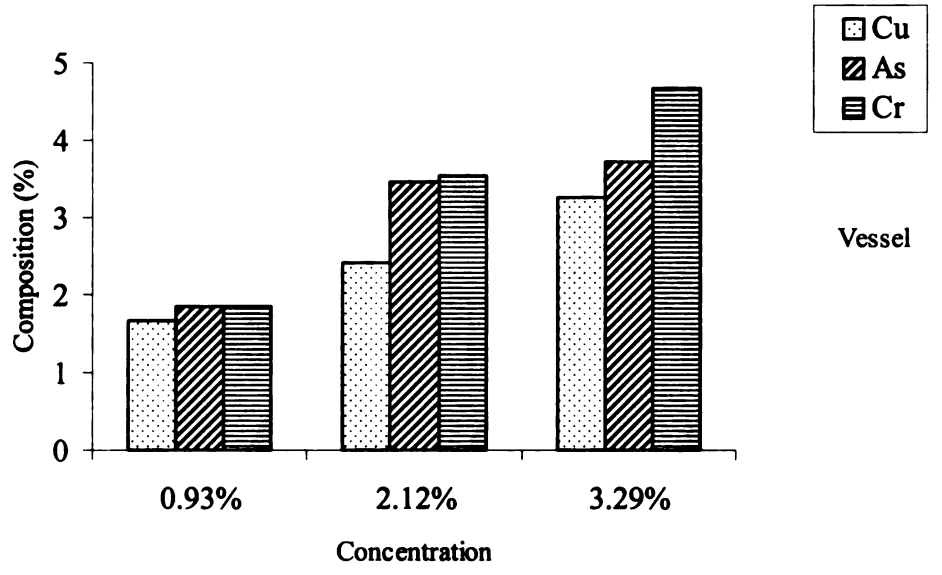
6.3.4 Influence of treating solution concentration on distribution of Cr, Cu, and As

The effect of an increase concentration on the microdistribution of CCA elements in individual cells cell types is shown in Figure 13 and 14. The increase of chromium, copper, and arsenic in vessel, ray, parenchyma and fiber cells with the increase of the strength of treating CCA solution is evident. The highest concentration of chromium,

copper, and arsenic were observed in the vessels treated with the highest solution strength (3%).

Treatment of samples with 0.93% and 2.12% CCA solution resulted in similar Cr, Cu, and As concentration in ray and parenchyma, but in vessel the increased of Cr, Cu, and As was about two fold, and in fibers the increased was about 1.5 fold. Increasing the solution concentration to 3.29% increased the concentration of CCA component to about two fold in ray and parenchyma and about 2.5 times in fibers. In vessels the increased in solution treatment to 3.29% increased the concentration of Cu and As to about two times and this is similar to 2.12% treatment but Cr increased was about 2.5 times.

Samples treated with different treating solution strength showed that the vessels produced the highest CCA element. This indicates that the main flow path of CCA preservative in rubberwood is the vessels (Levy and Greaves 1978; Greaves 1974) and it is consistent with results reported for most hardwood species impregnated with CCA (Nicholas and Siau 1973; Dickinson 1974; Levy and Greaves 1978). The ray cells consistently have high level of Cr, Cu and As suggesting that preservative penetration through ray cells accounted for a significant proportion of CCA components in rubberwood. Radial penetration of CCA through ray cells is common in pine species (Greaves 1974; Bodner and Pekny 1991). Hardwood species with ray cells significantly used for CCA penetration are *Fagus sylvatica* , *Eucalyptus regnans* and *E. maculata* (Greaves 1974). Maturbongs and Schneider (1996) showed that rays of 10 non-durable Indonesian hardwoods are well penetrated by CCA after pressure treatment, indicating that CCA penetrated through rays.



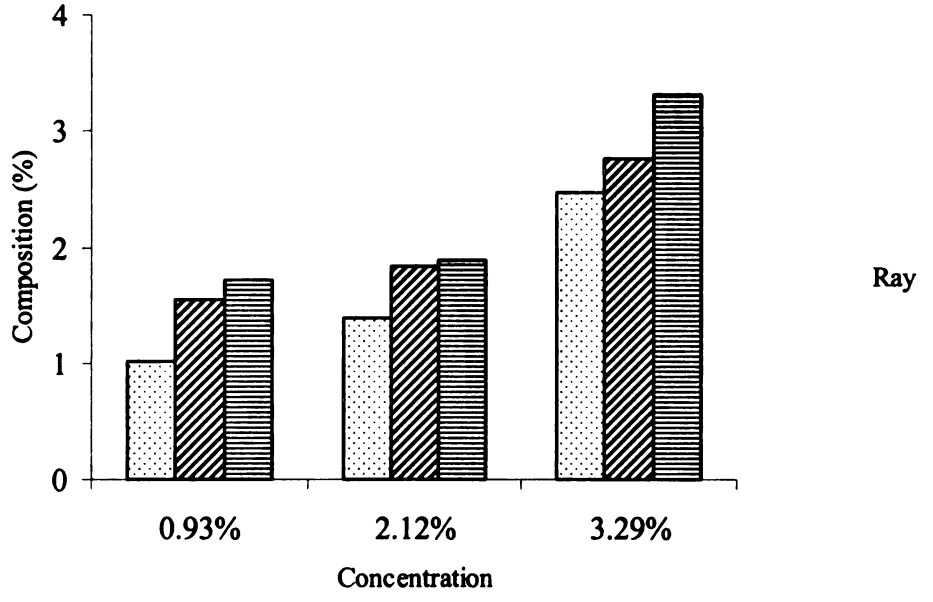


Figure 13. The effect of treating solution concentration on the Cr, Cu, and As composition in vessels and rays of CCA-treated rubberwood.

