



Faculty of Resource Science and Technology

**Soil contamination from wood extractives affecting plant seed
germination**

Tan Wei Khong

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

2013

Soil contamination from wood extractives affecting plant seed germination

Tan Wei Khong

(28481)

This project is submitted in partial fulfillment of the requirement for the Degree of Bachelor of Science with Honours in Plant Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

2013

APPROVAL SHEET

Name of candidate: Tan Wei Khong

Title of Dissertation: Soil Contamination from Wood Extractives Affecting Plant Seed Germination

Signature :

Supervisor's name : Associate Prof. Dr. Andrew Wong Han Hoy

Date :

Signature :

Coordinator's name : Dr. Rebicca

Date :

Declaration

I declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institute of higher learning.

.....

Tan Wei Khong

Plant Resource Science and Management

Department of Plant Science and Environment Ecology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Acknowledgement

First and foremost, I would like to express my sincere gratitude and appreciation to Associate Prof. Dr. Andrew Wong Han Hoy for his guidance, advise, assistance, and support throughout the course of this study. His wide knowledge and explanation on completing this thesis write up has been of great value experience.

My sincere thanks also goes to Associate Prof. Dr. Petrus Bulan for helping to getting the seed source from the beginning of this project and sharing about knowledge of seed testing.

Besides that, i would to thank the staff of Timber Research and Technical Training Centre (TRTTC), Kuching, Sarawak, Malaysia especially Mr. Pek Yaw Kee and Mr. Charles Riam for their assistance in processing of those wood samples into sawdust.

I would also like to thank Madam Lim Lee Lee, Senior Research Officer from Agriculture Research Center (ARC), Semengok, Kuching, Sarawak, Malaysia for her assistance and help in getting good quality seeds for this study.

My deepest appreciation also goes to all FRST laboratory staffs especially En. Mohd. Rizan and En. Syafudin for their help and assistance in providing the laboratory materials and equipments.

Last but no least, special thank goes to my dearest friends especially Mohd. Alifin, Godfred Eugene anak La-en, and Carlson Tawi anak Daud for their kind help and support throughout the course of this study.

Last, I would like to give big appreciation to my beloved family members for their moral support and financial assistance throughout my studies in UNIMAS.

Acknowledgement	i
Table of contents	ii
List of tables	v
List of figures	ix
List of plates	xii
Abstract/Abstrak	xiii
1.0 Introduction	1
2.0 Literature review	
2.1 Soil in Sarawak	4
2.2 Soil contamination	5
2.3 Wood extractives from heartwood and sapwood	7
2.4 Ecotoxicity testing	9
2.5 Plant growth analysis	10
2.5.1 Seed quality	10
2.5.2 Seed germination in contaminated environment	10
2.5.3 Seed moisture content	12
3.0 Materials and methods	
3.1 Study area	13
3.2 Soil preparation	13
3.3 Wood extractives preparation	13
3.4 Cold and hot water extractions	14

3.5 Dilution of extractives solutions	15
3.6 Seed quality tests	
3.6.1 Seed preparation	15
3.6.2 Moisture determination	16
3.6.3 Viability test	16
3.7 Phytotoxicity test for seed germination	
3.7.1 Tissue paper test	17
3.7.2 Oven-dry soil test	17
3.8 pH and total dissolved solids (TDS) determination from extractives	18
3.9 Statistical analysis	18
4.0 Results	
4.1 Cold and hot water extractions	19
4.2 Quality of wood extractives	
4.2.1 Quality of original concentration of wood extractives	21
4.2.2 Quality of increased concentration of wood extractives	23
4.3 Seed quality	
4.3.1 Moisture content determination	25
4.3.2 Seed viability test	25
4.4 Phytotoxicity test of seed germination	
4.4.1 Tissue paper test	
4.4.1.1 Acacia mangium extractives	27
4.4.1.2 Eusideroxylon zwageri extractives	31
4.4.1.3 Koompassia malaccensis extractives	35
4.4.1.4 Shorea macrophylla extractives	39

