



Faculty of Resource Science and Technology

**PHYTO-TOXICOLOGICAL EFFECTS OF CHROMATED COPPER ARSENATE
PRESERVATIVE AND WOOD EXTRACTIVES FROM SELECTED MALAYSIAN
TIMBER SPECIES**

Rafidah Binti Bakran (24857)

**Bachelor of Science with Honours
(Plant Resource Science and Management)**

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Rafidah Binti Bakran

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This project is submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

No portion of the work referred to this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

(Rafidah Binti Bakran)

Program of Plant Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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LIST OF ABBREVIATIONS

As	Arsenate
As ₂ O ₅	Arsenic pentoxide
CCA	Copper Chrome Arsenate
Cu	Copper Chrome Arsenate
CuSO ₄	Copper sulphate
EPA	Environmental Protection Agency
EC50	Effect of concentration
FDA	Food and Drug Administration
ISTA	International Seed Testing Association
LOSP	Light Organic Solvent Preservative
LD50	Lethal Death/ Lethal Dosage
LC50	Lethal concentration
Na ₂ Cr ₂ O ₂	Sodium dichromate
NOEC	No Observed Effect Concentration
OECD	Organization for Economic Cooperation and Development

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Phyto-toxicological Effects of Chromated Copper Arsenate Preservative and Wood Extractives from Selected Malaysian Timber Species

Rafidah Binti Bakran

Plant Resource Science and Management
Faculty of Resource Science and Management
Universiti Malaysia Sarawak

ABSTRACT

The wastewater and contaminated soil from wood extractives and both the wood-preservation and wood processing industries are considered significant pollutants because of their chemical components such as tannins, lignins, chlorinated compounds and wood preservation. This study evaluated the phyto-toxicity effects of wood extractives from four selected Malaysian timbers species and CCA (Chromated Copper Arsenate) on lettuce seed germination. The wood extractives were obtained using hot and cold water extractives. The concentration of both wood extractives for each species and CCA solution were diluted into three different concentrations. The seed germination method was employed to test the phytotoxicity of the wood extractives and CCA solution. The experimental result of this study indicates that concentration > 0.75 % w/w CCA caused < 50 % of the lettuce seed death and the mean of germination was 41.3 %. At concentration < 0.25 % w/w CCA, no observed effect concentration (NOEC) on lettuce seed germination was observed and the germinating rate was recorded as 96.3 to 88.8 %. The phyto-toxic effects of wood extractives for each species suggested that no observed effect concentration (NOEC) on the mean of seed germination.

Keywords: wood extractives, CCA preservative, phyto-toxicity, Malaysian timbers

ABSTRAK

Effluen dan tanah yang tercemar disebabkan oleh ekstraktif kayu dan industri bahan pengawetan kayu adalah faktor-faktor yang menyebabkan pencemaran disebabkan bahan-bahan kimia berbahaya yang terkandung didalamnya. Kajian ini dijalankan untuk menilai kesan fitotoksik empat spesies kayu balak Malaysia yang terpilih dan menguji fitotoksikan bahan pengawet kayu iaitu CCA. Ekstraktif kayu diperolehi daripada ekstraktif air panas dan sejuk. Kepekatan ekstraktif kayu bagi setiap spesies dan bahan pengawet kayu CCA dicairkan kepada tiga kepekatan yang berbeza. Kaedah percambahan biji benih digunakan untuk menyiasat fitotoksik ekstraktif kayu dan CCA. Keputusan eksperimen menunjukkan bahawa CCA berkepekatan > 0.75 % w/w telah menyebabkan < 50 % biji benih salad mati dan purata percambahan ialah 41.3 %. Manakala bagi CCA berkepekatan < 0.25 % w/w, tidak menunjukkan tanda-tanda fitotoksik terhadap biji benih dan percambahan sebanyak 96.3-88.8 % telah direkodkan. Tidak terdapat kesan kepekatan ekstraktif bagi setiap spesies kayu dicatatkan (NOEC) terhadap purata percambahan biji benih.

Kata kunci: ekstraktif kayu, bahan pengawet CCA, fitotoksikan, kayu balak Malaysia

1.0 INTRODUCTION

The increasing human population in nations is linked to the development of wood industries. However during the wood processing, the ecotoxicological effects of wood extractives and wood-treated preservative such as CCA (Copper Chrome Arsenate) that leads into the environment should be a major concern to individual and regulators especially with increasing awareness of environmental safety as well as public health and safety issues.

