ผลกระทบจากมลพิษทางอากาศต่อความหลากหลายของไลเคนและ

ความสัมพันธ์ทางพันธุกรรมของ *PYXINE COCOES* (SW.) NYL.

(PHYSCIACEAE) ในภาคตะวันออกเฉียงเหนือ

ของประเทศไทย

นางสาวแววดาว ดาทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาชีววิทยาสิ่งแวดล้อม
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2555

EFFECTS OF AIR POLLUTION ON LICHEN

DIVERSITY AND GENETIC RELATIONSHIP OF

PYXINE COCOES (SW.) NYL. (PHYSCIACEAE) IN

THE NORTHEAST OF THAILAND

Waewdao Dathong

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy in Environmental Biology

Suranaree University of Technology

Academic Year 2012

EFFECTS OF AIR POLLUTION ON LICHEN DIVERSITY AND

GENETIC RELATIONSHIP OF *PYXINE COCOES* (SW.) NYL.

(PHYSCIACEAE) IN THE NORTHEAST OF THAILAND

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

_____

(Asst. Prof. Dr. Nooduan Muangsan)

Chairperson

_____

(Asst. Prof. Dr. Nathawut Thanee)

Member (Thesis Advisor)

_____

(Prof. Dr. Arunrat Chaveerach)

Member

_____

(Assoc. Prof. Dr. Napat Noinumsai)

Member

_____

(Asst. Prof. Dr. Wanaruk Saipunkaew)

Member

_____   _____

(Prof. Dr. Sukit Limpijumnong)   (Assoc. Prof. Dr. Prapun Manyum)

Vice Rector for Academic Affairs   Dean of Institute of Science

แววดาว ดาทอง : ผลกระทบจากมลพิษทางอากาศต่อความหลากหลายของไลเคนและ
ความสัมพันธ์ทางพันธุกรรมของ *PYXINE COCOES* (SW.) NYL. (PHYSCIACEAE)
ภาคตะวันออกเฉียงเหนือของประเทศไทย (EFFECTS OF AIR POLLUTION ON
LICHEN DIVERSITY AND GENETIC RELATIONSHIP OF *PYXINE*
COCOES (SW.) NYL. (PHYSCIACEAE) IN THE NORTHEAST OF
THAILAND) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ณัฐวุฒิ ธานี, 216 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อประเมินความหลากหลายของไลเคนเปลือกไม้ และคุณภาพ
อากาศของภาคตะวันออกเฉียงเหนือของประเทศไทย พ.ศ. 2553 ด้วยวิธี Verein Deutscher
Ingenieure และศึกษาความสัมพันธ์ทางพันธุกรรมในไลเคน *Pyxine cocoes* โดยเปรียบเทียบระหว่าง
ทัลลัสที่เจริญในเขตเทศบาลกับนอกเขตเทศบาลนครนครราชสีมา ด้วยวิธี Randomly Amplified
Polymorphic DNA (RAPD) การศึกษาพบไลเคน 91 ชนิด 34 สกุล ของ 18 วงศ์ จากต้นมะม่วง 160
ต้น โดย 71 ชนิด พบบริเวณเขตเทศบาล ส่วนนอกเขตเทศบาลพบ 85 ชนิด ชนิดที่พบมากที่สุดคือ
Pyxine cocoes (32.40%) รองลงมาคือ *Chrysothrix xanthina* (8.65%) *Lecanora leprosa* (5.20%)
และ *Graphis* sp.1 (4.36%) ในขณะที่ *Hyperphyscia granulate Hypogymnia hypotrypa* และ
Hypotrachyna osseoalba พบน้อยที่สุด (0.01%) ไลเคนที่พบเฉพาะในเขตเทศบาลเท่านั้นมี 6 ชนิด
คือ *Graphis* sp.6 *Graphis* sp.12 *Graphis* sp.14 *Graphis* sp.16 *Graphis* sp.19 และ *Phyllopsora*
sorediata ในขณะที่ 20 ชนิดพบเฉพาะนอกเขตเทศบาล เช่น *Amandinea punctata Arthonia elegans*
Buellia erubescens และ *Cladonia fimbriata* ดัชนีความหลากชนิดนอกเขตเทศบาลเมืองมีค่าสูงกว่า
ในเขตเทศบาลอย่างมีนัยสำคัญ (F = 1.365 p< 0.05) การใช้ไลเคนทุกชนิดจำแนกคุณภาพอากาศ
สามารถแบ่งออกเป็น 4 กลุ่ม ในขณะที่ใช้เพียงไลเคนขนาดใหญ่ แสดงผลได้ชัดเจนกว่าโดยแบ่ง
ออกเป็น 5 กลุ่ม เมื่อวิเคราะห์ความสัมพันธ์ระหว่างจำนวนชนิดและความถี่ของไลเคนและปัจจัย
ทางสิ่งแวดล้อมด้วยวิธีการวิเคราะห์องค์ประกอบหลัก พบว่าปริมาณความถี่ของไลเคนที่วัดในแต่
ละแปลงมีความสัมพันธ์กับความสูงจากระดับน้ำทะเลปานกลาง (r = 0.243 p<0.01) แต่แปรผกผัน
กับระยะห่างระหว่างต้นไม้กับถนน (r = -0.264 p<0.01) ในขณะที่ค่าความอุดมของชนิดมีความแปร
ผันกับจำนวนประชากรในเทศบาล (r = -0.329 p<0.01) การศึกษาความสัมพันธ์ทางพันธุกรรมของ
Pyxine cocoes ด้วยเทคนิค RAPD พบว่ามีความสัมพันธ์ทางพันธุกรรมสูง การวิเคราะห์จัดกลุ่มแบ่ง
Pyxine cocoes ออกเป็น 2 กลุ่มหลักตามเขตพื้นที่ การศึกษานี้สรุปได้ว่าไลเคนสามารถใช้เป็นตัว

บ่งชี้คุณภาพอากาศได้ และมลพิษทางอากาศในเขตเทศบาลมีผลกระทบต่อความหลากหลายของไล
เคน ความถี่ของไลเคน และความสัมพันธ์ทางพันธุกรรมของไลเคน *Pyxine cocoes*

สาขาวิชาชีววิทยา   ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2555   ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

WAEWDAO DATHONG : EFFECTS OF AIR POLLUTION ON LICHEN
DIVERSITY AND GENETIC RELATIONSHIP OF *PYXINE COCOES*
(SW.) NYL. (PHYSCIACEAE) IN THE NORTHEAST OF THAILAND.
THESIS ADVISOR : ASSISTANT PROFESSOR NATHAWUT THANEE,
Ph.D. 216 PP.

LICHEN DIVERSITY, GENETIC RELATIONSHIP, PHYSCIACEAE, THAILAND

This research aimed to assess the epiphytic lichen diversity and air quality in
the Northeast of Thailand in 2010 using the Verein Deutscher Ingenieure (VDI)
protocol and to determine genetic variation in *Pyxine cocoes* found inside and outside
the Nakhon Ratchasima municipality using Randomly Amplified Polymorphic DNA
(RAPD). Altogether, 91 epiphytic lichen species belonged to 34 genera of 18 families
on 160 mango trees were identified. Seventy-one and 85 species were observed inside
and outside municipalities, respectively. The most lichen frequency encountered was
Pyxine cocoes (32.40%) followed by *Chrysothrix xanthina* (8.65%), *Lecanora leprosa*
(5.20%), and *Graphis* sp.1 (4.36%), whereas the three least frequently encountered
lichens were *Hyperphyscia granulate, Hypogymnia hypotrypa,* and *Hypotrachyna
osseoalba* (0.01%). Six lichen species, i.e., *Graphis* sp.6, *Graphis* sp.12, *Graphis*
sp.14, *Graphis* sp.16, *Graphis* sp.19 and *Phyllopsora sorediata* were found only
inside municipalities, whereas 20 lichen species, e.g. *Amandinea punctata, Arthonia
elegans, Buellia erubescen* and *Cladonia fimbriata* were found only outside
municipalities. The ecological indices showed that lichen diversity were significantly

higher for outside than inside municipalities (F = 1.365, p<0.05). However, the using of the frequency of all lichen taxa in each plot showed that air quality in this region was separated into four groups, depending on population number in each municipality. The group of air pollution can be separated clearly into five groups by using only macrolichens. The Principal Component Analysis (PCA) showed that the lichen frequency was correlated with altitude (r = 0.243, p<0.01), but negatively correlated with the distance from the road (r = -0.264, p<0.01). The species richness had negative correlation with population in each municipality (r = -0.329, p<0.01). The study of genetic relationship of *Pyxine cocoes* using RAPD showed high value. The samples of *Pyxine cocoes* were classified into two main groups depending on the locality using the Cluster Analysis. This work concluded that lichens could be used as bioindicators of air pollution and the air pollution in the city had an impact on lichen diversity, lichen frequency, and the genetic relationship in *Pyxine cocoes*.

School of Biology

Academic Year 2012

Student's Signature_____

Advisor's Signature_____

Co-advisor's Signature_____

Co-advisor's Signature_____

ACKNOWLEDGEMENTS

the School of Biology, they give me an opportunity to learn and understand deeply in Environmental Biology. I would also like to thank my friends and colleagues in the Ecology Group for their company, friendship, supporting, helping in fieldwork, and made the journey fun.

Importantly, I would like to thank my parents for believing in me and for being proud of me. Their unconditional love and support have given me the strength and courage. Without their support, love, and encouragement, this study would not have been possible.

Finally, I am very grateful to the Commission on Higher Education, Thailand, for the Ph.D. scholarship and Suranaree University of Technology for providing a grant. My Ph.D. would be not begun and achieved without these scholarships.

Waewdao Dathong

CONTENTS

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

°C	=	Degree celsius
%	=	Percentage
cm	=	Centimeter
g	=	Gram
Hr	=	Hour
Ka	=	Kilodallton
Kb	=	Kilobase
KC	=	Kilocycle
km	=	Kilometer
L	=	Liter
m	=	Meter
mg	=	Microgram
mm	=	Millimeter
mM	=	Millimolar
µl	=	Microliter
µm	=	Micrometer
µM	=	Micromolar
nm	=	Nanometer
Min	=	Minutes
s	=	Second

LIST OF ABBREVIATIONS (Continued)

AMOVA   =   Analysis of molecular variance

AQC   =   Air quality class

As   =   Arsenic

Bp   =   Base pair

CA   =   Cluster analysis

cDNA   =   Complementary DNA

DBH   =   Diameter at breast height

ddH_2O =   Double-distilled water

DNA   =   Deoxyribonucleic acid

EDTA   =   Ethylene diaminetetraacetic acid

F   =   Fluoride

GE   =   Gel electrophoresis

GPS   =   Geo-referencing positioning system

IAP   =   Index of atmospheric purity

LDV   =   Lichen diversity value

KOH   =   Potassium hydroxide

MDS   =   Non-metric multi-dimensional scaling

nrITS   =   Nuclear ribosomal internal transcribed spacer

nrSSU   =   Nuclear ribosomal small subunit

PCA   =   Principal component analysis

PCR   =   Polymerase chain reaction

LIST OF ABBREVIATIONS (Continued)

Pd	=	Paraphenylenediamine
PRIMER	=	Plymouth routines in multivariate ecological research
RAPD	=	Randomly amplified polymorphic DNA
Ta	=	Annealing temperature
TBE	=	Tris/Borate/EDTA
TLC	=	Thin layer chromatography
UPGMA	=	Unweighted pair group method with arithmetic mean
UV	=	Ultraviolet
VDI	=	Verein Deutscher Ingenieure

CHAPTER I

INTRODUCTION

1.1 Rationale of the Study

One reason explaining the sensitivity of lichens to pollutants is their complex structures with symbiosis relationship. However, somewhat paradoxically, this is also the key to their survival in very harsh environments. Lichens consist of a fungus (mycobiont) together with green algae or cyanobacterial partner (photobiont). The association between the fungus and the photobiont in lichens has been a successful relationship lasting at least 400 million years (Taylor, 1995), therefore, lichens are accepted as a pioneer species. In partnerships, the two species are able to withstand harsh environments e.g. *Rhizocarpon geographicum* (L.) DC. and *Xanthoria elegans* (Link) Th. Fr. have even survived in space (Sancho et al., 2007). Lichens are sensitive to air pollution, which mean that lichens decrease when the air is polluted. Lichens are used as bioindicator for air quality and either air pollution monitoring and environmental changes (Estrabou, Filippini, Soria, Schelotto, and Rodriguez, 2011; Rodriguez, Weller, Wannaz, Klumpp, and Pignata, 2011). The advantage of using lichens as indicators is lichens do not have a cuticle; all minerals and pollutants are directly absorbed into their thalli. Lichens have ability in bioaccumulation; this property was used to study the impact of pollutants in lichen thalli. Lichens grow very slow (about 3 mm in a year or less than), therefore, the studying of biomonitoring is acceptable. Some of the most significant threats to lichens are air pollution, global

is acceptable. Some of the most significant threats to lichens are air pollution, global warming, forestry habitat, habitat loss, and fragmentation of habitats.