4.4.2	Oven-dry soil test	
4.4.2.1	Acacia mangium extractives	43
4.4.2.2	Eusideroxylon zwageri extractives	47
4.4.2.3	Koompassia malaccensis extractives	51
4.4.2.4	Shorea macrophylla extractives	55
5.0	Discussion	
5.1	Cold and hot water extractives	60
5.2	Quality of extractives	61
5.3	Phytotoxicity test of wood extractives on seed germination	62
6.0	Conclusion	64
7.0	References	65
8.0	Appendices	71

List of tables

Table	Page
1(a) One-way ANOVA for different concentrations of cold <i>Acacia mangium</i> extractives on tomato seed germination	28
1(b) One-way ANOVA for different concentrations of hot <i>Acacia mangium</i> extractives on tomato seed germination	28
1(c) One-way ANOVA for different concentrations of cold <i>Acacia mangium</i> extractives on okra seed germination	29
1(d) One-way ANOVA for different concentrations of cold <i>Acacia mangium</i> extractives on okra seed germination	29
2(a) One-way ANOVA for different concentrations of cold <i>Eusideroxylon zwageri</i> extractives on tomato seed germination	32
2(b) One-way ANOVA for different concentrations of hot <i>Eusideroxylon zwageri</i> extractives on tomato seed germination	32
2(c) One-way ANOVA for different concentrations of cold <i>Eusideroxylon zwageri</i> extractives on okra seed germination	33
2(d) One-way ANOVA for different concentrations of hot <i>Eusideroxylon zwageri</i> extractives on okra seed germination	33
3(a) One-way ANOVA for different concentrations of cold <i>Koompassia malaccensis</i> extractives on tomato seed germination	36

3(b)	One-way ANOVA for different concentrations of hot <i>Koompassia malaccensis</i> extractives on tomato seed germination	36
3(c)	One-way ANOVA for different concentrations of cold <i>Koompassia malaccensis</i> extractives on okra seed germination	37
3(d)	One-way ANOVA for different concentrations of hot <i>Koompassia malaccensis</i> extractives on okra seed germination	37
4(a)	One-way ANOVA for different concentrations of cold <i>Shorea macrophylla</i> extractives on tomato seed germination	40
4(b)	One-way ANOVA for different concentrations of hot <i>Shorea macrophylla</i> extractives on tomato seed germination	40
4(c)	One-way ANOVA for different concentrations of cold <i>Shorea macrophylla</i> extractives on okra seed germination	41
4(d)	One-way ANOVA for different concentrations of hot <i>Shorea macrophylla</i> extractives on okra seed germination	41
5(a)	One-way ANOVA for different concentrations of cold <i>Acacia mangium</i> extractives on tomato seed germination	44
5(b)	One-way ANOVA for different concentrations of hot <i>Acacia mangium</i> extractives on tomato seed germination	44
5(c)	One-way ANOVA for different concentrations of cold <i>Acacia mangium</i> extractives on okra seed germination	45

5(d)	One-way ANOVA for different concentrations of hot <i>Acacia mangium</i> extractives on okra seed germination	45
6(a)	One-way ANOVA for different concentrations of cold <i>Eusideroxylon zwageri</i> extractives on tomato seed germination	48
6(b)	One-way ANOVA for different concentrations of hot <i>Eusideroxylon zwageri</i> extractives on tomato seed germination	48
6(c)	One-way ANOVA for different concentrations of cold <i>Eusideroxylon zwageri</i> extractives on okra seed germination	49
6(d)	One-way ANOVA for different concentrations of hot <i>Eusideroxylon zwageri</i> extractives on okra seed germination	49
7(a)	One-way ANOVA for different concentrations of cold <i>Koompassia malaccensis</i> extractives on tomato seed germination	52
7(b)	One-way ANOVA for different concentrations of hot <i>Koompassia malaccensis</i> extractives on tomato seed germination	52
7(c)	One-way ANOVA for different concentrations of cold <i>Koompassia malaccensis</i> extractives on okra seed germination	53
7(d)	One-way ANOVA for different concentrations of hot <i>Koompassia malaccensis</i> extractives on okra seed germination	53
8(a)	One-way ANOVA for different concentrations of cold <i>Shorea macrophylla</i> extractives on tomato seed germination	56