Crosby (1998) stated that from an ecological perspective, ecotoxicology is about environmental toxicology. It deals with the unfavorable effects of chemical substances on biological system within an ecosystem at the individual, population, or community (Suter, 1993). Ecosystems have structures that not only have living individuals but also their populations, communities, and non-living habitats (Crosby, 1998). Besides, ecosystems also represent the flow of energy and materials that arise in plant photosynthesis by primary producers, moving through macroconsumers (herbivore and carnivores) and on to microconsumers (scavengers) (Crosby, 1998). Therefore, if ecotoxicology alters any of these basic ecosystem structures and functions, serious environmental effects eventually follow (Crosby, 1998).

Although, wood has been used by mankind for millinea because of its excellent environmentally friendly materials properties (Hill, 2006), there are some problems arising with wood extractives by-product waste. Wood extractives from the heartwood of species, come from a large numbers of different compounds which can be extracted from wood by means of polar and non-polar solvents (Fengel & Wegener, 1989). Each wood of trees species have different content and composition of extractives (Fengel & Wegener, 1989). However, geographical site and the season are recognized as factors that cause some variations in

heartwood extractives compounds (Swan 1968; Dahm 1970). In this study, extractives from selected Malaysian hardwoods will be examined. Four Malaysian timbers species in which extractives will be extracted are Keruing (*Dipterocarp* sp.), Belian (*Eusidoroxyton zwegeri*), *Acacia mangium*, and Engkabang Jantong (*Shorea macrophylla*). These timbers are selected for being important species with potential hazard of extractives to the environment.

CCA preservative is one of the most used water-borne wood preservatives in Malaysia due to its high biocides efficacy (Vetter *et al.*, 2009). However, the disposal of CCA wastes and CCA treated wood materials after use is problematic (Yamamoto & Hong, 1988). The main problem of the broad-spectrum Cu-based wood preservatives is their susceptibility to leaching, inducing a subsequent potentially high ecotoxicity (Townsend *et al.*, 2005). While CCA preservatives are bound to the wood fiber, there are laboratory studies showing that this preservative do leach from CCA-treated wood over time when they were immersed in water (Townsend *et al.*, 2005). In another source, Vetter *et al.*, (2008) noted that a lot of these biocides contribute to leaching and threat on other living organisms and plants in surrounding soil or ground water discharges (river and streams). Therefore, this project was focused on the impact of using CCA wood preservation in terms of phyto-toxicological effects (phytotoxic test).

This project aim to assess phyto-toxicological impact of wood extractives and wood preservative CCA towards the quality of water by observing the survival of plant growth when this plant is exposed to such phyto-toxicity condition/environment stimulated in the laboratory. Seed germination growth and the radical length (root) were measured to examine the phyto-toxicity of wood extractives and CCA preservatives on plant growth.

In this research, the biological test was selected on crude extractives but no identification of extractives is possible. Both Khalil & Winder (2008) agreed that toxicity assessment of chemical mixture difficult as it is time consuming and needs detailed understanding of interaction between chemicals and information characteristics of the chemicals present. Biological test is based on the Australian and New Zealand Environment and Conservation Council (ANZECC) environmental guidelines for conservation and sustainable development, which recognizes a hierarchy of evidence, based assessment.

In general, this project will investigate whether a specific phyto-toxicology of wood extractives and CCA preservative affects the quality of ground water in wet soils and groundwater by using statement such as “Would the level of water quality in the river and groundwater interfere with the survival of other organism such as plant”. Thus, the main goals of this study are, firstly to investigate the phyto-toxicological effects of wood extractives from selected Malaysian species and CCA preservative contaminating ground water. Secondly, to understand the phyto-toxicological effects of wood extractives and CCA preservatives towards the survival of a representative biological plant.

2.0 LITERATURE REVIEW

2.1 Ecotoxicological Testing

Ecotoxicological tests methods for chemicals were developed within the last 30 years in order to tackle problems occurring when chemicals enter the environment. There are two terms that are commonly used when referring to the toxicity of a chemical namely NOEL (No Observed Effect Level) and LD50. NOEL is the maximum daily dose at which the response is zero. This means that the experimental animals do not appear to have any adverse effects with exposure to the chemical at the specific NOEL concentration. LD50 (Lethal Dose 50) is the dose of the chemical at which 50% of the animals or plants die. In this study, rate of germination and radical length of the seed were measured and the term LD50 will be used.