Thailand is located in the tropical zone; the climate of this region supports the high biodiversity. There are about 1,700 species found in Thailand (from lichen checklist) and 2,000 species were estimated in Thailand, although about 17,000 to 25,000 in the world (Sangvichien, Homchantara, Mongkolsuk, and Boonprakob, 2000; Pitakpong, 2009). The lichens have been observed in various purposes e.g. taxonomy, conservation, using as bioindicator of air quality and genetic variation. In case bioindicator, lichens presented a good result for Thailand as well as in the Northern Thailand (Wolseley and Aguirre-Hudson, 1991; Wolseley and Aguirre-Hudson, 1997a, 1997b; Thanwarat, 2005; Saipunkaew, Wolseley, Chimonides, and Boonpragob, 2007). For lichen surveying, many new species in Thailand were reported e.g. Pooprang et al. (2002), Jariangprasert and Anusarnsunthorn, (2005), Jariangprasert (2006), Tan et al. (2006) and Kalb et al. (2009) and this included the new lichen species of the world (Aptroot, Saipunkaew, Sipman, Sparrius, and Wolseley, 2007). There are 520 species lichens in Thailand identified in the forest, i.e., Khao Yai National Park in Nakhon Ratchasima province, Phu Hin Rong Kla National Park in Phitsanulok province. Moreover, 129 species are new taxa in Thailand and 6 species are new in the world (Buaruang, Mongkolsuk, and Manoch, 2009) and Phu Luang at Loei province, 275 species were identified, about 40 species are new in Thailand. There are a few data of lichens in the city e.g. Chiang Mai (Palee, 2002), Lampang (Pomphueak, 2005; Kanjoem, 2009), and vicinity of Chiang Mai (Saipunkaew, Wolseley, and Chimonides, 2005). In the northeast of Thailand, theuse of lichens as bioindicators was reported by Pitakpong et al. (2009), which

lichens were carried in Nakhon Ratchasima municipality area and reported that traffic had an effect on lichen diversity.

This research investigated lichens in the city habitats, where lichens might have influenced by population activity and focused entirely on lichens growing on mango trees (*Mangifera indica* L.), called epiphytic lichens. Epiphytic lichens are extremely sensitive to environmental perturbations (Nash, 1996). They have been identified as valuable indicators of environmental quality, particularly air quality, since as early as 1866 (Kricke and Loppi, 2002). Recent developments in the use of lichens as bioindicators of air quality have led to the development of the Index of Atmospheric Purity (IAP) 1968 (Kricke and Loppi, 2002). Subsequently a number of regional systems have been developed, and the Verein Deutcher Ingenieure (VDI) (Verein Deutcher Ingenieure, 1995) has been applied in various researches as a lichen mapping guideline (Brodeková , Gilmer, Dowding, Fox, and Guttov, 2006). The need for a general and widely applicable lichen based system for the determination of environmental stress within ecosystems leads to the development of the European guideline for mapping lichen diversity as an indicator of environmental stress. The concept of environmental stress is integrated with stress creating factors, such as atmospheric pollution, eutrophication, and climate change (Asta et al., 2008). The European guideline presents the first attempt to develop a unified guideline with application at European level with aim to provide a repeatable and objective strategy for mapping lichen diversity as an indicator of environmental changes.

Various studies have addressed the abundance and distribution of lichens in Thailand. However, the environmental status of the Northeast of Thailand has not been well-addressed using formal lichen based indices. Consequently, there is

potential for the development of new insights from the application of the European guideline for mapping lichen diversity as an indicator of environmental stress (Asta et al., 2002; Svoboda, 2007; Asta et al., 2008; Svoboda, Peksa, and Vesel, 2010) to the municipal area of each province. This research focused on the differences that arise in relation to epiphytic lichens in city habitats in the municipality of the northeast of Thailand and aimed to advance understanding of the factors that drive the sensitive and dynamic patterns for epiphytic lichen abundance and distribution in the Northeast of Thailand.

For the study of genetic relationship of *Pyxine cocoes* (Swartz) Nyl., one of tolerant species in the family Physciaceae was found both in the urban and suburban areas (Pimwong, 2002; Thanomsap, 2006). According to preliminary surveying, *P. cocoes* was the dominant species found both inside and outside municipal areas (Dathong, Thanee, and Saipunkaew, 2012). Although they survived, the air pollution may damage some genes therefore, the study of their genetic variation and using them as bioindicator for air quality in the municipality area is needed.

1.2 Research Objectives

1. To investigate and assess epiphytic lichens as bioindicators for air quality in the northeast of Thailand.

2. To examine the correlation between lichen diversity, air quality and factors influencing to lichen diversity in the northeast of Thailand.

3. To study the genetic relationship in *Pyxine cocoes* (Swartz) Nyl, which is differently affected by anthropogenic levels in Nakhon Ratchasima municipality Nakhon Ratchasima province, Thailand.

1.3 Hypotheses

1. The lichen diversity is higher for outside than in municipal areas of the northeast of Thailand.

2. Lichen diversity and distribution of lichens in the city have been affected by anthropogenic pollution levels.

3. Lichens can be used as bioindicator of air pollution in the city habitat of the northeast of Thailand.

4. *Pyxine cocoes* can be used to examine the genetic relationship which located at different anthropogenic in Nakhon Ratchasima municipality area.

1.4 Scope and Limitations

This research investigated epiphytic lichens on the mango trees in eight provinces in the northeast of Thailand in 2010 and genetic variation was determined in 2011 by selecting the specimens of *Pyxine cocoes* in the Nakhon Ratchasima province.

1.5 Expected Results

This study will provide knowledge on lichens the northeast of Thailand:

1. The research can provide lichen species and evaluate air pollution level using epiphytic lichens in the northeast of Thailand.

2. The results of this research will be useful for environmental management and provide value-added information for using lichens as bioindicator of air quality in the northeast of Thailand.

3. The result of genetic relationship can be used in animal and human health that can be changed the genes of organisms.

4. The results can be used for lichen conservation.

1.6 References

Aptroot, A., Saipunkaew, W., Sipman, H. J. M., Sparrius, L. B., and Wolseley, P. A. (2007). New lichens from Thailand, mainly microlichens from Chiang Mai. **Fungal Diversity.** 24: 75-134.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., and Van Haluwyn, C. (2008). **European guideline for mapping lichen diversity as an indicator of environmental stress.** [On-line]. Avilable: http://www.thebls.org.uk/content/documents/eumap.pdf.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., Van Haluwyn, C., and Wirth, V. (2002). Mapping lichen diversity as an indicator of environmental quality. In Nimis, P. L., Scheidegger C., and Welseley P. A. (eds.). **Monitoring with lichens-monitoring lichens.** London: Kluwer.

Brodeková, L., Gilmer, A., Dowding, P., Fox, H., and Guttov, A. (2006). An assessment of epiphytic lichen diversity and environmental quality in Knocksink Wood Nature Reserve, Ireland. **Natural History.** 223(3): 215-223.

Buaruang, K., Mongkolsuk, P., and Manoch, L. (2009). Morphology and anatomy of lichen family Parmeliaceae at Phu Hin Rongkla National Park. **Journal of Microscopy Society of Thailand.** 23(1): 20-24.

Dathong, W., Thanee, N., and Saipunkaew, W. (2012). The investigation of epiphytic lichen diversity in the Northeast of Thailand. In **Proceeding of the 7ᵗʰ International Association for Lichenologist**, Bangkok, Thailand.

Estrabou, C., Filippini, E., Soria, J. P., Schelotto, G., and Rodriguez, J. M. (2011). Air quality monitoring system using lichens as bioindicators in Central Argentina. **Environmental Monitoring and Assessment**. 182: 375-383.

Jariangprasert, S. (2006). New taxa of the lichen genus *Pertusaria* (Ascomycota) from Thailand. **Mycotaxon**. 96: 109-122.

Jariangprasert, S., and Anusarnsunthorn, V. (2005). Additional new taxa in the lichen genus *Pertusaria* (Lichenized Ascomycotina) from Thailand. **Mycotaxon**. 91: 279-292.

Jariangprasert, S., Archer, A. W., Elix, J. A., and Anusarnsunthorn, V. (2002). Two new species in the lichen genus *Pertusaria* (Ascomycotina) from Thailand. **Mycotaxon**. 83: 353-356.

Kalb, K., Buaruang, K., Papong, K., and Boonpragob, K. (2009). New or otherwise interesting lichens from the tropics, including the lichen genus *Ramboldia* in Thailand. **Mycotaxon**. 110(1): 109-123.

Kanjoem, R. (2009). **Lichen diversity and monitoring of sulfur dioxide around Mae Moh power plant area, Mae Moh District, Lampang Province**. M.S. thesis. Chiang Mai University Chiang Mai, Thailand.

Kricke, R., and Loppi, S. (2002). Bioindication: The I. A. P. Approach: Monitoring with lichens- monitoring lichens. In P. L. Nimis, C. Scheidegger, and P. A. Wolseley (eds.). **NATO Science Series IV** (Vol. 7, pp. 21-37). UK: Kluwer.

Nash, T. H. (1996). **Lichen biology**. UK: Cambridge University Press.

Palee, T. (2002). **Used of lichens as bioindicator for air pollution monitoring in town and urban of Chaing Mai province in 2001.** M.S.thesis. Chiang Mai University, Chiang Mai, Thailand.

Pimwong, S. (2002). **Distribution and frequency of lichen *Pyxine cocoes* Swartz and *Dirinaria picta* Swartz in Chiang Mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Pitakpong, A. (2009). **The use of lichen as an indicator for environmental monitoring in Nakhon Ratchasima municipality and Sakaerat Environmental Research Station.** M.S. thesis. Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Pomphueak, K. (2005). **Use of lichens as bioindicators for air quality monitoring in amphoe Mueang, Lampang.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Pooprang, T., Boonpragob, K., and Elix, J. A. (1999). New species and new records in the lichen family Parmeliaceae (Ascomycotina) from Thailand. **Mycotaxon.** 71: 111-127.

Rodriguez, J. H., Weller, S. B., Wannaz, E. D., Klumpp, A., and Pignata, M. L. (2011). Air quality biomonitoring in agricultural areas nearby to urban and industrial emission sources in Córdoba province, Argentina, employing the bioindicator *Tillandsia capillaris*. **Ecological Indicators.** 11(6): 1673-1680.

Saipunkaew, W., Wolseley, P. A., and Chimonides, P. J. (2005). Epiphytic lichens as indicators of environmental health in the vicinity of Chiang Mai city, Thailand. **The Lichenologist.** 37(4): 345-356.

Saipunkaew, W., Wolseley, P. A., Chimonides, P. J., and Boonpragob, K. (2007). Epiphytic macrolichens as indicators of environmental alteration in northern Thailand. **Environmental Pollution.** 146(2): 366-374.

Sancho, L. G., De La Torre, R., Horneck, G., Ascaso, C., De Los Rios, A., Pintado, A., Wierzchos, J., and Schuster, M. (2007). Lichens survive in space: Results from the 2005 lichens experiment. **Astrobiology.** 7(3): 443-454.

Sangvichien, E., Homchantara, N., Mongkolsuk, P., and Boonprakob, K. (2000). Lichen research in Thailand. **Mycologist.** 14(4): 151-152.

Svoboda, D. (2007). Evaluation of the European method for mapping lichen diversity (LDV) as an indicator of environmental stress in the Czech Republic. **Biologia.** 62(4): 424-431.

Svoboda, D., Peksa, O., and Vesel , J. (2010). Epiphytic lichen diversity in central European oak forests: Assessment of the effects of natural environmental factors and human influences. **Environmental Pollution.** 158(3): 812-819.

Tan, B. C., Vongkuna, K., Manachit, S., and Santanachote, K. (2006). New records of Thailand mosses collected from Chiang Mai province. **Tropical Bryology.** 27: 95.

Taylor, T. N. (1995). The oldest fossil lichen. **Nature.** 378: 244.

Thanomsap, K. (2006). **Distribution and frequency of lichen** *Pyxine cocoes* **Swartz and** *Lecanora* **cf.** *leprosa* **Fée in Lamphun city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Thanwarat, C. (2005). **The distribution and frequency of lichen** *Hyperphyscia adglutinata* **Florke and** *Lecanora* **cf.** *leprosa* **Fée in Chiang Mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Wolseley, P. A., and Aguirre-Hudson, B. (1991). Lichens as indicators of environmental change in the tropical forests of Thailand. **Global Ecology and Biogeography Letters.** 1: 170-175.

Wolseley, P. A., and Aguirre- Hudson, B. (1997a). The ecology and distribution of lichens in tropical deciduous and evergreen forests of northern Thailand. **Journal of Biogeography.** 24(3): 327-343.

Wolseley, P. A., and Aguirre- Hudson, B. (1997b). Fire in tropical dry forests: corticolous lichens as indicators of recent ecological changes in Thailand. **Journal of Biogeography.** 24(3): 345-362.

Verein Deutscher Ingenieure. (1995). Measurement and evaluation of phytotoxic effects of ambient air pollutants (emissions) with lichens. In **Mapping of lichens for assessment of the air quality,** VDI-Richtlinie 3799, Platt1/part 1: Düsseldorf.