8(b)	One-way ANOVA for different concentrations of hot <i>Shorea macrophylla</i> extractives on tomato seed germination	56
8(c)	One-way ANOVA for different concentrations of cold <i>Shorea macrophylla</i> extractives on okra seed germination	57
8(d)	One-way ANOVA for different concentrations of hot <i>Shorea macrophylla</i> extractives on okra seed germination	57
9	Lethal dose 50 of cold and hot extractives of each wood species on tissue paper and oven-dry soil test	59
10	Moisture content of okra and tomato seeds	73

List of figures

Figure		Page
1	Extractives content (%) of each wood species in cold and hot water extraction	20
2	Extractives concentration (g/ml) of each wood species in cold and hot water extraction	20
3	Total dissolved solids (μS) in original concentration of each wood species in cold and hot water treatment extraction	22
4	pH values in original concentration of each wood species in cold and hot water extraction	22
5	Total dissolved solids (μS) in increased concentration of each wood species in cold and hot water extraction	24
6	pH values in increased concentration of each wood species in cold and hot water extraction	24
7	Number of seed germinated per days	26
8(a)	Phytotoxicity effect of cold Akasia extractives on mean germination rate of tomato and okra seed in tissue paper test	30
8(b)	Phytotoxicity effect of hot Akasia extractives on mean germination rate of tomato and okra seed in tissue paper test	30

9(a)	Phytotoxicity effect of cold Belian extractives on mean germination rate of tomat and okra seed in tissue paper test	34
9(b)	Phytotoxicity effect of hot Belian extractives on mean germination rate of tomato and okra seed in tissue paper test	34
10(a)	Phytotoxicity effect of cold Kempas extractives on mean germination rate of tomato and okra seed in tissue paper test	38
10(b)	Phytotoxicity effect of hot Kempas extractives on mean germination rate of tomato and okra seed in tissue paper test	38
11(a)	Phytotoxicity effect of cold Engkabang extractives on mean germination rate of tomato and okra seed in tissue paper test	42
11(b)	Phytotoxicity effect of hot Engkabang extractives on mean germination rate of tomato and okra seed in tissue paper test	42
12(a)	Phytotoxicity effect of cold Akasia extractives on mean germination rate of tomato and okra seed in oven-dry soil test	46
12(b)	Phytotoxicity effect of hot Akasia extractives on mean germination rate of tomato and okra seed in oven-dry soil test	46
13(a)	Phytotoxicity effect of cold Belian extractives on mean germination rate of tomato and okra seed in oven-dry soil test	50
13(b)	Phytotoxicity effect of hot Belian extractives on mean germination rate of tomato and okra seed in oven-dry soil test	50

14(a)	Phytotoxicity effect of cold Kempas extractives on mean germination rate of tomato and okra seed in oven-dry soil test	54
14(b)	Phytotoxicity effect of hot Kempas extractives on mean germination rate of tomato and okra seed in oven-dry soil test	54
15(a)	Phytotoxicity effect of cold Engkabang extractives on mean germination rate of tomato and okra seed in oven-dry soil test	58
15(b)	Phytotoxicity effect of hot Engkabang extractives on mean germination rate of tomato and okra seed in oven-dry soil test	58
16	Lethal dose (LD) 50 of cold Akasia extractives on tomato germination in tissue paper test	73
17(a)	Lethal dose (LD) 50 of cold Belian extractives on tomato germination in tissue paper test	73
17(b)	Lethal dose (LD) 50 of hot Belian extractives on tomato germination in tissue paper test	74
18	Lethal dose (LD) 50 of cold Engkabang extractives on tomato germination in tissue paper test	74
19	Lethal dose (LD) 50 of hot Engkabang extractives on okra germination in oven-dry soil test	75

List of plates

Plate		Page
1	Soil sample before (A) and after (B) air-dry for several weeks	71
2	Fresh wood sawdust after grinding	71
3	Oven-dry sawdust for each wood species	72
4	Germination test of okra (left) and tomato (right) seeds in separate pedri dish	72