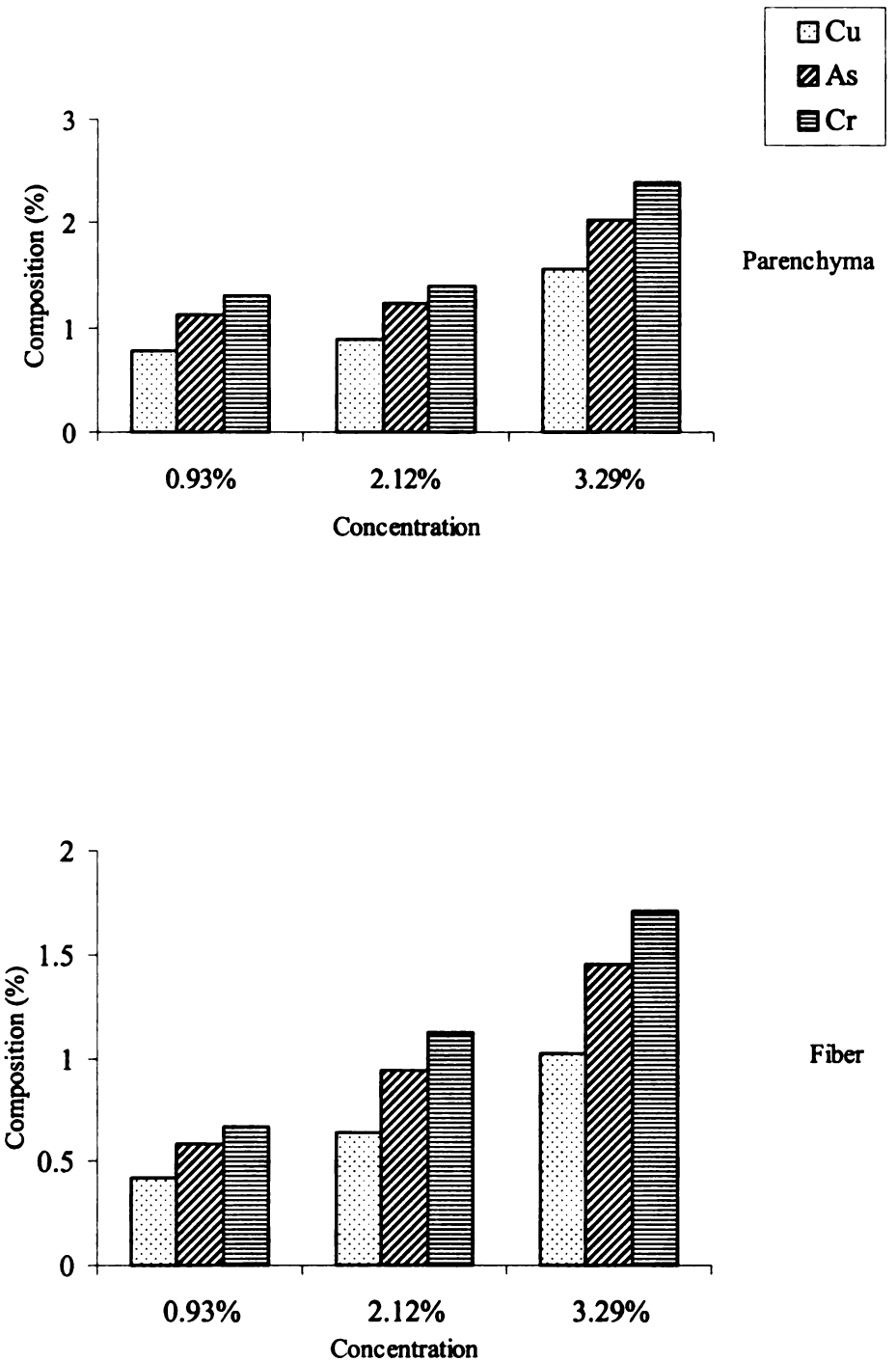


Figure 14. The effect of treating solution concentration on the Cr, Cu, and As composition in parenchyma and fibers of CCA-treated rubberwood.

CHAPTER 7

MICRODISTRIBUTION OF CCA ELEMENTS IN RUBBERWOOD (*HEVEA BRASILIENSIS* MUELL. ARG.) CELL WALLS

7.1 Introduction

Levy and Greaves (1978) reported that soft-rot attack of CCA-treated hardwoods is related to the poor and uneven microdistribution of copper in the S_2 layers of fibers. Later, Drysdale *et al.* (1980) suggested that the microdistribution patterns of preservative within cell walls was not the sole factor controlling soft-rot development, but the retention was also critical. Hardwood species may require high load of preservative to protect against soft-rot (Smith *et al.* 1996; Witheridge 1983) in the S_2 cell wall layer (Cooper 1998) because of their thicker S_2 layer compared to that of softwood species.

The retention and the microdistribution of CCA in wood cells are therefore of paramount importance to understand and predict the bioefficacy of CCA-treated hardwoods. In this study X-ray microanalysis was used to study the microdistribution of Cr, Cu, and As in CCA-treated rubberwood using semi-thin sections of 0.5 μm thick. The microdistribution of wood preservatives in hardwoods has been studied extensively in various ways. The microdistribution is defined by the quality and quantity of elements present in each microstructure of wood components.

Most of the research were done on chromated copper arsenate (CCA) preservative using scanning electron microscopy (SEM) in conjunction with X-ray microanalysis (Greaves 1974; Dickinson and Sorkhoh 1976; Greaves and Levy 1978; Greaves *et al.* 1982; Daniel and Nilsson 1987). Electron probe microanalyzer (EPMA) was also employed to study the distribution of CCB-treated red meranti (Salamah and Ani 1995).

Basically principles of electron probe microanalysis and scanning electron microscope X-ray microanalysis are similar. The differences between the two instruments are that in EPMA the region of the specimen to be analyzed is selected using the attached light microscope and the microanalysis is operated using high beam currents of 10^{-11} to 10^{-12} amperes. In SEM-X-ray microanalysis, the region of interest to be analyzed is selected using conventional SEM and the microanalysis is done using lower beam currents of 10^{-6} to 10^{-9} amperes. SEM-X-ray microanalysis has several advantages over EPMA. The low beam current in SEM-X-ray microanalysis reduces over heating of specimen, element migration and spot contamination (Greaves 1974; Ryan and Drysdale 1988).

Several researchers (Dickinson 1974; Drysdale *et al.* 1980; Ryan 1986; Newman and Murphy 1996) also used TEM in conjunction with X-ray microanalysis to study the microdistribution of CCA in wood cell walls. TEM provides higher spatial resolution and eliminates the complication encounters with the specimen electron interactions obtain with SEM. However, Daniel and Nilsson (1987) reported that the used of semi-thin sections of 0.5 μm in thickness with SEM yield useful information on the distribution of Cr, Cu, and As in treated wood. They concluded that SEM-X-ray microanalysis of semi-thin sections provide a convenient alternative to TEM-X-ray microanalysis for routine CCA microdistribution studies.

It is widely and generally accepted and established in the current literature that the middle lamella, the primary cell wall layer and the cell corners tends to retains high levels of Cr, Cu, and As than the adjacent S_2 layers (Daniel and Nilsson 1987; Drysdale *et al.* 1980; Greaves 1974). Drysdale *et al.* (1980) reported that increasing preservative retention do not result in proportional increase of Cr, Cu, As in the S_2 layers. Daniel and

Nilsson (1987) showed that the microdistribution of CCA in wood is closely related to the lignin distribution. All these studies used SEM and X-ray microanalysis observed high count per seconds of Cr, Cu, and As in the cell corner, middle lamella, and primary cell wall layers of CCA-treated wood.

In previous studies, no mention is made on the effect of the probable density difference within the different cell wall layers. The middle lamella (ML), cell corner (CC) and primary cell wall (P) are very rich in lignin compared to the secondary wall (S_2). The high retention of Cr, Cu, and As in ML, CC, and P have been thought to be due to the interaction with lignin.