CHAPTER II

REVIEW OF LITERATURES

2.1 Definition and Classification of Lichens

Lichens were included in Greek literature in about 300 BC by Theophrastus, primarily to describe of lichens that grow on the bark of olive trees (Hawksworth and Hill, 1984). From up to the end of the 16th century, the lichens were described based on their physical appearance and often incorrect as types of mosses or seaweeds. Until the beginning of the 18th century, the microscopes were advanced for enabling detailed anatomical studies of lichens, which revealed their special dual characters consisting of at least different two organisms of algal and fungal partners (Nash, 1996). Although in the 18th century lichens on the bark of trees and rocks that have recognized as physically joined algae and filaments of fungi (Hale, 1979). Indeed, the character of lichens was determined as comprising of algae and fungi living in a symbiotic relationship. This association provided a more specific explanation of the living arrangement between both partners, but in this case, it was difficult for finding a universal definition from the variability of fungal-algal associations and the range of symbiosis (Purvis, 2000; Orange, James, and White, 2001; Wolseley, 2002; Allaby, 2004; Gilbert, 2004). Therefore, the interpretation of lichens as an association of two organisms living in symbiotic relationship seems to be the most common dimension of these definitions. For example, in the dictionary of the fungi; lichens are defined as a stable self-supporting association of a fungus (mycobiont) and a green alga or

cyanobacterium (photobiont) (Kirk and Ainsworth, 2008). Moreover, the lichens are included as an ecologically obligate, stable mutualism between an ex-habitant or fungal partner and an inhabitant population of extracellular located unicellular or filamentous algal or cyanobacterial cells (Kirk and Ainsworth, 2008). Lichens consist of a fungus (mycobiont) and a photosynthetic green alga or blue-green alga (photobiont) (Nash, 1996). The photosynthetic partner produces food for the whole lichens and the fungus provides a stable, protective environment for the photosynthetic partner. The fungus forms the main lichen body, in most cases, the alga lies in layers between upper and lower fungal cortex (Hale, 1979). Lichens are completely different from bryophytes such as mosses and liverworts, which they often grow in the same conditions. The whole body of lichens called thallus; most grow as crusts (crustose), some are leafy (foliose), while others are shrubby (fruticose) and tiny scales (squamulose) (Nash, 1996) (Figure 2.1). The upper surface of many lichens bears special structures, which appear as miniature pots, or volcanoes with a minute pore at the tip. Some are brightly colored, others more muted, and many are black. All these structures are included as fruiting bodies as they forcibly discharge tiny spores, which become airborne (Figure 2.2 and 2.3). Lichens represent many colors: white, gray, black, yellow, and orange, sulfur, apple green, and pink or scarlet, the color can be changed with the changes of environment such as dry and wet conditions.
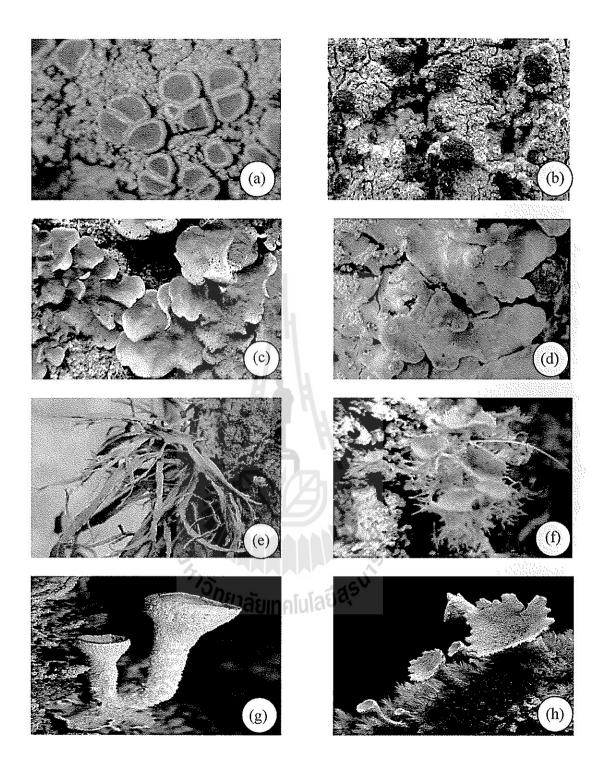
Figure 2.1 Lichens thallus types: crustose (a) *Lecanora* sp., (b) *Amandinea extunata*, foliose (c) *Parmotremar tinctorum*, (d) *Pyxine cocoes*, fruticose, (e) *Ramalina* sp., (f) *Teloshistes* sp., and squamulose, (g) *Cladonia* sp., (h) *Cladonia* sp.

Lichens are like other non-lichenized ascomycetes fungi, which produce sexual fruiting body containing spores. There are two types of the fruiting body, the shape with flattened or semi-globose discs (apothecia) and flask-shaped structures (perithecia) (Figure 2.2). The spores will be released from the mature fruiting body and finding the right algal partner to form new lichen therefore, this type of reproduction is unreliable. Ascospores vary in size, shape, and structure and may be colorless or brown (Figure 2.3). In other hand, some lichens increase their chance of achieving reproduction by producing special parts that become detached and grow into a new plant (propagules) containing both alga and a fungus called vegetative reproduction (Figure 2.4). The most common are powdery structures (soralia) that develop as pustules on the upper surface. These release small clumps of algal cells (soredia) held together by a web of fungal threads (hyphae). The other structures for vegetative reproduction, also unique to lichens, are isidia and they contain both alga and fungus (Gilbert, 2004). The classification of lichens is based on fungi; the nomenclature of fungi including lichen-forming fungi is governed by the International Code of Botanical Nomenclature (Kirk and Ainsworth, 2008).

2.2 Lichen Genetics

Lichens consist of two organisms; therefore, their genes are separated into two parts of fungi and algae. The fungi have developed a range of symbiotic interactions with plants (from single celled green alga to multicellular vascular plants) that have allowed them to diversify into an array of terrestrial niches (White, Bruns, Lee, and Taylor, 1990; Selosse and Le Tacon, 1998). The most successful symbioses, based on fungal species numbers alone, are the lichen symbiosis between a filamentous fungus

(mycobiont), and either a green alga, a cyanobacterium, or both (photobiont). Over 13,500 species of lichen-forming fungi have been described based on the transformative properties of the symbiosis to the fungal phenotype, comprising nearly one fifth of all known fungal species (Kirk and Ainsworth, 2008).
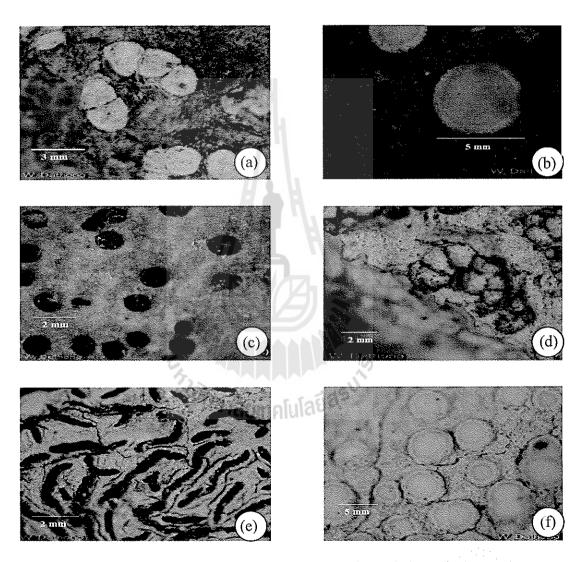
Figure 2.2 Fruiting bodies or vegetative reproduction of lichens: (a) *Arthonia* sp. (apothecia), (b) *Basidia* sp. (apothecia), (c) *Anthacothicium* sp. (perithecia), (d) *Arthonia* sp. (apothecia), (e) *Graphis* sp. (lirellate apothecia), (f) *Lecanora* sp. (lecanorine apothecia).
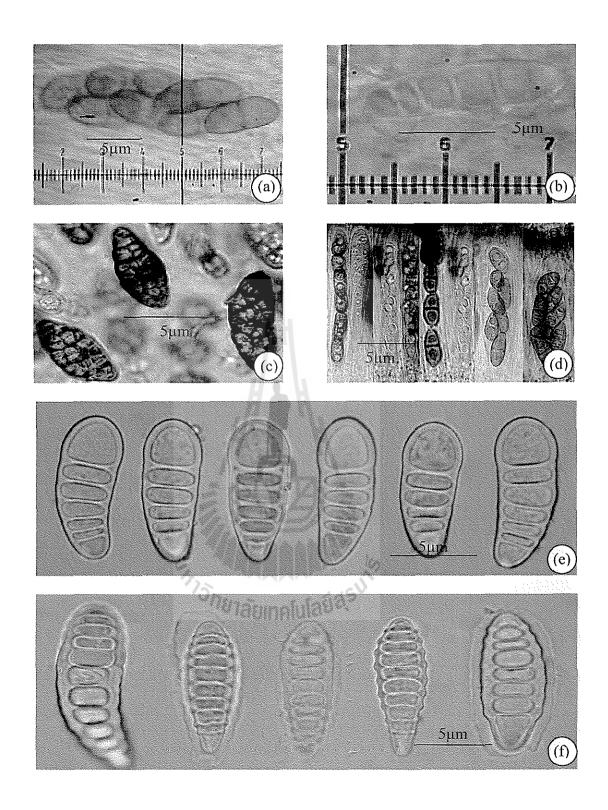
Figure 2.3 Spore characteristics (a) *Physcia* sp., (b) *Graphis* sp., (c) *Graphis* sp.1, (d)

Rinodina sp., (e) *Arthonia peraffinus*, (f) *Graphis* sp.3.
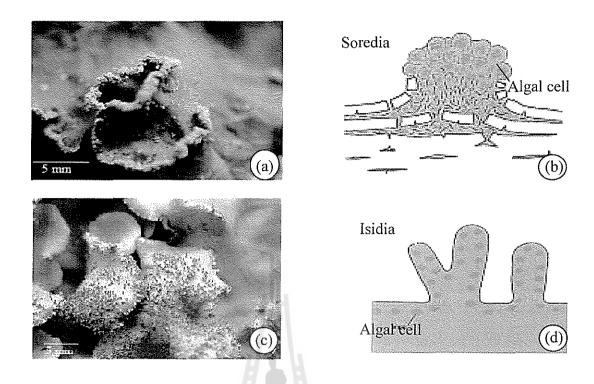
Figure 2.4 Asexual reproductive structures (a) closed up of soredia, (b) drawing of

soredia, (c) closed up of isidia, (d) drawing of isidia.

Source: http://picdb.thaimisc.com/p/pangfan/4304-5.jpg

Because extant lichen-forming fungal species diversity is highly concentrated

in one main clade of deep origin within the fungal tree of life, it is possible that

lichens represent the oldest mutualistic symbiosis between fungi and land plants

(James et al., 2006). The slow growth and genetic intractability of lichen symbionts

have prevented researchers from answering the most basic cellular and molecular

based questions about how mycobiont and photobiont partners find and recognize

each other, and how they maintain this relationship (Joneson, Armaleo, and Lutzoni,

2011). The initial stages in the development of lichen symbiosis can be routinely

examined in vitro through the axenic culturing of symbionts. In experimental

resynthesis, aposymbiotic cultures of mycobionts and photobionts are mixed and allowed to grow together on nitrocellulose filters over reduced nutrients (Armaleo, 1991; Bačkor and Fahselt, 2003). The development of a stratified lichen thallus follows four general developmental stages consisting of: pre-contact, contact and growth together, grows into a transitional pre-thallus, and growth into a stratified thallus (Galun, 1988). These stages are parts of a dynamic continuum that originates anywhere novel fungal–algal contacts are made (Armaleo and Clerc, 1991). The synchronized growth of the latest stage in lichen development cannot be regularly achieved in the lab, and for a reason the present study focuses on only the first two stages. The first two stages of development (pre-contact and contact) can be observed in vitro within 21 days, and the latter step is accompanied by a distinctive phenotype change in the fungus (Joneson and Lutzoni, 2009). The phenotypic change in *Cladonia grayi* consists of a switch from apical growth with long internodes, to reduced apical growth and internodes length with increased lateral branching (Joneson et al., 2011). Entry into lichen symbiosis is accompanied by changes in gene regulation and expression in the photobiont and mycobiont (Armaleo and Miao, 1999), although it is not yet knows at which step this genetic modification begins. The *Cladonia grayi* and *Asterochloris* sp. model system of symbiosis was used to explore the combined up regulated transcriptome of early lichen development using. At this time, expressed sequence tags (ESTs) and/or cDNA libraries are lacking for any closely related fungi or green algae to the lichen symbionts, and the annotation of any gene products relies on homology-based searches. The extracellular communication between symbionts prior to contact has generally been disregarded, as chemotropic signals do not appear to be exchanged (Ahmadjian, 1967).

2.3 Ecology of Lichens

Lichens play an important role in colonizing new surfaces among the metabolites excreted by some lichens are acids. Acids have the capacity to degrade the surfaces on which they are located, thus releasing minerals for uptake by the thallus. Acidic digestion has the effect of causing the slow disintegration of the surface, especially of limestone and other calcareous materials. Rusting of surfaces is probably unimportant in terms of the total uptake of minerals by lichens. Most minerals are extracted from solutes in rain or surface water flow (Baron, 1999). Lichens grow extremely slowly; any thallus may be many decades old. The outer edge is probably the only active component of the thallus, unless the lichens have started to overgrow it. The inner part is commonly inactive. Lichens have the potential to withstand a wide range of environments. Thus, they adapt rapidly to local and seasonal changes in temperature and water availability and found in bleak arctic and desert environments (Baron, 1999).

The thallus of lichens has the capacity to cope with the frequent aridity of the environment. Foliose will curl as the thallus dries, and then flatten as it rehydrates. Photosynthesis follows the pattern of wetting and drying. While changes in form enable a return from dehydration, the presence of trehalose, and possibly a range of polyols, is also important. These metabolites enable the cytoplasm to desiccate, while protecting the functionality of the enzymes. Thus, primary production of lichens is highly dependent on the moisture levels of the environment, but they can survive desiccation (Nash, 1976; Nash and Wirth, 1988; Nash, 1996).

The slow rate of growth and the reliance on minerals in rain or high humidity has consequences for survival of lichens in polluted environments. Lichens absorb all

minerals in the rain, and the presence of pollutants, including sulfur, will result in the decline of the thallus. Because of their sensitivity to pollutants, most lichens are uncommon in areas affected by acid rain and aerial pollutants (Ferry, Baddeley, and Hawksworth, 1973). However, some lichens grow on surfaces containing high concentrations of metals, and must be adapted to those metals: pollution of a single type is likely to select lichens that can tolerate the pollutant. In cities, the pollution profile is variable and changing over time. Thus, lichens are disappearing from cities (Nash, 1996).

Remnants of lichen communities within cities are associated with protected habitats. Churchyards for instance may house a wide diversity of lichens. Lichens are not welcome inhabitants of city where high pollution level; however, the capacity of lichens to rust the surface leads to loss of the structural integrity of stone and concrete. Attempts to remove lichens, and prevent the re-colonization of gravestones and other surfaces, are rarely successful (Baron, 1999).