2.2 Wood extractives from softwood and hardwood

Wood extractives are naturally occurring in timbers. Higher quantities of extractives are usually found in the heartwood than sapwood of species that form heartwood. These extractives are recognized as one of the matters that cause pollutant in the freshwater, receiving water and groundwater. Ek *et al.*, (2009) and Bajpai (2011) mentioned that some extractives have also been identified as main contributors to the toxicity of untreated effluent.

Softwoods and hardwoods have difference chemistry of their extractives, and also between wood species. Wood extractives can be classified according to their morphological site and function in the tree (Sjöström & Alén, 1999). Resin acids are only found in softwood, and the proportions between individual resin acids differ between wood species (Ek *et al.*, 2009; Sjöström & Alén, 1999). Besides that, fatty-acids composition between softwood and

hardwood also have different (Sjöström & Alén, 1999). Also, there is a variation between individual trees of the same species, depending on the age of the tree, genetic factors and growth condition (Ek *et al.*, 2009). Resin content between sapwood and heartwood also has differences and generally the slow growth of individual trees will content high resin.

There are also research that aimed at structural modifications of lignin due to its potential harm towards the environment (Bajpai, 2011). It is also a fact that wood with less lignin offers both economic and environmental advantages, because separating lignin from cellulose using harsh alkaline chemicals and high heat is costly and environmentally unfriendly (Bajpai, 2011). Waste lignin (like extractives) can lead to environment problems when such effluents are discharged into riverine systems unscrupulously.

Generally, extractives can be defined as the hydrophobic (lipophilic) components in wood that are extractable with neutral solvents (Ek *et al.*, 2009). These water-insoluble, lipophilic extractives called as wood resin. Wood resin can be defined as components that are soluble in liquids of low purity, e.g hexane and diethyl ether. But, sometimes extractives is used more widely where it is also include water-soluble substances, thus covering essentially all wood components other than cellulose, hemicelluloses, and lignin (Sjöström & Alén, 1999). Ek *et al.*, (2009) stated that wood extractives will contains four general main classes of lipophilic components namely triglycerides (fats and fatty acids), steryl esters and sterols, terpenoids (terpenes and polyisoprenes) and waxes (fatty alcohol and their esters with fatty acids). These main classes of lipophilic extractives in the softwood and some hardwood species such as *Eucalyptus* sp. will leach into wastewater during pulping and bleaching process (Bajpai, 2011). The major extractives compounds are triglycerides, resin acids, and steryl esters. According to (Bajpai, 2011) these lipophilic extractives are environmentally

significant because of their relative persistence and toxicity to fish and are responsible for a large part of acute toxicity of pulp and mill wastewater.

2.4 CCA preservative

CCA preservative was first developed in India and proved to be one of the most excellent wood preservative that was used in increasing amounts throughout most of the 20th century (Hill, 2006). Wood as organic material was also susceptible to deterioration by physical and chemical agents and microorganism such as insect, fungi, termites and so on. Thus, wood treated with preservatives such as CCA and LOSP (light organic solvent preservative) were used to protect wood from biodegradation. In Malaysia, timber in construction usually is treated with a water-based chemical preservatives containing CCA (Hong, 2005). According to Shibata *et al.*, (2004), the leachable arsenic from the CCA-treated wood may lead to groundwater contamination.

In previous studies of the effects of the use CCA-treated wood in playgrounds, it was conclude that children would have to ingest 10-30 kg of soil in the immediate area of the treated timber at one time for it to create a hazard (Henningsson and Carlsson, 1984). Based on this fact, many countries have either banned or restricted its use as wood-treated preservative to specific products or market. In the other hand, Frei & Albaiges (1986) in their study stated that the contamination of soil by the chemical components in the wood preservatives was more serious than expected. In their finding, it is revealed that, such soil contamination is the result of careless working habit, for example the sludge that accumulates on bottom of the treatments basin (saw dust, wood extractives, bark) during the preservation season leads to danger of leakage and leaching of CCA. Besides, the results from their studies

indicate that the wood preserving chemicals already in soil today, is an environmental problem, because there are little spontaneous biodegradation of preservatives (Frei & Albaigés, 1986).

In Turkey, a research was done by Erdin *et al.*, (1999) to investigate the contents and mobility of copper, chromium and arsenic in the soil where the CCA have been used to preserved the plant within that area. As the result from this study, it is found that leachability of As is highest near the impregnation cylinder. Plus, it is proved that Cr content in the beneath of piles of poles were highest and Cu mobility were low in deeper level of soil.

Therefore, it is essential to investigate the ecotoxicological effects of wood-treated preservatives on groundwater since they can contaminate the soil and threat an terrestrial and aquatic ecosystem.