A factor that is difficult to take into account is the density of the different part of the cell wall. Cell corner, ML, and P is rich in lignin which is a polymeric matrix richer in carbon than the cellulose. It can be theorized that lignin may be more conductive and more prone to produce X-rays during SEM-X-ray mcroanalysis than the cellulose. It can be also be postulated that since the S_2 layers have several orientation (Haygreen and Bowyer 1996) the emission of X-ray will be reduced and then less X-rays detected. To test this series of hypothesis, untreated and CCA-treated samples were analyzed for Cr, Cu, As, C and O using SEM-X-ray microanalysis. The objective of this study is to investigate the microdistribution patterns of Cr, Cu, As, C, and O in the cell walls of CCA-treated rubberwood.

7.2 Material and Methods

7.2.1 Wood treatment

The wood treatment was previously described in chapter 5.

7.2.2 Samples preparation for X-ray microanalysis

Small matchstick samples measuring 0.5 x 0.5 x 10.0 mm were cut from the outer portion (0 – 5 mm) of treated cubes to minimize the effects of uneven or poor penetration. The matchstick size samples were infiltrated, embedded in Spurr's resin and ultramicrotomed to obtain semi-thin section of 0.5 μm thick. The sections were mounted on tungsten 3 mm grid and centered on aluminum stub (Figure 15).

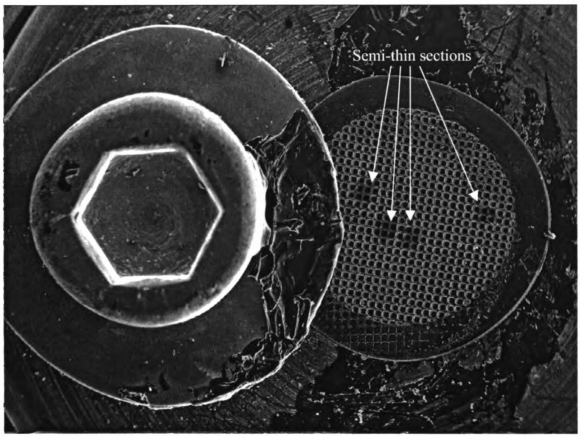


Figure 15. Semi-thin sections on a 3 mm tungsten grid centered on aluminum stub.

7.2.3 Scanning Electron Microscope – Energy Dispersive X-ray Analysis (SEM-EDXA)

The SEM-EDXA were performed using similar operating conditions described in Chapter 6. SEM-EDXA of thin sections of less than 0.15 μm is reported to have very little to negligible impact on the absorption (A) and fluorescence (F) factors (William and Carter 1996). The only correction factor that is important is Z. When 0.5 μm thick sections are used, as in this study, it can be postulated that the A and F factors exists but the effects are minimal. However, better precision can be achieved when all the Z, A, and F factors are included for corrections. Electron Flight Simulator Software (1998) was employed to estimate the volume of specimen-electron beam interactions of the semi-thin sections of 0.5 μm. The diameter over which X-ray microanalyses were conducted was approximately 0.300 – 0.375 μm (Figure 16).

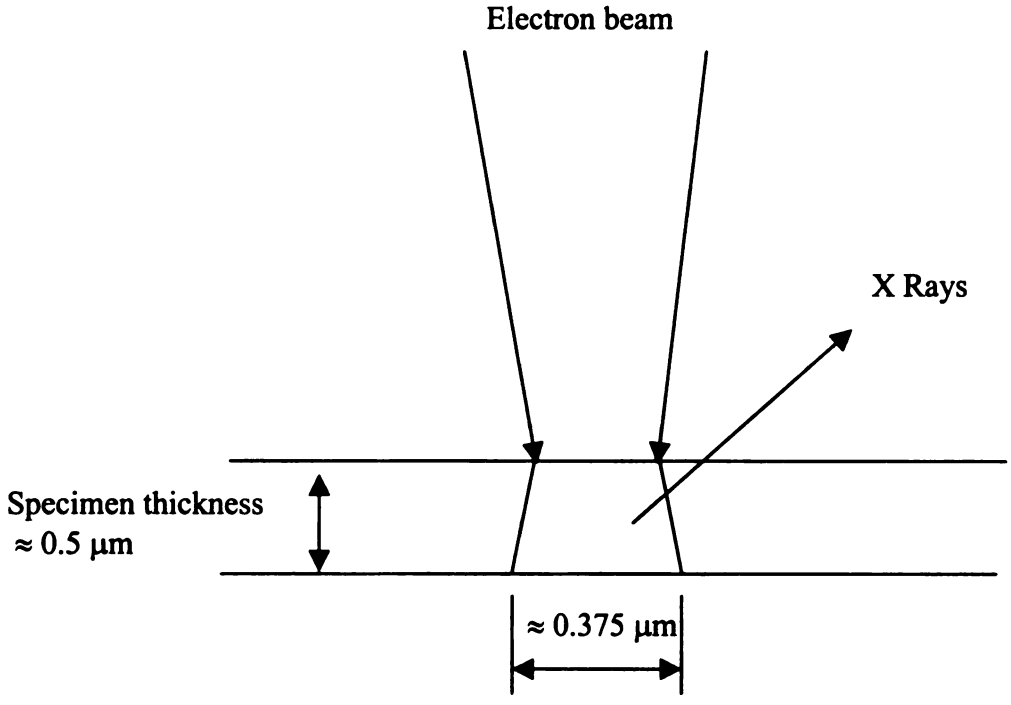


Figure 16. A schematic diagram of electron beam/specimen interaction.

The cell wall regions and their dimensions included in this study are summarized in Table 17.

Table 17. Dimension of cell layers in rubberwood.

Cell type	Morphological region	Dimension
Fiber	S_2 layer	4 – 7 μm
	fiber-to-fiber cell corner (FF_{CC})	2 – 3.5 μm
	fiber-to-fiber middle lamella (FF_{ML})	1 – 1.8 μm
Ray	S_2 layer	2.5 – 4 μm
	Fiber-to-ray cell corner (FR_{CC})	2 – 3.5 μm
	fiber-to-ray middle lamella (FR_{ML})	1 – 1.8 μm
Vessel	S_2 layer	2.5 – 8 μm
	fiber-to-vessel cell corner (FV_{CC})	2 – 3.5 μm
	fiber-to-vessel middle lamella (FV_{ML})	1 – 1.8 μm

The spot analyses were performed on cell corners (CC), middle lamella (ML) and S_2 cell wall layer of fibers, vessels and ray cells regions at a magnification of 5000 X (Figure 17). Twenty spot analyses were collected at each region during 100-second analysis time. To minimize variation, analyses were conducted on adjacent cell wall regions of the same cell from the same sections. Analyses of ML and CC region were done midway between adjacent cell wall. The S_2 layers were analyzed almost at the center of the layer to reduce the possible X-rays count from the primary cell wall (P)

Linescan analyses were performed on two adjacent fiber cell walls. The total acquisition time for each linescan was about 10-15 minutes depending on the thickness of the cell wall.