2.4 Factors Influencing Epiphytic Lichen Diversity

Lichens are classic pioneer and colonizers in a wide range of environments. They grow on the bark of temperate trees or as epiphytes on leaves of tropical rain forest trees. Lichens have specific requirements for their habitats. Although they can occur on a variety of substrates, each substrate must has the individual components in the right amounts that growing lichen needs. These requirements are water, air, nutrients, light, and substrates (Hale, 1969, 1979; Nash, 1996).

A large number of factors determine the development of particular assemblages of epiphytic lichens (Barkman, 1958; Brodo, Sharnoff, and Sharnoff,

2001; Orange et al., 2001). The influence of these factors on lichen development in a given ecosystem varies. Ranius et al. (2008) suggests that the natural distribution of epiphytic lichens is mainly influenced by the species of trees and microhabitats available. The most important microhabitat characteristics are humidity and availability of light through the canopy. In addition, general ecological factors such as climate, topography and geology as well as the prevailing land use, the extent of pollution, and the age and type of trees influence the occurrence of lichens (Orange et al., 2001). Coppins and Coppins (2002) also emphasizes the importance of the tree species has impact on epiphytic lichen community. In the diverse conditions that prevail in the urban ecosystems, trees and their epiphytes are subjected to varying degrees of exposure in terms of light, temperature, humidity, physical abrasion and the drying effects of wind (Coppins and Coppins, 2002; Ayrault, Clochiatti, Carrot, Daudin, and Bennett, 2007). Will-Wolf et al. (2002) suggest that forest age and the continuity of forest canopy are the critical factors for the development of epiphytic lichen communities. Indeed, forest structure affects the lichen distribution through its influence on light and the moisture regime. Gaps with low shrubby substrates or low branches on surrounding trees are important for lichen growth. Such microhabitats provide both high moisture, direct light, and represent diversity hotspots in moist forest climates. Forest edges differ from forest centers in both light and wind, which affect the moisture regime present (Will-Wolf et al., 2002). Tree age, the size and age of gaps, standing dead snags and the size, age and quantity of downed woody debris are all important microhabitat characteristics of lichen growth. However, Will-Wolf et al. (2002) also recognizes that lichen communities in forests co-variant most closely with climatic variables such as precipitation, moisture status, temperature, and

evapotranspiration. There are various factors have been highlighted in the literature as having a special significance in terms of influencing lichen abundance in forest ecosystems.

2.4.1 Dead Wood

The quantity of dead wood represents a particularly important host for epiphytic lichens in forests (Humphrey, Davey, Peace, Ferris, and Harding, 2002; Hauck, 2005). Indeed, the significance of dead wood for lichen biodiversity in forests has recently been recognized. Humphrey et al. (2002) noted that the dead woods were important microhabitat for lichen species-richness in semi-natural woods and plantations.

2.4.2 Air Quality

Epiphytic lichen diversity differs between forests with a high degree of atmospheric pollution and those with a low pollution status (Hauck, 2005). In forests with moderate or low atmospheric pollution, the microclimatic factors (light, water availability, physical substrate properties, substrate age, stand history, and competition) are found to have a greater importance than in forests with high atmospheric pollution, where pollutants are the dominant factor.

2.4.3 Relative Humidity

Lichen abundance is particularly sensitive to change in relative humidity (Frahm, 2003). Indeed, lichen habitats are characterized by 20-30% shorter wet phases and accordingly longer phases of desiccation than bryophyte habitats. Generally, lichens are present in higher numbers in the crowns of trees and bryophytes at the trunks. Fox et al. (2001) has reported that the location of lichens on trees is important in relation to relative humidity. Stems and trunks that rise

perpendicularly from the ground tend to have a continuous cover of bryophytes. In contrast, those growing at an angle to the ground have a denser cover of bryophytes on the wet surface while lichens dominate on the drier surfaces. Lichens are more tolerant of desiccation, and require dry periods for the purposes of metabolism (carbon assimilation).

2.4.4 Tree Bark Characteristic and pH

The nature of the bark is an important factor for epiphytic lichen development. The physical and chemical properties of bark vary between different tree species. Bark properties also vary on a single mature tree depending on the age of the bark. A range of bark related parameters include the presence of other epiphytes, nutrient status, water holding capacity, and buffer capacity all of which are important determinants of lichen development (Coppins and Coppins, 2002; Coppins. 2002; Larsen et al., 2007). However, the pH of the bark is one of the most significant parameters and has been studied intensively by various workers (Barkman, 1958, 1969; James, Hawksworth, and Rose, 1977). Although bark is a solid material and cannot strictly have a pH, bark pH refers to the pH of unbuffered aqueous solution in contact with the bark (Kricke and Loppi, 2002). Barkman (1958) has reported on the kind of epiphytic communities that may be present on the bark of trees with different pH levels. The importance of bark pH has been further emphasized in the classification of British lichen communities by James et al. (1977). The pH of tree bark varies naturally between different species of trees and is influenced by pollution (Nimis and Wolseley, 2002; Wolseley, 2002).

2.5 Benefits of Lichens

Lichens have been used for various purposes since the past for example lichens in food, they consist of no actual carbohydrate or even cellulose and lichens in which considered as a good gradient. In the northern hemisphere, *Cetraria islandica* (Island moss) had taken as food and as a medicine to better food digestion in a body. Besides, it was mixed with flour for making sea biscuit (Thailand Graduate Institute of Science and Technology, 2002). For medicine, the ancient Egyptians used lichens as ingredients in medicines and herbs as in the 15th Century, people used lichens for treatment such as *Usnea barbata, Lobaria pulmonaria, Xanthoria parietina,* and *Peltigera canina,* etc. In Thailand, people in local areas have used lichens as herbal medicine, i.e., *Usnea* spp. (Thailand Graduate Institute of Science and Technology, 2002). *Umbilicaria esculenta* was reported that it produced substances that can inhibit the growth of the HIV virus (Brodo et al., 2001). The principal limitation in developing the economic potential of lichens is their slow growth rate in culture. Large –scale collecting in the field is unacceptable in many parts of the world because the need to conserve natural resources.

2.6 Lichens as Bioindicators of Air Pollution

Lichens are used as biomonitors of air pollution and either biomonitoring of environmental changes. Many lichens are long-lived organisms with high habitat specificity. They can be used to estimate species diversity and habitat potential at all times of the year. Lichens are sensitive organisms; their responses to environmental change may include biodiversity, morphology, physiology, and accumulation of pollutants, etc. Such responses can be used as indicators for many complex factors,

from climatic change to pollution (Nimis and Wolseley, 2002). In particularly, lichens are highly effective biological indicators of specific air pollution; responding to their effects at the cellular, individual, population, and community levels. Lacking a protective cuticle and roots, they absorb substances from the atmosphere directly through their thalli. Lichens are widely distributed, occurring in all terrestrial ecosystems, and collectively covering (in many cases dominating) about 8% of the Earth's land surface. Globally, they play an important biogeochemical role in the retention and distribution of nutrients and trace elements usually accumulating lead, cadmium and other heavy metals of environmental concern (Purvis, Seaward, and Loppi, 2007). As such, they have been widely used to assess trace element atmospheric contaminants. The advantages of using lichens over conventional air sampling techniques are that lichens are perennial and can be found in various conditions. They also present easy sampling, low cost and the possibility of monitoring wide areas. Besides that, lichens do not have root systems and thus they are able to uptake elements and accumulate them in their tissues. The high degree of trace element accumulation enables the determination of several elements with high precision and accuracy. Consequently, several papers have been published on monitoring trace elements using lichens in different geographic areas (Conti and Cecchetti, 2001; Carreras and Pignata, 2002; Loppi, Giordani, Brunialti, Isocrono, and Piervittori, 2002; Garty, Tomer, Levin, and Lehr, 2003; Bergamaschi et al., 2004; Yenisoy-Karaka and Tuncel, 2004). Lichens are various in their sensitivity to SO_2 pollution; in general, crustose and squamulose lichens are least sensitive, foliose lichens are more sensitive, and fruticose lichens are most sensitive. However, the different species are also different in sensitivity.

Lichens show remarkable differences with respect to their sensitivity to heavy metals. Some species are highly tolerant to high concentrations of transition metals including copper (Cu) (Purvis and Halls, 1996; Purvis, 2000; Purvis et al., 2007), iron (Fe) (Hauck, 2005; Paul, Hauck, and Leuschner, 2009), and manganese (Mn) (Paul and Hauck, 2006; Paul et al., 2009). The Cu- and Fe-tolerant lichens include hyperaccumulors inhabiting metal-rich rock and slag (Branquinho, Matos, Vieira, and Ramos, 2011). Other lichen species respond with reduced net photosynthesis or nitrogen fixation, chlorophyll degradation, and damage of thylakoids and plasma lemmas to relatively small amounts of heavy metals (Garty, Tamir, Cohen, Lehr, and Goren, 2002; Garty et al., 2003).

Lichens are among the most frequently used biomonitors of atmospheric pollution (Van Herk, 2001; Nimis and Wolseley, 2002; Jeran et al., 2007) as well as they have even been proved useful as indicators of human health (Gombert, Asta, and Seaward, 2004). Classic lichen-based monitoring has generated pollution maps showing areas largely devoid of epiphytic lichens, the so-called lichen deserts, in and around cities, for example, in Great Britain (Hawksworth and Rose, 1970), the Netherlands (Barkman, 1958), and in many other countries. Richardson and Dalby (1992) stated that lichens could also indicate past pollution by faded or abnormal coloring and patchiness in the center of the thallus (Table 2.1).

Table 2.1 Some lichens indicative of different levels of air pollution.

Highly polluted	Moderately polluted	Slightly polluted	No pollution
Hypogymnia physodes	*Evernia prunastri*	*Parmelia caperata*	*Usnea subfloriden*
Xanthoria parietina	*Foraminella ambigua*	*Graphis scripta*	*Parmelia perlata*
Lecanora dispersa	*Lecanora chlarotera*	*Bryoria fucescens*	*Degelia plumbea*
Diploicia canescens	*Ramalina farinacea*	*Physconia distorta*	*Ramalina fraxinea*
Lepraria incana	*Lecidella elaeochroma*	*Opegrapha varia*	*Teleoschistes flavicans*

Surveying of lichen types in various areas is the basic data on lichen types and regularly survey in the future to observe changes of lichen types. The quantity of accumulation of air pollutant substances in lichens can be examined by transplantation lichen thalli from good air quality to the polluted areas. Lichens have some characters appropriate to be indicators of air quality such as no protecting cell-layer. Therefore, they can directly obtain pollutants, slowly grow up, and have a long lifetime. In a year, crustose and foliose groups radically grow up for only 0.5-5.0 mm. As for fruticose groups, they lengthwise grow up to 1-2 cm (Nash and Wirth, 1988; Nash, 1996). Moreover, it is realized that lichens are living things with more sensitivity to air pollution (Ferry et al., 1973). Factors influence lichen growth includes humidity, light, temperature, receiving of nutrients from outside, season variance and annual season variety (Hale, 1967, 1979).

The use of lichens as bioindicators is based on the concept that there is a close, sensitive, and the dynamic relationship between lichen communities and their environments. The derivation of meaningful outcomes from the application of such indices requires the monitoring of change in epiphytic lichen communities under a

strictly standardized sampling protocol. Several lichen diversity indices have been developed during the last four decades. Lichens have been identified as indicators of environmental quality, particularly air quality, since as early as 1866 (Kricke and Loppi, 2002). Recent developments in the use of lichens as bioindicators for air quality lead to the development of the Index of Atmospheric Purity (IAP) 1968 (LeBlanc and De Sloover, 1970; Kricke and Loppi, 2002). The Lichen Mapping Guideline 3799 (Verein Deutscher Ingenieure, 1995) was applied for tropical lichens surveying (Saipunkaew et al., 2007).

2.7 Lichen Researching

It has been more than 200 years that scientists have studied on the relationship of lichen growth and pollutions and on the capabilities of lichens used as indicating biological indices in different countries worldwide. The following evidences appeared at primary age, in 1970, E. Darwin noted about the incapability of lichens in growing up near the areas around metal melting machines on Anglesey North Wales Island. As for the restudy by W. Borer in 1812, it was observed that lichens were more difficultly found in areas where the air was unclean (Hawksworth and Rose, 1970; Ferry et al., 1973).

Hawksworth and Rose (1970) studied on basis of living patterns, structures, and kinds of lichens on adhering substrates used as representatives of the studied areas in order to make air quality maps using lichens. Later, *Lecanora conizaeoides* lichen was used as an indicator of pollutant accumulation in industrial factory areas in Frederiksvaerk, Denmark. It was reported that heavy metal concentrations in *L. connizaeoides* were various according to spaces from pollution resources i.e. more

heavy metal concentrations in *L. connizaeoides* were found in the areas near industrial factories comparing to the areas far away. Rossbach et al. (1999) found the correlation of metal accumulated capacity in lichens and dust level in the air. For other pollutants, Frati et al. (2006) studied lichens as indicators of ammonia and nitrogen around pig farms in Italy and found that appropriate lichen to be as index indicating ammonia pollution was *Physconia grisea*. As for the *Xanthoria parietina* and *Flavopararmelia caperata*, they accumulated more nitrogen when ammonia concentration was higher. On the other hand, other factors had an influence on lichen diversity e.g. bark's acidity (Larsen et al., 2007). Lichen diversity was investigated around the world, the results were increased, and level of studying was deeper than in the past. The molecular methods were used to identify lichens to species and subspecies, and are applied lichens as much useful equipment for air quality treatments (Zoller, Lutzoni, and Scheidegger, 1999).