Soil contamination from wood extractives affecting plant seed germination

Tan Wei Khong

Program of Plant Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Abstract

Wood extractives are non-cell wall component which usually contain only 1-5 % or up to 20 % in wood. It can be solubled in non-polar organic solvent and in water. Therefore, it can be extracted by using water, acetone, ether or ethanol. Wood extractives in trees function as a protecting barrier from bugs, decay and termite attack. In this study, soil moisture content was around 0.02 % and the percentage of seed germination for okra and tomato seeds were 90 % and 80 % respectively. Furthermore, seed moisture content in okra seeds was 12.04 % while tomato seeds contained 20 % of moisture content. The germination rate of okra and tomato seeds in phytotoxicity test varied depending on the quality of seeds and concentration of extractives used. However, almost all of the tests showed no significant different of the seed germination in different concentration of cold and hot extractive solutions. Hence extractive are mainly environmentally natural chemicals in relation to generally no effect on okra and tomato seed germination.

Keywords: wood extractives, phytotoxicity test, seed germination rate

Abstrak

Ekstraktif kayu merupakan sejenis komponen yang bebas sel dinding dan biasanye mengandungi 1-5 % ataupun mengadungi sehingga 20 % dalam kayu. Ekstraktif kayu boleh larut dalam pelarut organik bebas kutub and air. Oleh itu, ekstraktif kayu boleh berpisah dengan menguna air, acetone, eter, ataupun etanol. Ekstraktif kayu berfungsi sebagai halangan untuk pokok sendiri daripada pepijat, reput, dan serangan anai-anai. Dalam penyelidikan ini, kandungan kelembapan tanah adalah lebih kurang 0.02 % dan kadar percambahan benih tomato dan bendi masing-masing adalah 90 % dan 80 %. Tambahan pula, kelembapan biji benih bendi adalah 12.04 % manakala tomato adalah 20 %. Percambahan biji benih sama ada tomato atau bendi dalam ujian phytotoxicity adalah berbeza dan bergandung kepada kualiti biji benih serta kepekatan ekstraktif yang diguna. Walau bagaimanapun, lebih kurang semua ujian yang dijalankan menunjukkan tiada perbezaan yang ketara antara percambahan biji benih dalam berbeza kepekatan ekstraktif sejuk dan panas yang diguna. Oleh itu, ekstraktif merupakan bahan-bahan kimia alam semula jadi berhubung dengan tiada kesan pada percambahan benih bendi dan tomato secara am.

Kata kunci: ekstraktif kayu, ujian phytotoxicity, kadar percambahan biji benih

1.0 Introduction

Environment pollution has been discussed for many years by scientists around the world. It can be occurring naturally or deliberately by human beings. There are many types of pollution such as soil pollution, water pollution, air pollution and sound pollution. However, soil contaminated by wood extractives will be the main topic to discuss in this study.

Soil pollution can define as the soil being contaminated by chemicals. For example, chemicals like heavy metals such as arsenic, mercury, and lead, or organic compounds where the amount in soil is over a certain permissible level. Usually soil pollution is also normally caused by human activities such as excess use of pesticides and agrochemicals by farmers, domestic dumping by household, and release of industrial waste into terrestrial areas. Other than normal pollutants such as pesticides and agrochemicals, domestic dumping and industrial waste, wood extractives are suspected have the ability to make soil contaminate. Wood extractives may leach from logs and timbers especially when they are stored outdoors for long periods on log ponds, wet soils after heavy rainfall or when extractive wastes from pulping industries contaminate ground water.

Wood extractives or resins are non-cell wall components which are soluble in nonpolar organic solvent and water (Sjostrom, 1993). Wood extractives can be divided into tannins, polyphenolics, coloring matter, essential oils, fats, resins, waxes, gums and starch. In general, total extractives content of wood depends on species, growing conditions and harvest season. It can be extracted or removed by solvents without altering the structure of cellulose or lignin (Donegen et al., 1999). Examples of solvents used are ether, water, methanol and ethanol. According to Rowe and Conner (1979), if extractives are extracted with hot water, there has a slight, slow degradation of the lignocellulosic cell wall whereas

there is no degradation of lignocellulosic cell wall in cold water. Although they contain low molecular mass in a wood but they provide great functions to woods such as colour, odour, taste, decay and insect resistance, density, hygroscopicity, flammability, and dimensional stability.