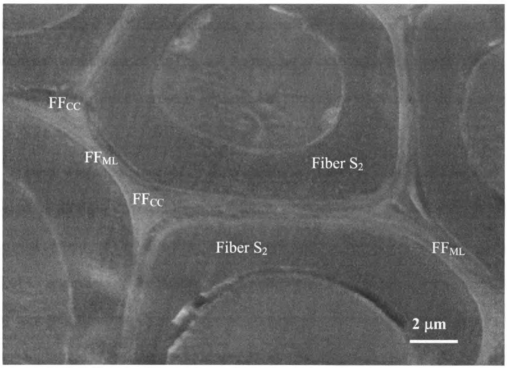


Figure 17. SEM micrograph of semi-thin (0.5 μm) section showing fiber-to-fiber cell corner (FF_{CC}), fiber-to-fiber middle lamella (FF_{ML}), and fiber S_2 layer regions used for analyses. (Magnification 5000X).

7.3 Results and Discussion

7.3.1 Microdistribution of Cr, Cu, and As

The elemental composition and proportion of Cr, Cu, and As of various cell wall regions detected from semi-thin sections of rubberwood are shown in Table 18. Each value is the average of 20 analysis points, automatically expressed by the Noran Vantage software as a percentage of Cr, Cu, and As present at each region analyzed. The proportion from different region is expressed as percentages of the total composition of Cr, Cu, and As.

There was a high level of Cr compare to Cu and As in all cell wall regions analyzed for all three different treating solution strengths. The S_2 layer of fibers exhibited the lowest amount of Cr while fiber to vessel cell corner (FV_{CC}) contained the highest level of Cr regardless of the treating solution strength. Arsenic was present in slightly lower concentration than Cr in all regions. Arsenic was the lowest in the S_2 layer of fibers and the highest in FV_{CC}. Copper appeared to have the lowest level in all regions analyzed compared to Cr and As. Similarly, as with Cr and As, the Cu level was the lowest in S_2 layer of fiber and the highest in FV_{CC}.

Different regions have different elemental composition at various solution strengths. High concentration of Cr, Cu, and As were observed in the CC followed by the ML region. The FV_{CC} and ML appeared to have the highest level of Cr, Cu, and As. The S_2 layer of vessels contained higher amount of Cr, Cu and As compared to the S_2 layers of rays and fibers. The high concentration of Cr, Cu and As in the CC of rubberwood is in agreement with studies reported for CCA-treated *Betula verrucosa* using SEM-EDXA (Daniel and Nilsson 1987), *Betula alba* and *Alsotnia scholaris* using TEM-EDXA (Ryan and Drysdale 1988).

Table 18. Average elemental composition and proportion of various cell morphological regions in CCA-treated rubberwood.

Morphological region*	Treating solution strength	Elemental composition (wt%)			Elemental proportion
		Cr	Cu	As	Cr : Cu : As
Fiber S_2	0.93	0.57	0.29	0.43	44 : 22 : 33
	2.12	0.99	0.52	0.94	40 : 21 : 38
	3.29	1.52	0.89	1.60	38 : 22 : 40
FF_{CC}	0.93	0.95	0.47	0.91	41 : 20 : 39
	2.12	1.85	0.81	1.72	42 : 19 : 39
	3.29	3.37	1.60	3.09	42 : 20 : 38
FF_{ML}	0.93	0.85	0.38	0.78	42 : 19 : 39
	2.12	1.81	0.78	1.65	43 : 18 : 39
	3.29	2.98	1.49	2.75	41 : 21 : 38
Ray S_2	0.93	1.38	0.64	1.01	46 : 21 : 33
	2.12	2.41	1.10	2.01	44 : 20 : 36
	3.29	3.35	1.71	2.60	44 : 22 : 34
FR_{CC}	0.93	1.93	0.79	1.48	46 : 19 : 35
	2.12	4.58	2.01	4.22	42 : 19 : 39
	3.29	6.77	3.09	4.90	46 : 21 : 33
FR_{ML}	0.93	1.83	0.77	1.39	46 : 19 : 35
	2.12	4.21	1.58	3.16	47 : 18 : 35
	3.29	6.35	2.65	4.56	47 : 20 : 34
Vessel S_2	0.93	1.82	0.91	1.79	40 : 20 : 40
	2.12	3.38	1.75	3.08	41 : 21 : 38
	3.29	4.50	2.04	4.09	42 : 19 : 38
FV_{CC}	0.93	2.01	0.91	1.88	42 : 19 : 39
	2.12	5.21	2.78	4.90	40 : 22 : 38
	3.29	7.51	3.45	6.50	43 : 20 : 37
FV_{ML}	0.93	1.98	0.89	1.75	43 : 19 : 38
	2.12	4.80	2.50	4.40	41 : 21 : 38
	3.29	6.95	3.20	5.48	44 : 20 : 35

* Fiber S_2 - Fiber's S2 layer; FF_{CC} – Fiber-to-fiber cell corner; FF_{ML} – Fiber-to-fiber middle lamella; Ray S_2 - Ray's S_2 layer; FR_{CC} – Fiber-to-ray cell corner; FR_{ML} – Fiber-to-ray middle lamella; Vessel S_2 - Vessel's S_2 layer; FV_{CC} – Fiber-to-vessel cell corner; FV_{ML} – Fiber-to-vessel middle lamella.

In CCA-treated *Cryptomeria japonica,* Lee *at al.* (1992) noted that Cr, Cu, and As were located within the ML and CC in greater concentration than in the secondary wall. The present study also showed that Cr, Cu, and As were accumulated in the ML, CC, and the primary wall in agreement with published literature.

The cell corner and middle lamella are rich in lignin (Fengel and Wegener 1983; Saka and Goring 1985). It has been suggested that lignin is one of the fixation sites for Cr, Cu, and As. The microdistribution of CCA components in treated wood correlated well with the distribution of lignin in wood (Daniel and Nilsson 1982, 1987). Petrič *et al.* (2000) reported that lignin is the preferential site for copper and other metal ions. However, Thomason and Pasek (1997) suggested that copper from a copper ethanolamine solution react with the carboxyl groups of hemicellulose. Using Fourier transform infrared spectroscopy (FTIR), Zhang and Kamdem (1999) concluded that the carboxyl groups in hemicellulose and phenolic hydroxyl, and ester groups of lignin are the major bonding sites for copper. Craciun and Kamdem (1997) proposed that Cu interact with carboxyl groups of hemicellulose and lignin.