In 1899-1900, the lichens were surveyed in Thailand by a Danish botanist. After that, in 1901, Vainio from Finland published first lichens collection of 95 species in 29 genera at Chang Island in Trad province, Thailand; some of them were new species of the world. Then, in 1921, Vainio reported on lichens in Doi Suthep areas and in 1962, Sato investigated lichens again at Doi Suthep and Doi Inthanon. They added many lichen species of Thailand. In 1930, Poaulson reported results of a lichen survey in the Tao Island, Suratthani province. Later, in 1964, Hale and Kurokawa made a survey and collected lichen samples in Thailand (Wolseley and Aguirre-Hudson, 1991). The samples, later on, were kept in the Smithsonian Institute, Washington D.C., and the Tokyo National Museum. In addition, in 1978, Warncke collected lichens samples in the northern part of Thailand and assigned Yoshimura to

examine species and strains of those samples. Those samples, later on, were collected at the Arthus University in Denmark and at Kochi Gakun College in Japan (Thailand Graduate Institute of Science and Technology, 2002).

The studying on lichens in Thailand by Thai botanists began in 1990 in order to use lichens as indicators of air pollution in Bangkok, under the support of Ramkhamhaeng University. At that time, researchers needed to send lichen samples abroad for examining of their scientific names. Nimis and Wolseley (2002) studied on vertical lichen distribution under the dense and clear canopies of *Cratoxylum* sp. and *Schima wallichii* in the second batch forest of the Khao Yai National Park. They observed that the area under clear canopy have higher lichen diversity than the dense canopy. They also found that foliose lichens was found greater than crustose at every high level of tree trunk, but was not found in areas under dense canopies. The lichens found in the light intensity level were *Dirinaria* sp., *Graphis* sp., *Parmptrema* sp. and *Pyxine* sp. whilst the area under dense canopy was *Coccocarpia* sp. (Nimis and Wolseley, 2002).

Palee (2002) conducted a continuum study inside and outside Chiangmai City in 2001 and found the correlation of pollution and quantity of chlorophyll and pheophytin in lichen thallus, this result was confirmed by Asta et al. (2008). Thanomsap (2006) reported that *Pyxine cocoes* distributes overall studied areas, both in and outside the city, but higher in city areas of Lamphoon province. However, Thanwarat (2005) reported the distribution and frequency of *Hyperphycia adglutinata* Flörke which were higher in the city areas of Chiangmai province with rather high pollution, but and *Lecanora* cf. *leprosa* Fée was low. *Lecanora* cf. *leprosa* tended to be used as good indicators of air quality and *H. adglutinata* tended to be used as

minor indicators of air quality. Jariangprasert and Anusarnsunthorn (2005) reported lichen genus *Pertusaria* (Lichenized, Ascomycotina) from Thailand, whilst the Lecanoraceae was reported by Papong and Lumbsch (2011). For using lichen as bioindicator, there were a few reserch using lichens as bioindicator for air pollution, Saipunkaew et al. (2007) reported the air pollution inside Chiang Mai city was low than outside, this was supported the reported that study of Polyiam and Boonpragob (2005) found that lichen diversity was incresed when the distance was located far from Bangkok to Khao Yai National Park. They reported that the main factors affected lichen distribution were light, humidity, temperature, and wind (Boonpragob, Konpab, and Pornprom, 2003; Polyiam and Boonpragob, 2005). Pomphueak (2005) used lichens to evaluate of air quality in city and around Lampang city. Twenty-one species were found and then air quality was classified into eight air quality levels and concluded that NO_2 influenced lichen diversity in the studied areas.

2.8 Lichen Conservation

Lichens are useful as bioindicator of environmental quality, therefore, it was sampling around the world. Lichens were used in a commercial so it might be a cause of extinction in some species as same as many other living things on this planet. In the other hand, lichens are diversely affected by air pollution and human activities. Mostly, this is a result of habitat destruction by atmospheric and aquatic degradation (Fritz, Niklasson, and Churski, 2009). In other words, the polluted air and water can be made some organisms that line almost entirely on air and water with direct relationships are continuing decreasing. Although maintaining large areas of viable habitat in nature reserves, and protection against damage is important, ultimately only

purification of the air and water will bring lichens back to their full potential (Fritz, Gustafsson, and Larsson, 2008). For example, old- growth temperate forests support a diverse array of epiphytic macro lichens and bryophytes (Peterson and McCune, 2001; Price and Hochachka, 2001) whose diversity can exceed that of vascular plants in the same forest (McCune, 2000). Kantvilas and James (1987) found that reduction in size of native forest stands, especially rainforest, could destroy lichens and their habitats. The lichens were conserved by various countries, e.g., Kantvilas (2000), Binder and Ellis (2008), and Rame (2010), lists of species were available in Table 2.2.

Table 2.2 List of lichen conservation in some countries (Kantvilas, 2000; Binder and

Ellis, 2008; Rame, 2010).

Country	Lichen taxa	Lichen taxa
USA (2010)	*Cladonia perforate*	*Gymnoderma lineare*
UK (2010)	*Bryoria furcellata*	*Lecidea inops*
	Buellia asterella	*Nephroma arcticum*
	Caloplaca luteoalba	*Pannaria ignobilis*
	Caloplaca nivalis	*Parmelia minarum*
	Catapyrenium psoromoides	*Parmentaria chilensis*
	Catillaria laureri	*Peltigera lepidophora*
	Cladonia stricta	*Pertusaria bryontha*
	Collema dichotomum	*Physcia tribacioides*
	Gyalecta ulmi	*Pseudocyphellaria lacerate*
	Heterodermia leucomelos	*Psora rubiformis*
	Heterodermia propagulifera	*Solenopsora liparina*
	Lecanactis hemisphaerica	*Squamarina lentigera*
	Lecanora achariana	*Teloschistes flavicans*
Scotland (2004)	*Catapyrenium rufescens*	*Pycnora leucococca*
	Collemopsidium caesium	*Pyrenocollema caesium*
	Fuscopannaria atlantica	*Pyrenula acutispora*
	Hypocoenomyce leucococca	*Pyrenula microtheca*
	Lecidea porphyrospoda	*Ramonia azorica*
	Myochroidea porphyrospoda	*Topeliopsis azorica*
	Placidium rufescens	*Vahliella atlantica*
Australia (2000)	*Hypotrachyna laevigata*	*Xanthoparmelia jarmaniae*
	Parmotrema crinitum	*Xanthoparmelia molliuscula*
	Punctelia subflava	*Xanthoparmelia vicaria*
		Xanthoparmelia willisii

2.9 References

Ahmadjian, V. (1967). A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. **Phycologia.** 6(2): 127-160.

Allaby, M. (2004). **Dictionary of plant sciences.** UK: Oxford University Press.

Armaleo, D. (1991). Experimental microbiology of lichens - mycelia fragmentation, a novel growth chamber, and the origins of thallus differentiation. **Symbiosis.** 11(23): 163-177.

Armaleo, D., and Clerc, P. (1991). Lichen chimeras: DNA analysis suggests that one fungus forms two morphotypes. **Experimental Mycology.** 15(1): 1-10.

Armaleo, D., and Miao, V. (1999). Symbiosis and DNA methylation in the *Cladonia* lichen fungus. **Symbiosis.** 26(2): 143-163.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., and Van Haluwyn, C. (2008). **European guideline for mapping lichen diversity as an indicator of environmental stress.** UK: British Lichen Society.

Ayrault, S., Clochiatti, R., Carrot, F., Daudin, L., and Bennett, J. P. (2007). Factors to consider for trace element deposition biomonitoring surveys with lichen transplants. **Science of the Total Environment.** 372(2-3): 717-727.

Backor, M., and Fahselt, D. (2003). Cellulose-acetate disks as novel substrate for the resynthesis and culture of lichens. **The Bryologist.** 106(3): 439-442.

Barkman, J. J. (1958). **Phytosociology and ecology of cryptogamic epiphytes.** The Netherlands: Van Gorcum.

Baron, G. (1999). **Understanding lichens.** USA: Richmond.

Bergamaschi, L., Rizzio, E., Giaveri, G., Profumo, A., Loppi, S., and Gallorini, M. (2004). Determination of baseline element composition of lichens using samples from high elevations. **Chemosphere.** 55(7): 933-939.

Binder, M. D., and Ellis, C. J. (2008). Conservation of the rare British lichen *Vulpicida pinastri*: changing climate, habitat loss and strategies for mitigation. **The Lichenologist.** 40(01): 63-79.

Boonpragob, K., Konpab, P., and Pornprom, P. (2003). **Use of lichens as indicators of air quality from Bangkok city in the Khao Yai National Park.** [On-line]. Available: http://www.ru.ac.th/lichen/publications/STT29.html.

Branquinho, C., Matos, P., Vieira, A. R., and Ramos, M. M. P. (2011). The relative impact of lichen symbiotic partners to repeated copper uptake. **Environmental and Experimental Botany.** 72(1): 84-92.

Brodo, I. M., Sharnoff, S. D., and Sharnoff, S. (2001). **Lichens of North America.** USA: Yale University.

Carreras, H. A., and Pignata, M. A. L. (2002). Biomonitoring of heavy metals and air quality in Cordoba city, Argentina, using transplanted lichens. **Environmental Pollution.** 117(1): 77-87.

Conti, M. E., and Cecchetti, G. (2001). Biological monitoring: Lichens as bioindicators of air pollution assessment--a review. **Environmental Pollution.** 114(3): 471-492.

Coppins, A. M., and Coppins, B. J. (2002). **Indices of Ecological Continuity for Woodland Epiphytic Lichen Habitats.** UK: Twayne.

Coppins, B. J. (2002). **Checklist of lichens of Great Britain and Ireland.** UK: Twayne.

Ferry, B. W., Baddeley, M. S., and Hawksworth, D. L. (1973). **Air pollution and lichens.** (Vol. 389). UK: Athlone Press.

Fox, H., Cullen, M., Little, D. J., Ciaurriz, P., Ryan, D., Dwyer, R., and Boyle, G. M. (2001). Vegetation monitoring and botanical survey of Brackloon Wood, Westport, County Mayo. **Forest Ecosystem Research Group Report** (31):1-91.

Frahm, J. P. (2003). Climatic habitat differences of epiphytic lichens and bryophytes. **Cryptogamie Bryologie.** 24(1): 3-14.

Frati, L., Caprasecca, E., Santoni, S., Gaggi, C., Guttova, A., Gaudino, S., Pati, A., Rosamilia, S., Pirintsos, S., and Loppi, S. (2006). Effects of NO_2 and NH_3 from road traffic on epiphytic lichens. **Environmental Pollution.** 142(1): 58-64.

Fritz, Ö., Gustafsson, L., and Larsson, K. (2008). Does forest continuity matter in conservation? – A study of epiphytic lichens and bryophytes in beech forests of southern Sweden. **Biological Conservation.** 141(3): 655-668.

Fritz, Ö., Niklasson, M., and Churski, M. (2009). Tree age is a key factor for the conservation of epiphytic lichens and bryophytes in beech forests. **Applied Vegetation Science.** 12(1): 93-106.

Galun, M. (1988). Recognition in fungus alga interaction in lichens. **Phytoparasitica.** 16(2): 190-190.

Garty, J., Tamir, O., Cohen, Y., Lehr, H., and Goren, A. I. (2002). Changes in the potential quantum yield of photosystem II and the integrity of cell membranes relative to the elemental content of the epilithic desert lichen *Ramalina maciformis*. **Environmental Toxicology and Chemistry.** 21(4): 848-858.

Garty, J., Tomer, S., Levin, T., and Lehr, H. (2003). Lichens as biomonitors around a coal-fired power station in Israel. **Environmental Research.** 91(3): 186-198.

Gilbert, O. (2004). **Lichens naturally Scottish.** Perth: Scottish Natural Heritage.

Gombert, S., Asta, J., and Seaward, M. R. D. (2004). Assessment of lichen diversity by index of atmospheric purity (IAP), index of human impact (IHI) and other environmental factors in an urban area (Grenoble, southeast France). **Science of the Total Environment.** 324(1-3): 183-199.

Hale, M. E. (1967). **The biology of lichens.** Dubuque: WC Brown.

Hale, M. E. (1969). **How to know the lichens: The pictured key nature series.** (Vol. 5). Dubuque: WC Brown.

Hale, M. E. (1979). **How to know the lichens.** Dubuque: WC Brown.

Hauck, M. (2005). Epiphytic lichen diversity on dead and dying conifers under different levels of atmospheric pollution. **Environmental Pollution.** 135(1): 111-119.

Hawksworth, D. L., and Hill, D. J. (1984). **The lichen-forming fungi.** California: Glasgow Blackie & Son.

Hawksworth, D. L., and Rose, F. (1970). Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. **Nature.** 227(5254): 145-148.

Humphrey, J. W., Davey, S., Peace, A., Ferris, R., and Harding, K. (2002). Lichens and bryophyte communities of planted and semi-natural forests in Britain: The influence of site type, stand structure and deadwood. **Biological Conservation.** 107(2): 165-180.

James, P. W., Hawksworth, D. L., and Rose, F. (1977). Lichen communities in the British Isles: A preliminary conspectus. In Seaward, M. R. D. (ed.). **Ecology** (pp. 295-413). UK: Academic Press.

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., Celio, G., Gueidan, C., Fraker, E., and Miadlikowska, J. (2006). Reconstructing the early evolution of fungi using a six gene phylogeny. **Nature.** 443(7113): 818-822.

Jariangprasert, S., and Anusarnsunthorn, V. (2005). Additional new taxa in the lichen genus *Pertusaria* (Lichenized Ascomycotina) from Thailand. **Mycotaxon.** 91: 279-292.

Jeran, Z., Mrak, T., Jacimovic, R., Batic, F., Kastelec, D., Mavsar, R., and Simoncic, P. (2007). Epiphytic lichens as biomonitors of atmospheric pollution in Slovenian forests. **Environmental Pollution.** 146(2): 324-331.