Therefore, plant seed germination test on contaminated soil will be assayed to test the impact of wood extractives. Seed germination is a process of emergence of radicle and root from seed. During this process, several requirements must be fulfilled to induce germination, which are oxygen, light, temperature, water and humidity. If any one of these requirements is not fulfilled, then seed will remain dormant. Nevertheless, although these five criteria are fulfilling, if the seeds are not viable, germination will not occur too.

In general, quality of seeds can be evaluated by test for seed purity, viability, and moisture content. Seed purity refers to the percentage of purity in seed. Seed viability is the ability of the seed to germinate and produce normal seedling whereas seed moisture content refers to the percentage of moisture in seed. Testing in seed quality is very important because it will affect the productivity and income for farmers or accuracy of an assay. Hence, it is necessary to test the seeds in this project before sowing or doing a seed germination experiment. Seed viability and moisture content will be used to test the seed quality in this project.

Studying all these effects is a part of ecotoxicity studies. According to Truhaut's (1977) cited in Relyea and Hoverman (2006) definition of ecotoxicity is the branch of toxicology concerned about the effect of pollutants on the constituents of an ecosystem. Ecotoxicological test for chemical in environment has been developed by using No Observed Effect Concentration (NOEC) and Lethal Dose (LD) 50. NOEC refers to the

maximum daily dose at which the response is zero whereas LD_{50} is the dose of chemical at which 50 % of the animals or plants die.

Finally, objectives of this project are to assay the phytotoxicity effect of hot- and cold-water extraction from four selected Malaysian timbers at different concentration on the seed germination and to establish LD_{50} values for seed germinating on tissue paper and soil.

2.0 Literature review

2.1 Soil in Sarawak

Actually soil is the unconsolidated materials between the surface of the earth. It used as growing media for plants and habitat for some microorganisms and animals. It also can be classified into three types of layer if vertically view, which is topsoil or surface soil, subsoil, and last is weathering rock. These three soil layers have their own characteristics such as color, texture, depth, soil temperature and structure. Different composition of textures or structures can pose different properties of soil such as level of water and nutrient retention in soil and depth of root penetration.

Soil can be divided into 12 classes, which is Alfisols, Andisols, Aridisols, Entisols, Gelisols, Histosols, Inceptisols, Mollisols, Oxisols, Spodosols, Ultisols, and Vertisols (Soil Survey Staff, 2010). Different types of soil have their unique characteristics and their functions as well. Organic soil in Sarawak, Malaysia can be classified as peat soil or Histosols. USDA defines that organic soil is more than half of the upper 80 cm layer of the soil (Soil Survey Staff, 1998). However, classification of organic soil in Sarawak is different compared to USDA Taxonomy due to surface vegetation is used to classify the organic soils in Sarawak instead of degree of decomposition. Soil Division of Sarawak referred to organic soil as the soils that have more than and equal to 50 cm and within 100 cm or more than twice of mineral soils overlying bedrock within 50 cm (Teng, 1996 as cited in Mohamed Murtedza et al., 2002).

The physical and chemical properties of undisturbed forest soils in Kuching such as red-yellow podzolic, grey-white podzolic, gley, podzols and peat soil were evaluated by Wong et al. (2012) in relation for the wood decay varieties exposed to the soil. In this study, they found that red-yellow podzolic at 84 % moisture content had highest mean

fungal decay mass loss as compared to grey-white podzolic, grey soil, podzols, and peat soil. Besides that, they also found that white rot (*Pycnoporus sanguineus*) poses highest mean mass loss on podzols at 43 % moisture content and soil macronutrients such as nitrogen, calcium and magnesium have significant relationship to soft rot (*Chaetomium globosum*), white rot (*Pycnoporus sanguineus*), and Brown rot (*Gloeophyllum trabeum*).