7.3.2 Distribution of carbon and oxygen

The X-ray counts for carbon and oxygen in the S_2 fiber layer and the adjacent cell corner and middle lamella of untreated and CCA-treated rubberwood are presented in Table 19. The count rate of carbon and oxygen counts of S_2 layer of fiber, CC, and ML were expressed in ratios with S_2 layer of fiber count rate as 1.0. The ratio was calculated as follow:

$$\text{Ratio value} = \frac{\text{Carbon or oxygen count rate of cell wall region}}{\text{Carbon or oxygen count rate of } S_2 \text{ layer}}$$

The count rates of carbon and oxygen in the ML and CC were higher than that of the S_2 of the adjacent fiber cells. Carbon ratio values of ML and CC were higher than the S_2 layer in both treated and untreated samples (Table 19). Oxygen count rate are relatively similar between CC and ML. The results indicate that higher amount of carbon and oxygen present in the middle lamella and cell corner. However, studies on wood component distribution strongly suggested that high lignin content and low cellulose contents are found in the middle lamella and cell corner (Sjostrom 1981; Fengel and Wegener 1983). Since wood lignin has a higher molecular weight than cellulose (Fengel and Wegener 1983; Eaton and Hale 1993), thus at any unit area or volume of the middle lamella and the cell corner have higher density than the S_2 layer. Hence, the result of this study suggests that density of the cell wall region might influence the microdistribution of carbon and oxygen.

Table 19. Count rate of carbon and oxygen for cell corner, middle lamella, and the adjacent S_2 layer of fiber of untreated and CCA-treated rubberwood.

CCA retention (kg/m^3)	Average X-ray counts						Ratio*					
	S_2		CC		ML		S_2		CC		ML	
	C	O	C	O	C	O	C	O	C	O	C	O
Control	4689	723	6541	814	7455	795	1	1	1.4	1.1	1.6	1.1
4.1	4459	661	6632	758	7685	821	1	1	1.5	1.1	1.7	1.2
10.5	4528	520	6406	838	6954	835	1	1	1.4	1.6	1.5	1.6
14.5	4218	596	5281	848	5451	833	1	1	1.3	1.4	1.3	1.4

* Mean X-ray counts are expressed in ratios with S_2 carbon and oxygen counts as 1

** S_2 –S_2 layer of fiber; CC – cell corner; ML – middle lamella

7.3.3 Comparison between the distribution of carbon and oxygen and distribution of Cr, Cu, and As

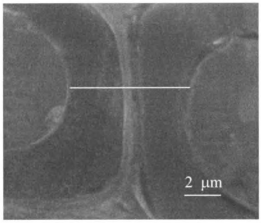
The distribution patterns of carbon and oxygen in untreated rubberwood cell walls of fiber observed by using X-ray linescan are shown in Figure 18. The SEM-EDXA linescans of CCA-treated rubberwood, counts corrected for the background for carbon, oxygen, Cr, Cu, and As are presented in Figures 18 to 21. The count rate was assumed to be proportional to the concentration of each element per unit volume since all the data were from the same sample (Russ 1984; Goodhew and Humphreys 1988). The counts for carbon and oxygen were low in the S_2 layer and high in the middle lamella in both untreated and CCA-treated samples.

The linescans show that Cr, Cu, and As were concentrated in the middle lamella and on the lumen surface. The linescans show that count rates increases from the lumen area toward the lumen surface then decreases across the S_2 layer and then increases again in the middle lamella region. This suggests that higher concentration of Cr, Cu, and As were present in the lumen surface and middle lamella region as compare to the S_2 layer.

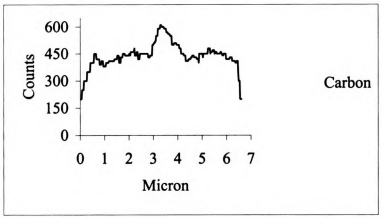
The count rate of Cr, Cu, and As increases with the concentration of treating solution. However, no significant effect of increased treating solution concentration was observed on the carbon and oxygen count rates. The linescan curve suggested that the distribution of Cr, Cu, and As were similar to the distribution of carbon and oxygen. High Cr, Cu, and As levels were observed in the region with high carbon and oxygen level. Thus, the results of this study suggest that density of ML might influence the microdistribution of CCA component in wood.

Figure 18. SEM-EDXA linescan of untreated rubberwood. (A) SEM micrograph of transverse surface showing a line across two adjacent fibers used for linescan analyses. (B) Carbon distribution and (C) oxygen distribution across two adjacent fiber cells.

(A)


2 µm

(B)


600
450
300
150
0
Counts
0 1 2 3 4 5 6 7
Micron
Carbon

(C)

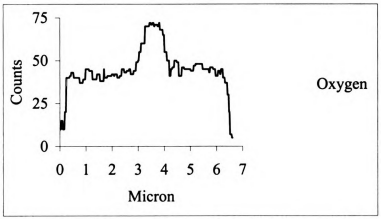
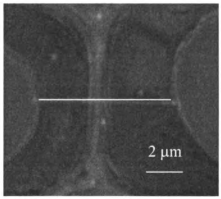
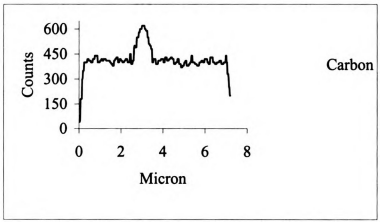

75
50
25
0
Counts
0 1 2 3 4 5 6 7
Micron
Oxygen

Figure 19. SEM-EDXA linescan analyses of rubberwood treated with 0.93% CCA solution. (A) SEM micrograph of transverse surface showing a line across two adjacent fibers used for linescan analyses. (B) Carbon distribution, (C) oxygen distribution, (D) chromium distribution, (E) copper distribution, and (F) arsenic distribution across the two adjacent fiber cells.

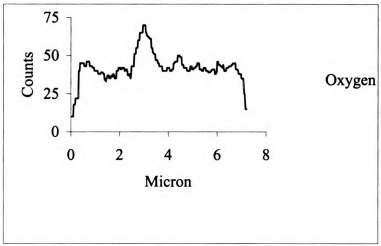
(A)


2 µm

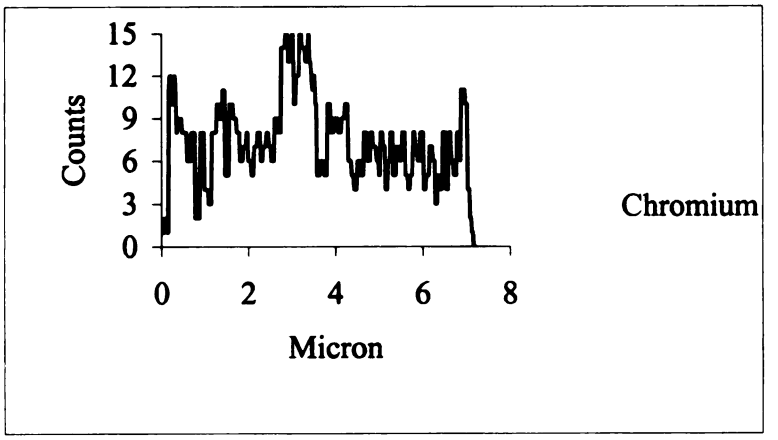
(B)