Joneson, S., Armaleo, D., and Lutzoni, F. (2011). Fungal and algal gene expression in early developmental stages of lichen-symbiosis. **Mycologia.** 103(2): 291-306.

Joneson, S., and Lutzoni, F. (2009). Compatibility and thigmotropism in the lichen symbiosis: A reappraisal. **Symbiosis.** 47(2): 109-115.

Kantvilas, G. (2000). Conservation of Tasmanian lichens. **Forest Snow and Landscape Research.** 75: 357-367.

Kantvilas, G., and James, P. W. (1987). The macrolichens of Tasmanian rainforest: Key and notes. **The Lichenologist.** 19(1): 1-28.

Kirk, P. M., and Ainsworth, G. C. (2008). Ainsworth & Bisby's dictionary of the fungi. UK: Cabi.

Kricke, R., and Loppi, S. (2002). Bioindication: The I.A.P. Approach: Monitoring with lichens- monitoring lichens. In Nimis, P. L., Scheidegger, C., and Wolseley, P. A. (eds.). **NATO Science Series IV** (Vol. 7, pp. 21-37). The Netherlands: Kluwer.

Larsen, R. S., Bell, J. N. B., James, P. W., Chimonides, P. J., Rumsey, F. J., Tremper, A., and Purvis, O. W. (2007). Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. **Environmental Pollution.** 146(2): 332-340.

LeBlanc, F., and De Sloover, J. (1970). Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. **Canadian Journal of Botany.** 48: 1485-1496.

Loppi, S., Giordani, P., Brunialti, G., Isocrono, D., and Piervittori, R. (2002). **Identifying deviations from naturality of lichen diversity for bioincidations purposes.** The Netherlands: Kluwer.

McCune, B. (2000). Lichen communities as indicators of forest health. **The Bryologist.** 103(2): 353-356.

Nash, T. H. (1976). Lichens as indicators of air pollution. **Naturwissenschaften.** 63(8): 364-367.

Nash, T. H. (1996). **Lichen biology.** USA: Cambridge University Press.

Nash, T. H., and Wirth, V. (1988). **Lichens, bryophytes and air quality.** USA: Cramer.

Nimis, P. L., and Wolseley, P. A. (2002). **Monitoring with lichens: Monitoring lichens.** The Netherlands: Kluwer.

Orange, A., James, P. W., and White, F. J. (2001). **Microchemical methods for the identification of lichens.** UK: British Lichen Society.

Palee, T. (2002). **Used of lichens as bioindicator for air polltion monitoring in town and urban of Chaing Mai province in 2001.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Papong, K., and Lumbsch, H. T. (2011). A taxonomic survey of *Lecanora* sensus *tricto* in Thailand (Lecanoraceae; Ascomycota). **The Lichenologist.** 43(04): 299-320.

Paul, A., and Hauck, M. (2006). Effects of manganese on chlorophyll fluorescence in epiphytic cyano-and chlorolichens. **Flora-Morphology, Distribution, Functional Ecology of Plants.** 201(6): 451-460.

Paul, A., Hauck, M., and Leuschner, C. (2009). Iron and phosphate uptake in epiphytic and saxicolous lichens differing in their pH requirements. **Environmental and Experimental Botany.** 67(1): 133-138.

Peterson, E. B., and McCune, B. (2001). Diversity and succession of epiphytic macrolichen communities in low-elevation managed conifer forests in western Oregon. **Journal of Vegetation Science.** 12: 511-524.

Polyiam, K., and Boonpragob, K. (2005). **Ecological strategies of epiphytic lichen communities in the tropical rain forest at Khao Yai National Park, Thailand.** [On-line]. Available: http://www.ru.ac.th/lichen/publications/STT 29.html.

Price, K., and Hochachka, G. (2001). Epiphytic lichen abundance: effects of stand age and composition in coastal British Columbia. **Ecological Applications.** 11(3): 904-913.

Purvis, O. W., and Halls, C. (1996). A review of lichens in metal- enriched environments. **The Lichenologist.** 28(6): 571-601.

Purvis, O. W., Dubbin, W., Chimonides, P. D. J., Jones, G. C., and Read, H. (2008). The multi-element content of the lichen *Parmelia sulcata*, soil, and oak bark in relation to acidification and climate. **Science of the Total Environment.** 390(2–3): 558-568.

Purvis, O. W., Seaward, M. R. D., and Loppi, S. (2007). Lichens in a changing pollution environment: An introduction. **Environmental Pollution.** 146(2): 291-292.

Purvis, O.W. (2000). **Lichens.** UK: Natural History Museum.

Ranius, T., Johansson, P., Berg, N., and Niklasson, M. (2008). The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. **Journal of Vegetation Science.** 19(5): 653-662.

Ramel, G. (2010). **Lichen conservation.** [On-line]. Avilable: http://www.earthlife.net /lichens/conservation.html.

Richardson, D. H. S., and Dalby, C. (1992). **Pollution monitoring with lichens.** UK: Richmond.

Rossbach, M., Jayasekera, R., Kniewald, G., and Thang, N. H. (1999). Large scale air monitoring: lichen vs. air particulate matter analysis. **The Science of the Total Environment.** 232(1-2): 59-66.

Saipunkaew, W., Wolseley, P. A., Chimonides, P. J., and Boonpragob, K. (2007). Epiphytic macrolichens as indicators of environmental alteration in northern Thailand. **Environmental Pollution.** 146(2): 366-374.

Scott, L. G. (2007). Lichens: An illustrated guide to the British and Irish species. **Botanical Journal of the Linnean Society.** 154(2): 290-290.

Selosse, M. A., and Le Tacon, F. (1998). The land flora: A phototroph-fungus partnership? **Trends in Ecology and Evolution.** 13(1): 15-20.

Thailand Graduate Institute of Science and Technology. (2002). **History of Lichens.** [On-line]. Available: http://eduarea.bkk2ict.net/bio variety/1lichen/ body_1.3. html.

Thanomsap, K. (2006). **Distibution and freqeuncy of lichen** *Pyxine cocoes* **Swartz and** *Lecanora* **cf.** *leprosa* **Fée in Lampun city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Thanwarat, C. (2005). **The distribution and frequency of lichen** *Hyperphyscia adglutinata* **Florke and** *Lecanora* **cf.** *leprosa* **Fée in Chiang mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Van Herk, C. (2001). Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time. **The Lichenologist.** 33(05): 419-442.

Verein Deutscher Ingenieure. (1995). Measurement and evaluation of phytotoxic effects of ambient air pollutants (emissions) with lichens. In **Mapping of lichens for assessment of the air quality,** VDI-Richtlinie 3799, Platt1/part 1: Düsseldorf.

White, T. J., Bruns, T., Lee, S., and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds.). **PCR protocols: A guide to methods and applications** (pp. 315-322). USA: Academic Press.

Will-Wolf, S., Esseen, P. A., and Neitlich, P. (2002). **Monitoring biodiversity and ecosystem function: Forests.** The Netherlands: Kluwer.

Wolseley, P. A. (2002). Using lichens on twigs to assess changes in ambient atmospheric conditions. In Nimis, P. L., Scheidegger, C. and Wolseley, P. A. (eds.). **Monitoring with lichens-monitoring lichens** (Earth and Environmental Sciences, Vol. 7, pp. 291-294). The Netherlands: Kluwer.

Wolseley, P. A., and Aguirre-Hudson, B. (1991). Lichens as indicators of environmental change in the tropical forests of Thailand. **Global Ecology and Biogeography Letters.** 1: 170-175.

Yenisoy-Karaka, S., and Tuncel, S. G. (2004). Geographic patterns of elemental deposition in the Aegean region of Turkey indicated by the lichen, *Xanthoria parietina* (L.) Th. Fr. **Science of the Total Environment.** 329(1-3): 43-60.

Zoller, S., Lutzoni, F., and Scheidegger, C. (1999). Genetic variation within and among populations of the threatened lichen *Lobaria pulmonaria* in Switzerland and implications for its conservation. **Molecular Ecology.** 8(12): 2049-2059.

CHAPTER III

ASSESSMENT OF EPIPHYTIC LICHEN DIVERSITY
AND AIR QUALITY IN THE NORTHEAST OF
THAILAND

3.1 Abstract

Epiphytic lichen diversity and air quality in the northeast of Thailand in 2010 were assessed using the Verein Deutscher Ingenieure (VDI) method and the European guideline for mapping lichen diversity as an indicator of environmental stress protocol. Sixteen sample plots were located in eight municipalities of eight provinces. Two sampled plots of inside and outside municipalities were selected in each municipality. Ten mango trees (*Mangifera indica* L.) were selected in each plot. Frequencies of lichens in each tree were recorded using a grid frame; a grid with 20 x 50 cm^2. This grid frame comprised of 10 squares of 10 x 10 cm^2. It was placed on the tree trunk where the most lichen thallus appeared. Altogether, 91 epiphytic lichens were recorded from 160 mango trees; 71 inside and 85 outside municipalities, respectively. *Pyxine cocoes* had highest in frequency and distribution followed by *Chrysothrix xanthina, Lecanora leprosa,* and *Graphis* sp.1, whereas the three lowest species were *Hyperphyscia granulate, Hypogymnia hypotrypa,* and *Hypotrachyna osseoalba.* Twenty species were observed outside municipalities and 6 lichen species were found only inside municipalities. The ecological indices showed that lichen

diversity outside the municipal areas were significantly higher than in the municipal areas (F = 1. 365, p<0.05). The cluster analysis based on all factors and all lichen taxa separated the study sites into four groups. However, the use of macrolichens showed clear result for five groups. This work concluded that lichen diversity outside the municipalities was higher than inside the municipalities and they can use to assess air qualities in the northeast of Thailand, as well as macrolichens are reliable.

3.2 Introduction

Lichens are symbiotic organisms composed of a fungus (mycobiont) with a green algal or cyanobacterial partner (photobiont). The lichens are identified depending on their mycobiont: Kingdom of Fungi. The most of mycobiont is ascomycetes. Lichens are accepted as biomonitoring of environmental changes and bioindicator of air quality (Hale, 1979), especially sulfur dioxide arising from industrial and domestic fuel burning (Saipunkaew, Wolseley, Chimonides, and Boonpragob, 2007). Lichens studies were included various levels and purposes e.g. conservation, taxonomy and using as bioindicators of air pollution (Freitas, Costa, Marques, and Da Silvamanuela, 2011; Nascimbene, Brunialti, Ravera, Frati, and Caniglia, 2010). From an eco-toxicological perspective, contaminants are all chemical compounds that are fundamentally released into the environment because of anthropogenic activities, which cause harm to the organisms as well as human health and related with long-term chronic effects on health. (Conti and Cecchetti, 2001; Larsen et al., 2007). In addition, Nash (1996) reported a high-level of lung cancer has correlated with the biodiversity of lichens because of atmospheric pollution. The study on lichens vary from simply observing lichen species for recording the lichen

species diversity or investigating phytosociological relationships between different lichen species and environmental conditions and also using species to detect amount of pollutant (Grangeon, Guédron, Asta, Sarret, and Charlet, 2012). Mapping lichen diversity is the basis of using lichens to assess air quality in many countries and is especially useful where direct measurement of air pollutant concentrations is impossible. The lichen diversity value (LDV) and index atmospheric purity (IAP) are applied to assess air quality and to monitor environmental alteration in several countries e.g., Italy (Loppi and Frati, 2006), France (Gombert, Asta, and Seaward, 2004), Ireland (Brodeková, Gilmer, Dowding, Fox, and Guttov, 2006), London (Larsen et al., 2007), Portugal (Freitas et al., 2011), Brazil (Käffer et al., 2011), and Thailand (Saipunkaew et al., 2007).

An update and review of the species of lichens found in Thailand is presented in Aptroot et al. (2007). Recent developments in the use of lichens as bioindicators of air quality have led to the development of the Index of Atmospheric Purity (IAP). For the lichen mapping guideline, various researchers applied these guidelines for assessing the environmental quality (Gombert et al., 2004; Giordani, 2007). The need for general and widely applicable lichen based system for the determination of environmental stress within ecosystems led to the development of the European guideline for mapping lichen diversity as an indicator of environmental stress (Asta et al., 2008). The European guideline has been recently applied in a number of major studies (Pinho et al., 2008). Various studies have addressed the abundance and distribution of lichens in many areas of the Northeast of Thailand especially in the forest (Pooprang, 2001; Sparrius and Saipunkaew, 2005), but lichens inside the city have not been investigated. Moreover, the environmental status of this region has not

been well-addressed using formal lichen based indices. Consequently, there is potential for the development of new insights from the application of the European guideline for mapping lichen diversity as an Indicator of environmental stress to the Northeast of Thailand. This research aimed to assess the epiphytic lichen diversity and distribution across the municipality habitats and to evaluate the air quality using lichens as ecological bioindicators.

3.3 Materials and Methods

3.3.1 Study Sites

3.3.1.1 General Sets of the Study Area

The northeast of Thailand is located at the Korat Plateau, bordered by the Mekong river (along the border with Laos) to the north and east, Cambodia to the southeast and the Prachinburi mountain in the south of Nakhon Ratchasima province to the west, separated from northern and central Thailand by the Phetchabun mountain range (Figure 3.1).

3.3.1.2 Geographical Description

The northeastern covers 155,000 km, it is roughly coterminous with the Korat Plateau, which tilts from the Phetchabun mountain range in the west of the region (the location of several national parks) down towards the Mekong river (Geo-Informatics Centre for the Development of Northeast Thailand, 2008). The plateau consists of two main plains: the southern Khorat plain is drained by the Mun and Chi rivers, whilst the Loei drains the northern Sakhon Nakhon plain and Songkhram rivers. Phu Phan mountains separate two plains. The soil is mostly sandy, with substantial salt deposits (Thai Meteorological Department, 2010).