Ultisols and Oxisols were the among the mineral soils in the state of Sarawak that can be used as arable land due to their higher ability of having higher reservoir and sequester of carbon in soil compared to other types of soils (Padmanabhan et al., 2010). They also showed that Histosols or peat soil can hold highest percentage of the total soil organic carbon (SOC).

2.2 Soil contamination

A guideline which was published by Department of Environment, Malaysia (2009) listed that agricultural land, chemical manufacturing and storage, fertilizer manufacturing, pesticides and herbicides formulation, packaging and / or distribution, sawmill as well as wood treatment or disposal can potentially pollute soils and groundwater. Nevertheless, different countries have their own criteria for determining soil contamination. Research of Beyer (1990) stated that different countries have their own criteria for the degree of soil contamination. For example in Japan, Japanese Environmental Administration has developed a criteria for identifying polluted soil due to chemicals. The criteria of polluted soil are arsenic (20 ppm), cadmium (9 ppm), lead (600 ppm), and mercury (3 ppm) (Beyer, 1990).

Effect of contaminated soil can be classify into three classes, which are: (i) significant harm to human health, (ii) significant possibility of significant harm to human health, and (iii) significant harm and significant possibility of harm to non-human

receptors (Department for Environment, Food and Rural Affairs, 2012). Flora and fauna are grouped in the class of significant harm and significant possibility of harm to non-human receptors.

An assay of groundwater contamination in a phytoremediation site by Waters (2003) showed that tree tissue analysis can be used to locate the creosote contamination areas and monitor the remediation progress. In addition, Waters (2003) also showed that tissues from bud, bark, and twigs have the greatest predictive ability of creosote contamination area. However, bud tissue does not have comparable predictive ability.

There are various techniques or protocols to identify soil contamination and one of the techniques is using earthworm avoidance test. Earthworm avoidance test can be a useful bioassay to contaminated soil. In a study of Artuso et al. (2011), they used five ecologically different earthworm species to test the avoidance response to contaminated soils. At the end of their study, they showed that *Allolobophora chlorotica*, *Eisenia fetida*, and *Lumbricus terrestris* were attracted by low concentration of biosolids (2 ton hectare⁻¹) whereas they were avoided at concentration of 20 ton hectare⁻¹. Hence earthworms are sensitive enough to reflect different toxicities of biosolids by avoidance response. However, another bioassay on contaminated soil by Moura et al. (2007) showed that there was no significant difference between the test substrate and the basin soil based on earthworm mortality studies. Other test organisms could also be used such as plant seeds, fishes or scrimps.

2.3 Wood extractives from heartwood and sapwood

Heartwood and sapwood can be distinguished by their physical appearance and position in wood. Heartwood usually is darker in color and inner part of the wood whereas sapwood is lighter in color and outer part of the wood. In a healthy tree, sapwood consists of living cells which are conduct water from roots to other parts of the tree. However, cells in the inner part of the sapwood are dead (Bowyer et al., 2007). On the other hand, heartwood only consists of dead cells but they provide structural support to the trees.

Extractives are located in heartwood and sapwood but the concentration and biotoxicity are different in these two regions with the heartwood extractives being normally toxic and the sapwood contains more nutrients to wood degrading organisms. The concentration of extractives in heartwood and sapwood depends on several factors. Gutierrez et al. (1998) showed that the total wood extractives in *Eucalyptus globulus* varied during seasoning which decrease significantly from first month until third month. Furthermore, macro- and micro-distribution of extractives in tree stem are significantly different with positions either vertically or radially (Debell et al., 1999 cited in Grabner et al., 2005; Gartner et al., 1999; Gierlinger & Wimmer, 2004), cell location (Hillis, 1971 cited in Grabner et al., 2005), and positions within earlywood and latewood (Cote et al., 1966 cited in Grabner et al., 2005). However, micro-distribution of extractives is less documented because it is difficult to accurately assess extractive content in situ (Taylor, 2002).

Studies of Cote et al. (1966), Hillis's (1971), and Gierlinger and Wimmer's (2004) also showed that extractives content in larch species gradually increase from pitch to bark, highest in heartwood and sapwood boundary, and followed by an immediate drop to almost zero in sapwood. Another study of Grabner et al. (2005) showed that the total extractive