600
450
300
150
0
Counts
0
2
4
6
8
Micron
Carbon

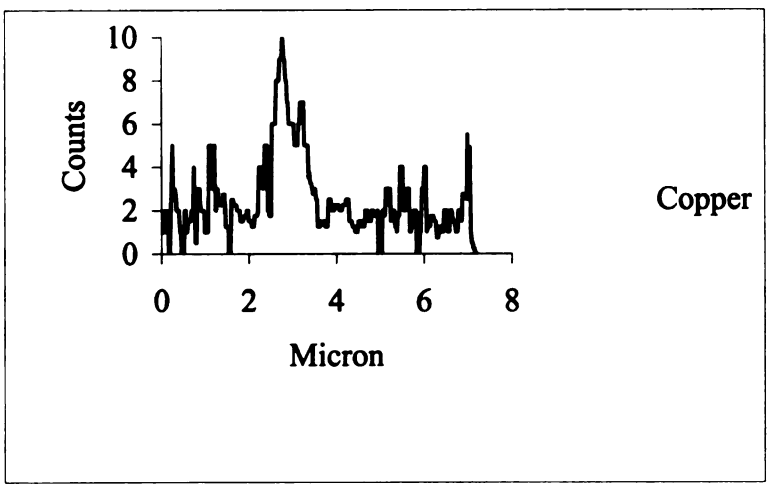
(C)


75
50
25
0
Counts
0
2
4
6
8
Micron
Oxygen

(D)


15
12
9
6
3
0
Counts
0
2
4
6
8
Micron
Chromium

(E)


10
8
6
4
2
0
Counts
0
2
4
6
8
Micron
Copper

(F)

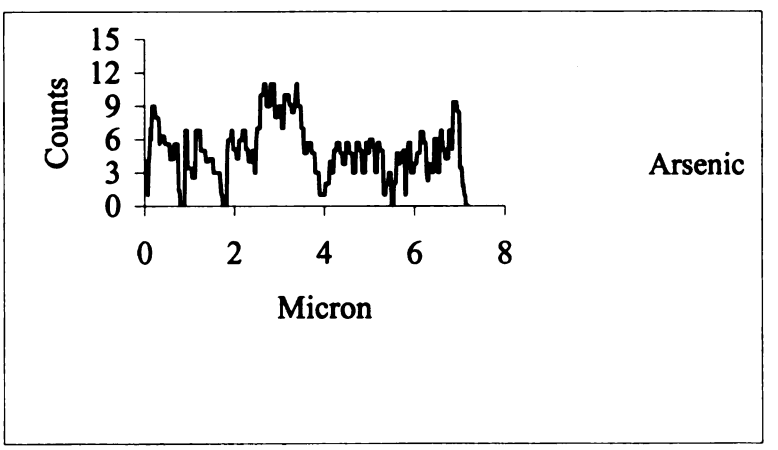
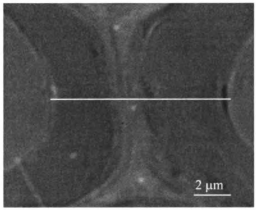
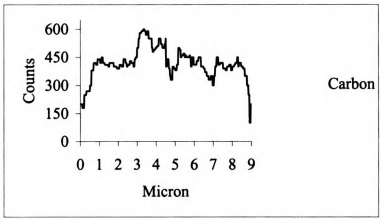

15
12
9
6
3
0
Counts
0
2
4
6
8
Micron
Arsenic

Figure 20. SEM-EDXA linescan analyses for rubberwood treated with 2.12% CCA solution (A) SEM micrograph of transverse surface showing a line across two adjacent fibers used for linescan analyses. (B) Carbon distribution, (C) oxygen distribution, (D) chromium distribution, (E) copper distribution, and (F) arsenic distribution across the two adjacent fiber cells.

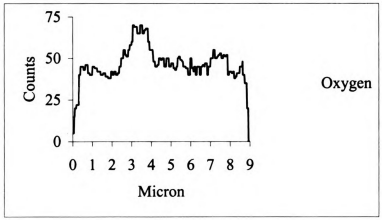
(A)


2 μm

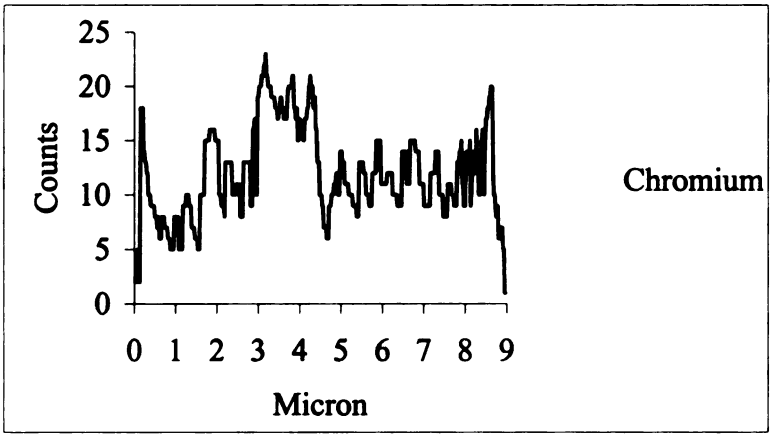
(B)


600
450
300
150
0
Counts
0 1 2 3 4 5 6 7 8 9
Micron
Carbon

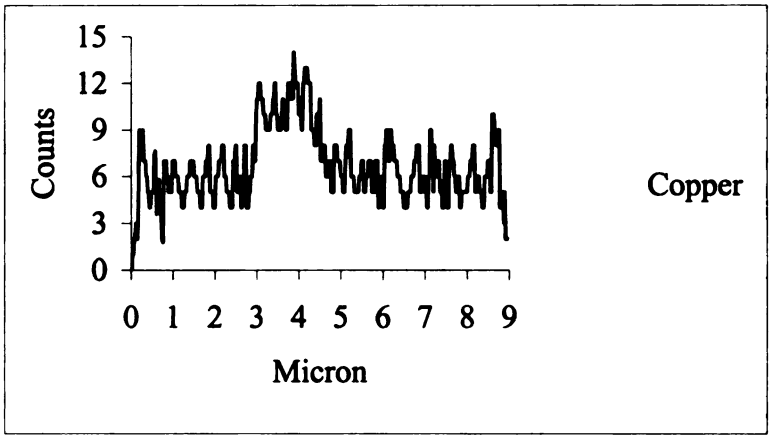
(C)


75
50
25
0
Counts
0 1 2 3 4 5 6 7 8 9
Micron
Oxygen

(D)


25
20
15
10
5
0
Counts
0 1 2 3 4 5 6 7 8 9
Micron
Chromium

(E)


15
12
9
6
3
0
Counts
0 1 2 3 4 5 6 7 8 9
Micron
Copper

(F)