3.3.1.3 Climate Description

The average annual temperature range is from 30.2°C to 19.6°C. The highest temperature was recorded 43.9°C at Udon Thani, the lowest was -1.4°C at Sakhon Nakhon Agro Station (Cooper, Rajatasereekul, Immark, Fukai, and Basnayake, 1999). Rainfall in this region is unpredictable, but is concentrated in the rainy season from May to October. Average annual precipitation varies from 2,000 mm in some areas to 1,270 mm in the southwestern provinces including Nakhon Ratchasima, Buriram, Maha Sarakham, Khon Kaen and Chaiyaphum (Ratanopad and Kainz, 2006). There are three seasons around the year; the rainy season begins with occasional short but heavy showers, eventually rain very heavily for longer periods almost every day, usually in the late afternoon or at night until it ends abruptly at the onset of the winter. The other seasons are the cool season from October to February, and the hot season from February to May with its sudden peak of high temperatures in April (Thai Meteorological Department, 2010).

3.3.2 Selection of Sampling Plots

The sampling strategy adapted from the VDI method (Verein DeutscherIngenieure, 1995) and the European guideline for mapping lichen diversity as an indicator of environmental stress(Asta et al., 2002;Svoboda, 2007).The lichen specimens were collected from eight provinces of the Northeast of Thailand including Nakhon Ratchasima, Chaiyaphum, Khon Kaen, Loei, Ubon Ratchathani, Yasothon, Nong Khai, and Sakhon Nakhon. Two plots of 1x1 km^2: one inside municipality where lichens have usually been affected by high pollution and one outside municipality in the southern municipality area where lichens may not be affected by air pollution from the city (Figure 3.2).
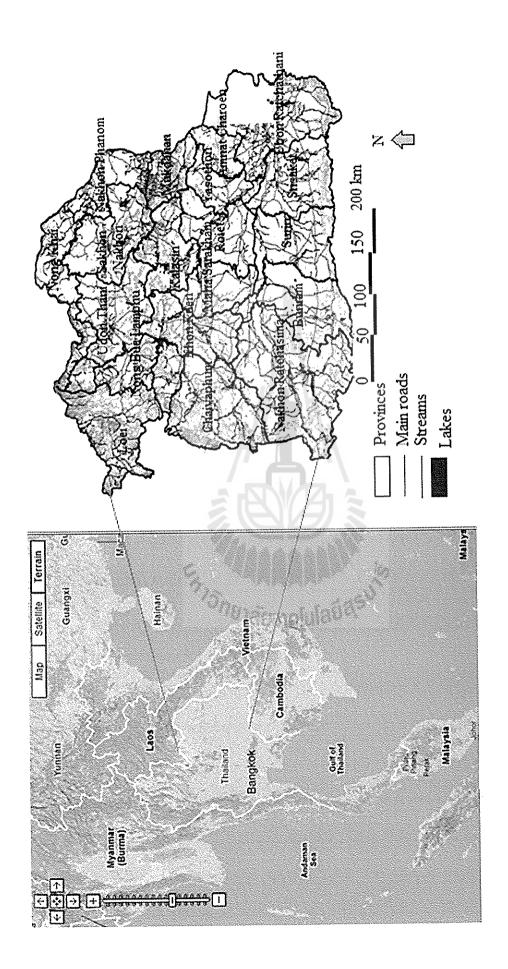
Figure 3.1 Map of the northeast of Thailand.
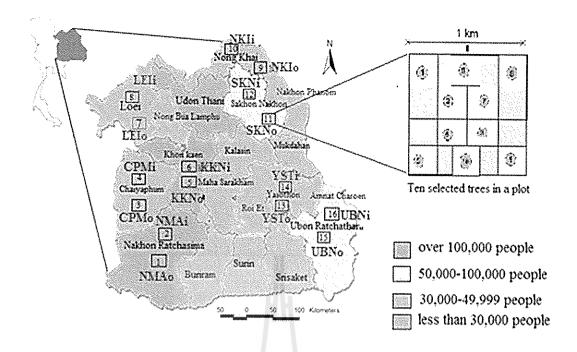
Figure 3.2 Sixteen sampling plots in eight provinces of the Northeast of Thailand; CPM = Chaiya phum, NMA = Nakhon Ratchasima, UBN = Ubon Ratchathani, KKN = Khon Kaen, NKI = Nong Khai, YST = Yasothon, LEI = Loei, SKN = Sakhon Nakhon, i = inside municipality, o = outside municipality.

3.3.3 Sampling Period

Sampling took place over a period of January-December 2010.

3.3.4 Sampling within a Sample Plot

The selected sample plots were divided into equal quadrat of 1x1 km. Ten mango trees were then selected in each plot (Figure 3.3). The tree sampled according to defined set of criteria derived from Asta et al. (2002, 2008) and Brodeková (2006). These criteria specified that the sample tree should be a tree species with found in all study areas; mango tree was selected for this work followed

Saipunkaew et al. (2005). A freestanding tree showing no evidence of damage or interference by human or animals; a tree with a trunk circumference from 50 to 150 cm at a height of 150 cm and an inclination of less than 10° from vertical. Trees of the different trunk circumferences and different ages were considered for sampling as they naturally occurred within the environment because it must have impact to epiphytic lichens on tree trunks. They were located as close as possible to the center of the sampling plot (Figure 3.3). The position of selected trees within a sample plot was mark using the survey map. A Geo-referencing Positioning System-GPS 12 GARMIN was used to indicate sampling plots.

3.3.5 Surveying the Epiphytic Lichen Diversity

Epiphytic lichen diversity was surveyed on the selected trees, using a surveying quadrat followed the Verein Deutscher Ingenieure (VDI) method (Verein Deutscher Ingenieure, 1995). This quadrat consisted of four quadrat segments; each at 50 cm in height and 20 cm in width, each quadrat segment was subdivided into 10 quadrat squares of 10 x 10 cm^2 (Figure 3.4). The surveying quadrat was placed on the tree trunk at the side where the most lichen presented at 100 cm above ground level. The presence of lichen species was recorded in each quadrat square. A list of lichen species and their frequencies in one quadrat segment were recorded. Lichen samples were identified in the field if possible whilst the unidentified specimens were taken to laboratory for further identification.
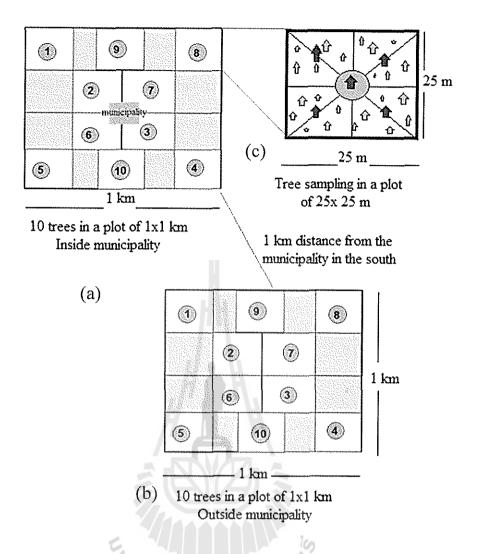
Figure 3.3 Selection of trees for sampling within a sampled plot: (a) a plot located

in municipalities with 10 selected mango trees, (b) a plot located outside

municipalities with 10 selected mango trees, (c) sampling trees in a

plot.

3.3.6 Laboratory Work

The collected lichen samples were identified in the laboratory using an

Olympus microscope BH-2 for identification of microlichens and an Olympus

Binocular stereomicroscope (SZH 10) with magnifications from x7 to x70 for

identification of macrolichens and cutting cross sections. The insights of thalli, fruiting organs, spores and other characteristics were measured using a built in micrometer. Images for microscopic identification were recorded using a digital camera (Canon EOS D30) attached to the microscopes. Spot color reaction tests were applied for distinguishing some of the problematic lichen species using 10% solution of potassium hydroxide (K), 5% solution of sodium hypochlorite (C) and the Steiner's Stable Pd solution of paraphenylenediamine (Pd). Species identification following the lichen determination keys (Hale, 1969; Kantvilas and James, 1987; Sipman, 2003).
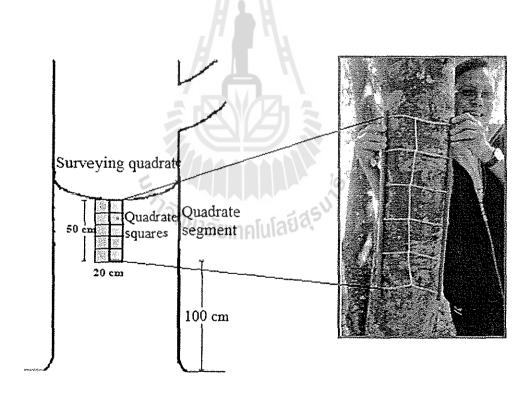
Figure 3.4 Surveying quadrat (adapted from Asta et al., 2002).

3.3.7 Ecological Indices

The lichen diversity was compared between inside and outside municipalities using ecological indices as follows:

3.3.7.1 Species diversity index

The Shannon-Wiener Diversity Index (H') was used to establish alternative estimates of species diversity at inside and outside municipalities.

$$H' = \sum_{i=1}^{s} p_i \ln p_i \qquad (3.1)$$

Where: s   is the number of species

p$_i$ is the proportion of individuals or the abundance of the ith species expressed as a proportion of total cover

ln   is log base e

Multiply the proportion (p_i) of each species in the simple times the natural log of that same value (ln p_i), then sum ($\sum$) the value of each species, and finally multiply by minus 1. The value of H' is highest when species are equally abundant.

3.3.7.2 Evenness (E)

Separate the effect of different population size (numbers of individuals within species) from species diversity (number of species) (Ludwig and Reynolds, 1988).

$$E = \frac{e^{H'}}{s} \qquad (3.2)$$

Where: E   is the evenness

e   is 2.7 (constant value)

H'   is the value of the Shannon- Weaver Information Function

S   is the number of species in the sample

3.3.7.3 Species Richness

Species richness is the number of species in a sample or study
site.

3.3.7.4 Similarity of Lichen Diversity

The Sørensen s coefficient was calculated for expressing
similarity in species composition between inside and outside municipality, and in each
province using formula:

$$\text{Sørensen's coefficient} = \frac{2a}{2a+b+c} \qquad (3.3)$$

Where: a   is number of species common to both quadrats/samples

b   is number of species in quadrat/sample 1

c   is number of species in quadrat/sample 2

3.4 Results

3.4.1 Lichen Diversity in the Northeast of Thailand

Altogether, 91 lichen taxa were recorded, belonged to 34 genera and 17 families from 160 mango trees in the northeast of Thailand (Table 3.1), the pictures of taxa were presented in Appendix D. The most of lichen species recorded was 64.86% crustose lichens followed by 34.86% foliose and 0.28% squamulose, but fruticose was not found in this study. *Pyxine cocoes* was the highest of total frequency followed by *Chrysothrix xanthina, Lecanora leprosa,* and *Graphis* sp.1, and the three lowest lichen frequencies were *Hyperphyscia granulate, Hypogymnia hypotrypa,* and *Hypotrachyna osseoalba.* The genera with the highest number of species were *Ghaphis* (26 species) followed by *Arthonia* (6 species), *Lecanora* (4 species) *Pyxine* (4 species) and *Physcia* (3 species) (Table 3.1). Two species: *C. xanthina* and *P. cocoes* were found in all studied plot. Eighty-five and 71 species found outside and inside municipalities, respectively. Twenty and six lichen species were found only outside and inside municipality, respectively (Table 3.2). Eighteen species were found specifically in one municipality, i.e., *Graphina* sp. was recorded in Nakhon Rachasima, *Buellia erubescens* was observed in Chaiyaphum, *Graphis* sp.22 was recorded in Sakhon Nakhon, *Graphis* sp.19 were found in Ubon Ratchathani, seven species, ˉi.e., *Amandinea extent, Caloplaca displace, Caloplaca diplacioides, Caloplaca gambiensis, Graphis* sp.12, *Graphis* sp.15, and *Pyrenula coffins* were found only in Loei and six species were found only in Nong Khai, i.e., *Graphis* sp.10, *Graphis* sp.11, *Graphis* sp.17, *Graphis* sp.20, *Hyperphyscia lavida, Hypogymnia hypotrypa,* and *Hypotrachyna osseoalba* (Table 3.2).

Table 3.1 Lists of lichen species in the northeast of Thailand.

Thallus type	Family	Genus	Species
Crustose	Arthoniaceae	*Arthonia*	*Arthonia catenatuta*
			Arthonia cinnabarina
			Arthonia cytodes
			Arthonia elegans
			Arthonia incospicua
			Arthonia tumidula
		Cryptothecia	*Cryptothecia candida*
			Cryptothecia punctosorediata
	Bacidiaceae	*Bacidia*	*Bacidia pallidocarnea*
	Chrysothrichaceae	*Chrysothrix*	*Chrysothix candelaris*
			Chrysothrix xanthina
	Crocyniaceae	*Crocynia*	*Crocynia pyxinoid*
	Graphidaceae	*Graphina*	*Graphina* sp.
		Graphis	*Graphis* sp.1
			Graphis sp.2
			Graphis sp.3
			Graphis sp.4
			Graphis sp.5
			Graphis sp.6
			Graphis sp.7
			Graphis sp.8
			Graphis sp.9
			Graphis sp.10
			Graphis sp.11
			Graphis sp.12
			Graphis sp.13
			Graphis sp.14
			Graphis sp.15

Table 3.1 Lists of lichen species in the northeast of Thailand (Cont.).