2.5 Biological test for ecotoxicological assessment

Ecotoxicological assessment is commonly carried under laboratory condition to measure effects on the target organisms exposed to a chemical in different concentrations in specific time. Ecotoxicological test is also possible to be carried out on the the actual's habitat of the selected species. Therefore, representative species are used as surrogate for laboratory testing.

An ecotoxicological test is performed under assumed worst-case conditions and is designed to determine the effects of one or repeated applications of the test substances over a wide range of concentrations. The test result is usually summarised as the LC50- or LD50-value. i.e the concentration or dose at which 50% of the test organism die. Braunbeck *et al.*, (1998) noted that death as endpoint in toxicological study was unambiguous parameters for the individual.

Also, literature review on the ecotoxicological selection criteria noted that preferred tested organism in toxicity test should have been widely used in previous toxicity test and their use in broad contaminant test such as herbicide bioassays, heavy metal screening, salinity or mineral stress test, or allelopathy studies, indicates sensitivity to a wide variety of stressors (Kumar *et al.*, 2010; Sunahara, 2002). Most ecotoxicologist agreed with these selection criteria.

2.5.1 Plant Growth Analysis

Plant growth analysis is a common method to compare statistically measured responses of plant to the experimental treatment (Moore *et al.*, 1986). Evan (1972) and Hunt (1982) agreed that this technique allow certain biologically-important growth functions to be obtained from raw data, for example relative growth rate, root: shoot ratio, specific nutrient absorption rate and specific nutrient among others.

2.5.2 Seed Germination

Seed germination is the process that begins with imbibitions of the water and ends with the start of elongation of the embryonic axis (Bewley & Black 1985). Generally, in toxicity test emergence of the radical are used as indicators of germination (Callow, 1997).

Moore *et al.*, (1986) stated that germination experiments are usually carried out in the laboratory where the light, temperature and water supply can be controlled. Seeds will be applied to various nutrients by applying solutions of the required compositions to Petri dishes and filter paper and the seeds. Effects of the nutrients can be quantified by measuring the time taken for 50% of the seeds to germinate (Grime *et al.*, 1981).

Callow (1997) in his book stated that seed germination and root elongation test are the acute phytotoxicity test and these methods are widely used in ecotoxicity research. In previous study by Jungel *et al.*, (1999), germination and growth test were applied in ecotoxicological assessment of CCA preservatives and their actives ingredients by means of germination test using cress. This study concluded that germination test give helpful knowledge on a possible environmental risk assessment including problem related with leaching of wood extractives and wood-treated preservatives to the environment.

There are many species have been recommended for ecotoxicity test using seed germination and root elongation (Wang & Keturi, 1990). However, for this project, lettuce seeds were used instead of other suggested plant species. Lettuce is a test species that was suggested by EPA and FDA (Wang & Keturi, 1990). Lettuce species also was suggested by OECD (1984) for environmental assessment. A study was carried out to find plant species suitable for testing metal effluent toxicity, on the basis of high germination rate, long shelf life, and high sensitivity to toxicity (Wang & Keturi, 1990). In this experiment, ten plant species were compared: cabbage, carrot, cucumber, lettuce, Japanese millet, white proso millet, oat, rice, tomato, and wheat, and the results revealed lettuce is a plant species most suitable for toxicity testing.

3.0 MATERIALS AND METHODS

3.1 Area of Study

The experiments for this project were carried out at the Wood Bio-deterioration and Protection Laboratory, Faculty of Resource Science and Technology, University Malaysia Sarawak (UNIMAS).

3.2 Wood sample

Four selected wood species of Malaysian hardwoods were used in the study. They were Keruing (Dipterocarpus) heartwood, Belian (*Eusidoroxyton zwageri*) heartwood, *Acacia mangium* heartwood, and engkabang (*Shorea macrophyla*) undifferentiated heartwood/sapwood substrates. The sawdust of all these species were obtained by grinding in a Wiley Mill and used to obtain their wood extractives by cold and/or hot water extraction in the laboratory.

3.3. Wood extractives preparation

3.3.1 Oven-dry sample preparations

Each timber species was cut into wood slivers of match stick dimensions. These match sticks then were grinded into sawdust. The wood sawdust were sieved using sieve that have fraction of a particle size varying between 0.5 and 1.0 mm. 50 g of sawdust was weighed for each species and was oven-dried at 105 °C for 24 hours to obtain the oven-dry sawdust. Then, the samples were cooled in a desiccator before weighing.