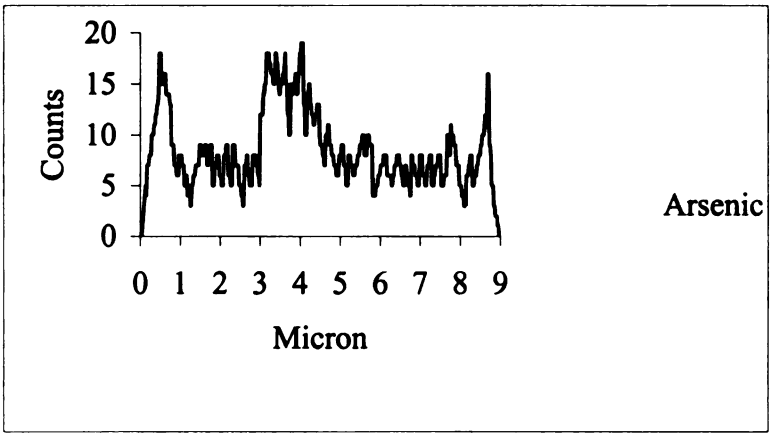
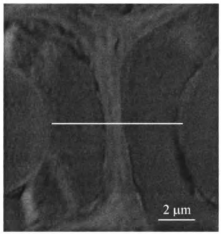
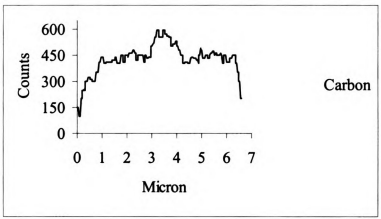

20
15
10
5
0
Counts
0 1 2 3 4 5 6 7 8 9
Micron
Arsenic

Figure 21. SEM-EDXA linescan analyses for rubberwood treated with 3.29% CCA solution (A) SEM micrograph of transverse surface showing a line across two adjacent fibers used for linescan analyses. (B) Carbon distribution, (C) oxygen distribution, (D) chromium distribution, (E) copper distribution, and (F) arsenic distribution across the two adjacent fiber cells.

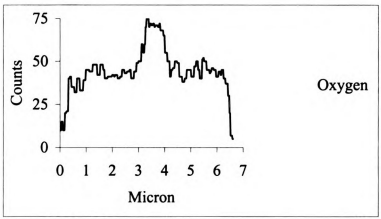
(A)


2 µm

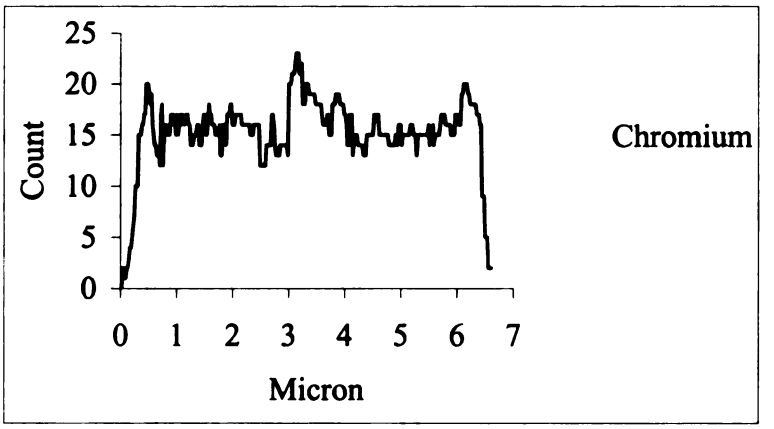
(B)


Counts
600
450
300
150
0
0 1 2 3 4 5 6 7
Micron
Carbon

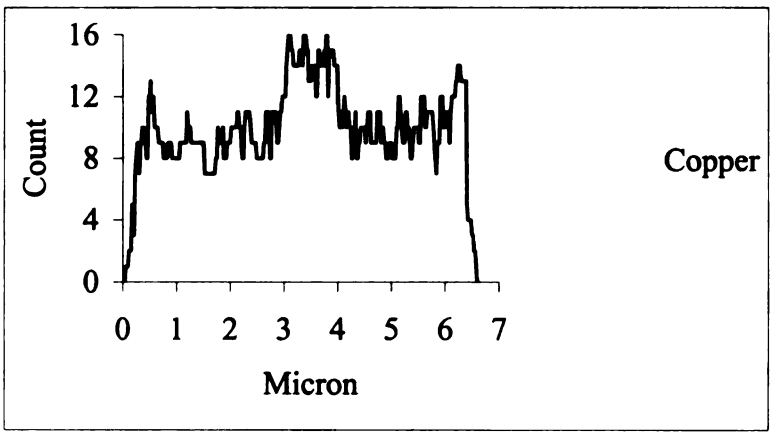
(C)


Counts
75
50
25
0
0 1 2 3 4 5 6 7
Micron
Oxygen

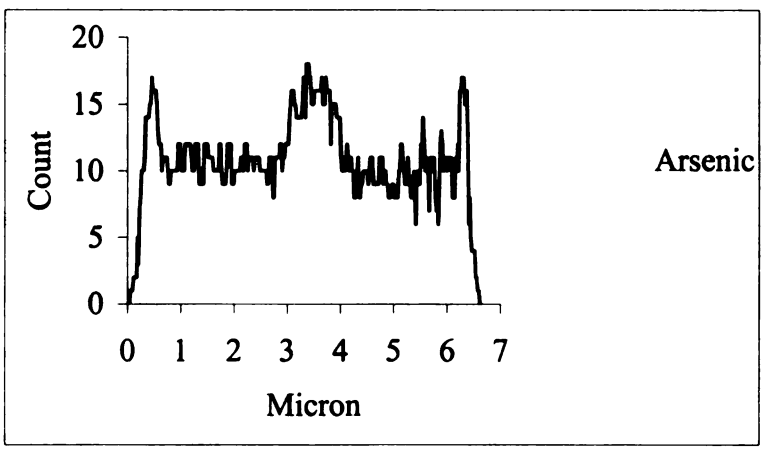
(D)


25
20
15
10
5
0
Count
0 1 2 3 4 5 6 7
Micron
Chromium

(E)


16
12
8
4
0
Count
0 1 2 3 4 5 6 7
Micron
Copper

(F)


20
15
10
5
0
Count
0 1 2 3 4 5 6 7
Micron
Arsenic

7.3.4 Cr, Cu, and As distribution in relation to decay fungi attack

According to Hedley *et al.* (1990) after the Bethell or full cell process, the cell lumina are filled with preservative solution. As the wood dries, more preservative ingredients are deposited on the lumen wall. Deposition of CCA components in the lumen surface was also reported in various softwood and hardwood species (Newman and Murphy 1996; Drysdale *et al.* 1980; Greaves 1974).

It was noticed earlier that CCA-treated rubberwood at retention of 4.1 kg/m^3 and 10.5 kg/m^3 were not satisfactorily protected from soft-rot fungi (chapter 5). At these retentions, SEM-EDXA data showed that the average Cu elemental composition in the S_2 layer of fibers were 0.29% and 0.52%, respectively. Acceptable control of soft-rot was achieved at CCA retention of 14.5 kg/m^3. At this level of retention, the elemental composition of Cu in the S_2 layer of fiber was 0.89% which correspond to 3 times higher than the Cu level in 10.5 kg/m^3 retention. The loading of Cu in rubberwood treated at 14.5 kg/m^3 retention was sufficient to prevent soft-rot attack in rubberwood in laboratory test.

7.3.5 Influence of CCA solution strength on Cr, Cu, and As distribution

The composition of Cr, Cu, and As in the cell wall, middle lamella, and cell corner increase with increasing treating solution strength (Table 18). However, the relative proportion of each element in all regions seems to have little variation with increasing CCA solution strengths. Both chromium and arsenic were present in larger proportion in all regions. The increases in the amount of Cr, Cu, and As with increasing treating solution strength in S_2 layers of fiber, ray and vessel is not as much as in cell corner and middle lamella. The S_2 layer of fiber shows the lowest increased in all three

CCA elements. The increase of Cr, Cu, and As with increase treating solution strength was also observed in *Pinus radiata*, *Betula alba*, *Alstonia scholaris* using TEM-X-ray analysis (Ryan and Drysdale 1988). Drysdale *et al.* (1980) did not observe any increases in the amount of Cr, Cu, and As with increased treating solution strength in S_2 layer of tracheid in *Pinus sylvestris* using EMMA. According to Ryan *et al.* (1988), Drysdale *et al.* (1980) used a short analyses time of 10 seconds per spot, which may be not sufficient to obtain a low noise background. In this study, 100 seconds analysis time per spot was used for every cell types and morphological regions.

CHAPTER 8

CONCLUSIONS

Based on the observations made in the study of chemical constituents in rubberwood, hollocellulose averaged 77.5%, of which 37.2% was hemicellulose and α-cellulose, respectively. Klason lignin content averaged 18.5%, whereas extractives content averaged 6% and ash 1%. Rubberwood vessels are large ranging from 150 to 300 μm and its frequency of occurrence was 3-4 vessels/mm^2. The vessel cell wall varies from 2.5 to 8 μm thick in which the thickest part of the wall was the vessel cell walls adjacent to each other. Fiber cell wall of rubberwood was thick ranging from 4 to 7 μm and fiber pits were confined to radial walls. Examination of parenchyma and ray cell revealed the presence of starch grains suggesting that rubberwood can easily be decay or colonized by fungi.

Retention and penetration of CCA after full cell treatment are influence by the pressure duration on rubberwood. Good penetration and retention were achieved after four hours of pressure (1240 kPa). This finding suggests that rubberwood was not easily treatable contrary to many studies stating that rubberwood is highly permeable to CCA treatment.

The result on the fluid pathways showed that anatomical features influence the penetration of CCA in rubberwood. Longitudinal permeability was the highest followed by radial and tangential permeability. Pre-treatment steaming was beneficial to the CCA penetration and retention. The substantial improvement in the retention and penetration of CCA suggests that pre-treatment steaming can be used to improve the permeability of rubberwood blocks.

The laboratory soil-block test showed that rubberwood was a nondurable species. Weight losses due white rot and brown rot decay fungi were 1.5 times higher than that of soft rot. Exposure of the test fungi to varying retention of CCA resulted in marked reduction in weight loss. Treatment of rubberwood with CCA at retention of 4.1 kg/m^3 reduced the weight loss to about 10% after 12-week exposure to all test fungi. A higher CCA retention of 14.5 kg/m^3 was required to protect against *Irpex lacteus, Trametes versicolor*, *Gloeophyllum trabeum*, *Postia placenta*, *Chaetomium globosum,* and *Phialophora* sp. not to exceed 2%. Based on the threshold values (Table 13), protection against all test fungi can be achieved with CCA retention of 14.7 kg/m^3. Field test is strongly recommended to validate this result.

There were differences in Cr, Cu and As microdistribution between cell types in rubberwood. Chromium, copper, and arsenic were high in the vessels. Fiber recorded the lowest level of CCA elements. Fibers, which constitute about 62% of rubberwood, contain about 0.42%, 0.63%, and 1.02% of copper in CCA-treated wood samples with retention of 4.1 kg/m^3, 10.5 kg/m^3, and 14.5 kg/m^3, respectively. The microdistribution of the individual CCA components in wood cells differ in the order of Cr > As > Cu similar to the treating solution. The concentration of Cr, Cu, and As in the vessels followed by rays suggests that vessels are the main pathway of CCA flow and the ray cells are the secondary pathway in rubberwood. This study showed that the increase in the solution strength of the treating solution resulted in the increase in the amount of CCA elements. The proportion of Cr, Cu, and As did not vary much among cell types and treating solution strength, suggesting that CCA solution did not disproportionate during their application to rubberwood.

Regardless of treating solution strength, the highest levels of Cr, Cu, and As were found in fiber-to-vessel cell corner (FV_{CC}) and fiber-to-vessel (FV_{ML}). The lowest level was detected in the S_2 layer. The concentration of Cr, Cu, and As in cell wall and morphological region increased with increasing treating solution strength. The variation of proportion of Cr, Cu, and As in all region analyzed regardless of treating solution concentrations were insignificant. Linescan analyses showed that Cr, Cu, and As were present on the surface of cell lumen. The results showed that even though the S_2 layer of fiber contains only about 0.9% copper after treatment with CCA retention of 14.5 kg/m^3, its still effective preventing decay fungi attack. This suggests that uneven distribution of CCA in wood structure did not affect the performance of CCA-treated wood rather the well-treated cell corner, middle lamella, and lumen surface prevent decay.

Using spot and linescan analyses of semi-thin section, the distribution of carbon, oxygen chromium, copper, and arsenic can be compared. Spot analyses showed that the composition of C, O, Cr, Cu, and As were higher in the middle lamella and cell corner than in the S_2 layer. Linescan analyses also showed that higher counts were obtained from middle lamella compared to the adjacent S_2 layer of fiber. The density of cell wall regions might influence the microdistribution of Cr, Cu, and As in CCA-treated rubberwood.

CHAPTER 9

RECOMMENDATIONS

Recommendations for further study can be summed up as the followings:

(1) More study is required to assess the effect of pre-treatment steaming on the strength properties of rubberwood.

(2) Since wood in actual use is exposed to many decay hazards, field test is strongly recommended to validate this result.

(3) More studies on microdistribution of preservative in hardwoods should be carried on other wood preservative system in order to obtain complete understanding of preservative efficacy.

(4) Future studies on preservative microdistribution in wood should employ SEM-EDXA or TEM-EDXA in combination with UV microscopy or any *in situ* chemical analyses method. This will elucidate the relationship between microdistribution of preservatives elements and distribution of wood chemical components.

(5) Further research is needed to determine the relationship between the distributions of carbon, oxygen and CCA elements in other wood species.

(6) More research is needed to determine how the ratios of Cr, Cu, and As affect and thus provide protection against wood destroying organism.

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