Thallus type	Family	Genus	Species
Crustose	Graphidaceae	*Graphis*	*Graphis* sp.16
			Graphis sp.17
			Graphis sp.18
			Graphis sp.19
			Graphis sp.20
			Graphis sp.21
			Graphis sp.22
			Graphis sp.23
			Graphis sp.24
			Graphis sp.25
			Graphis sp.26
		Sarcographa	*Sarcographa* sp.
	Lecanoraceae	*Lecanora*	*Lecanora achrosa*
			Lecanora helva
			Lecanora leprosa
			Lecanora tropica
	Physciaceae	*Amandinea*	*Amandinea extunata*
			Amandinea punctata
		Rinodina	*Rinodina intrasa*
			Rinodina cinereovirescens
	Pyrenulaceae	*Anthracothecium*	*Anthracothecium prasinum*
		Pyrenula	*Pyrenula confinis*
	Ramalinaceae	*Phyllopsora*	*Phyllopsora sorediata*
	Ramalinaceae	*Lecanographa*	*Lecanographa atropunctata*
		Opegrapha	*Opegrapha stirtinii*
	Stereocaulaceae	*Lepraria*	*Lepraria atrotomentosa*
	Teloschistaceae	*Caloplaca*	*Caloplaca diplacia*
			Caloplaca diplacioides

Table 3.1 Lists of lichen species in the northeast of Thailand (Cont.).

Thallus type	Family	Genus	Species
Crustose	Teloschistaceae	*Caloplaca*	*Caloplaca gambiensis*
	Thelotremataceae	*Porina*	*Porina eminentior*
			Porina internigrans
	Tricotheriaceae	*Ocellularia*	*Ocellularia crocea*
		Myriotrema	*Myriotrema microporellum*
	Trypetheliaceae	*Laurera*	*Laurera benguelensis*
			Laurera megasperma
		Trypethelium	*Trypethelium eluteriae*
			Trypethelium tropicum
			Sterile crust sp.1
			Sterile crust sp.2
			Sterile crust sp.3
Foliose	Caliciaceae	*Dirinaria*	*Dirinaria appleanata*
			Dirinaria confluens
			Dirinaria pica
	Parmeliaceae	*Hypogymnia*	*Hypogymnia hypotrypa*
		Hypotrachyna	*Hypotrachyna osseoalba*
		Parmotrema	*Parmotrema praesorediosum*
			Parmotrema tinctorum
	Physciaceae	*Buellia*	*Buellia erubescens*
		Hyperphyscia	*Hyperphyscia adglutinata*
			Hyperphyscia adsendes
			Hyperphyscia flavida
			Hyperphyscia granulate
		Physcia	*Physcia atrostriata*
			Physcia damidiata
			Physcia poncinsii
		Pyxine	*Pyxine coccifera*
			Pyxine cocoes
			Pyxine consocians
			Pyxine subcinerea
Squamulose	Cladoniaceae	*Cladonia*	*Cladonia crispate*
			Cladonia fimbriata
			Cladonia glauca

Table 3.2 The presence and frequency of epiphytic lichen species; F = frequency, TF = total frequency, P = presence, TP = total presence (* only outside, **only inside).

Lichen species	Inside municipality		Outside municipality		%TF	%TP
	%F	%P	%F	%P		
Amandinea extunata	0.47	0.26	0.20	0.06	0.31	0.14
*Amandinea punctata**			0.45	0.19	0.26	0.11
Anthracothecium prasinum	1.99	1.65	1.24	0.54	1.56	0.95
Arthonia catenatuta	1.00	0.55	0.35	0.25	0.62	0.40
Arthonia cinnabarina	1.06	0.66	0.89	0.47	0.97	0.56
Arthonia cytodes	0.53	0.15	0.45	0.17	0.48	0.16
Arthonia incospicua	1.13	0.78	0.35	0.10	0.68	0.40
*Arthonia elegans**			0.59	0.43	0.34	0.25
Arthonia tumidula	2.59	1.79	0.89	0.42	1.62	0.98
Bacidia pallidocarnea	0.80	0.33	0.30	0.09	0.51	0.19
Buellia erubescens *			0.20	0.06	0.11	0.03
Caloplaca diplacia	1.53	1.01	1.98	1.50	1.79	1.30
Caloplaca diplacioides	0.53	0.29	0.64	0.37	0.60	0.35
Caloplaca gambiensis	1.66	2.06	0.64	0.47	1.08	1.10
Cryptothecia candida	1.00	0.64	0.30	0.13	0.60	0.35
Cryptothecia punctosorediata	0.27	0.15	0.15	0.04	0.20	0.09
Chrysothix candelaris	0.73	0.40	1.93	0.94	1.42	0.72
Chrysothix xanthina	8.91	11.06	5.94	6.49	7.21	8.65
*Cladonia fimbriata**			0.15	0.04	0.09	0.02
*Cladonia crispate**			0.15	0.04	0.09	0.02
*Cladonia glauca**			0.20	0.06	0.11	0.03
Crocynia pyxinoid	0.20	0.06	0.35	0.30	0.28	0.14
Dichosporidium boschianum	1.13	0.47	0.64	0.28	0.85	0.37
Dirinaria applanata	2.53	1.97	2.97	1.85	2.78	1.99
*Dirinaria confluens**			0.69	0.61	0.40	0.35
Dirinaria pica	3.32	3.06	3.12	3.03	3.21	3.11
Graphina sp.*			0.20	0.12	0.11	0.07
Graphis sp.1	3.66	3.03	4.66	5.42	4.23	4.36
Graphis sp.2	3.59	2.48	3.42	2.65	3.49	2.61
Graphis sp.3	1.40	0.77	2.58	1.28	2.07	1.09
Graphis sp.4	0.33	0.09	1.93	1.80	1.25	1.03
Graphis sp.5	0.13	0.04	0.30	0.09	0.23	0.07
Graphis sp.6**	0.53	0.18			0.23	0.08

Table 3.2 The presence and frequency of epiphytic lichen species; F = frequency, TF

= total   frequency, P = presence, TP = total presence (* only outside,

**only inside) (Cont.).

Lichen species	Inside municipality		Outside municipality		%TF	%TP
	%F	%P	%F	%P		
Graphis sp.7	0.66	0.37	0.50	0.36	0.57	0.39
Graphis sp.8	0.60	0.17	0.30	0.17	0.43	0.19
Graphis sp.9	1.13	0.42	0.10	0.03	0.54	0.20
Graphis sp.10*			0.30	0.09	0.17	0.05
Graphis sp.11*			0.25	0.22	0.14	0.12
Graphis sp.12**	0.80	1.10			0.34	0.50
Graphis sp.13	0.13	0.07	1.19	0.69	0.74	0.43
Graphis sp.14**	0.73	0.61			0.31	0.27
Graphis sp.15*			0.74	0.86	0.43	0.50
Graphis sp.16**	0.66	0.28			0.28	0.12
Graphis sp.17*			0.25	0.14	0.14	0.08
Graphis sp.18*			0.59	0.17	0.34	0.10
Graphis sp.19**	0.33	0.18			0.14	0.08
Graphis sp.20	0.80	0.44	0.30	0.26	0.51	0.37
Graphis sp.21	0.07	0.02	0.25	0.07	0.17	0.05
Graphis sp.22*			0.30	0.09	0.17	0.05
Graphis sp.23	0.27	0.07	0.35	0.20	0.31	0.14
Graphis sp.24	0.86	0.48	0.05	0.01	0.40	0.20
Graphis sp.25	0.53	0.44	0.59	0.69	0.57	0.58
Graphis sp.26	0.33	0.18	0.79	1.15	0.60	0.61
Hyperphyscia adglutinata	4.72	4.56	1.14	0.66	2.67	2.19
Hyperphyscia adsendes	0.13	0.04	0.45	0.26	0.31	0.14
*Hyperphyscia flavida**			0.25	0.22	0.14	0.12
*Hyperphyscia granulate**			0.05	0.01	0.03	0.01
*Hypogymnia hypotrypa**			0.05	0.01	0.03	0.01
*Hypotrachyna osseoalba**			0.05	0.01	0.03	0.01
Laurera benguelensis	0.86	0.36	0.64	0.94	0.74	0.47
Laurera megasperma	0.73	0.61	0.59	0.43	0.65	0.52
Lecanographa atropunctata	1.06	0.59	1.04	0.91	1.05	0.76
Lecanora achrosa	0.60	0.25	2.08	1.70	1.45	1.02
Lecanora leprosa	4.26	4.30	4.31	5.85	4.29	5.20
Lecanora helva	0.20	0.06	1.78	1.38	1.11	0.72
Lecanora tropica	1.40	0.51	3.12	2.16	2.38	1.34
Lepraria atrotomentosa	0.47	0.13	0.05	0.01	0.23	0.07
Myriotrema microporellum	0.07	0.02	0.15	0.04	0.11	0.03

Table 3.2 The presence and frequency of epiphytic lichen species; F = frequency, TF = total frequency, P = presence, TP = total presence (* only outside, **only inside) (Cont.).

Lichen species	Inside municipality		Outside municipality		%TF	%TP
	%F	%P	%F	%P		
*Ocellularia crocea**			0.59	0.17	0.34	0.10
Opegrapha stirtinii	0.27	0.15	1.04	0.61	0.71	0.41
Parmotrema praesorediosum	0.27	0.07	0.64	0.19	0.48	0.14
Parmotrema tinctorum	0.33	0.09	1.73	0.84	1.14	0.50
*Phyllopsora sorediata***	1.32	0.92			0.57	0.41
Physcia atrostriata	0.66	0.28	0.64	0.31	0.65	0.30
Physcia damidiata	1.51	1.05	0.10	0.03	0.71	0.36
Physcia poncinsii	1.38	0.90	1.09	0.85	1.22	0.89
Porina eminentior	0.39	0.11	0.79	0.54	0.62	0.36
Porina internigrans	0.33	0.09	0.10	0.03	0.20	0.06
*Pyrenula confinis**			0.15	0.04	0.09	0.02
Pyxine cocoes	16.33	36.38	12.38	27.93	14.14	32.40
Pyxine coccifera	1.78	1.65	3.02	3.87	2.50	2.91
Pyxine consocians	2.44	2.04	1.39	1.35	1.85	1.69
Pyxine subcinerea	0.46	0.51	1.88	1.64	1.28	1.18
Rinodina intrasa	0.86	0.32	0.40	0.29	0.60	0.31
Rinodina cinereovirescens	3.82	3.06	3.52	4.09	3.66	3.46
Sarcographa sp.	1.65	0.92	1.39	1.61	1.50	1.25
Sterile crust sp.1	0.26	0.15	0.89	0.69	0.62	0.45
Sterile crust sp.2	0.13	0.04	0.35	0.30	0.26	0.15
Sterile crust sp.3	0.13	0.04	0.10	0.03	0.11	0.03
Trypethelium eluteriae	1.25	0.87	3.22	1.87	2.38	1.47
Trypethelium tropicum	0.66	0.24	3.17	2.61	2.10	1.43

3.4.2 Lichen Frequency and Presence

The frequency illustrated of each lichen species found in the Northeast of Thailand found that *Pyxine cocoes* was the highest of frequency (TF = 14. 14%) and presence (32.40%) followed by *Chrysothrix xanthina*, *Lecanora leprosa*, and *Graphis* sp.1, respectively whilst, *Hyperphyscia granulate* and *Hypogymnia hypotrypa* and

Hypotrachyna osseoalba were the lowest (TF = 0.03%, TP = 0.01%) (Table 3.5). Comparing between in sampling plots, it was found that the total of lichen frequency was the highest on the outside the municipality of Loei province for 442, followed by outside the municipality of Nakhon Ratchasima province for 380, and outside the municipality of Sakhon Nakhon for 349 (Table 3.3). The data of lichen species and lichen frequency in each plot was shown in Appendix C. For species, the plot outside the municipality of Loei was the highest for 42 species followed by inside the municipality of Loei province for 41 species; the lowest was inside and outside the municipality of Khon Kaen province for 14 species. Two species of *Chrysothrix xanthina* and *Pyxine cocoes* were observed in all sample plots (Table 3.3).

3.4.3 Ecological Indices

3.4.3.1 Diversity Indices

The Shannon-Wiener Diversity Index (H') computed a value of 3.6871 for outside municipalities and 3.4969 for inside municipalities. For each province, the highest of lichen diversity was in Loei province and lowest in Ubon Ratchathani for 3.6967 and 2.3295, respectively. The value of lichens diversity was compared among groups of inside and outside municipalities. The result showed that the lichen diversity was highest in outside Loei municipality for 3.5112, whilst inside Loei municipality showed the highest value for 3.47 (Table 3.4). The differentiation between inside and outside municipalities was distinguished by using one way-ANOVA which showed significant difference between inside and outside the municipalities (F = 0.580, p < 0.05).

Table 3.3 Lists of lichen species in the northeast of Thailand in a plot; in = inside municipality and out = outside municipality.

NO.	Lichen species	NMA		CPM		KKN		LEI		SKN		NKI		YST		UBN		Total
		out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	
1	Amandinea extunata	0	0	0	0	0	0	6	7	0	0	0	0	0	0	0	0	13
2	Amandinea punctata	6	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	13
3	Anthracothecium prasinum	2	0	3	0	0	0	4	6	0	6	4	9	9	0	3	2	48
4	Arthonia catenatuta	0	5	0	0	0	0	8	6	0	0	5	4	0	0	0	0	28
5	Arthonia cinnabarina	0	0	2	4	0	0	6	0	0	1	3	3	2	0	5	8	34
6	Arthonia cytodes	2	1	0	0	0	1	5	2	0	0	2	4	0	0	0	0	17
7	Arthonia incospicua	3	0	0	0	0	0	0	11	0	3	0	3	0	0	4	0	24
8	Arthonia tumidula	2	19	0	0	1	0	2	4	2	4	0	7	11	4	0	0	56
9	Arthonia elegans	0	0	0	0	0	0	7	0	5	0	0	0	0	0	0	0	12
10	Buellia erubescens	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	4
11	Bacidia pallidocarnea	3	6	0	0	0	0	3	6	0	0	0	0	0	0	0	0	18
12	Caloplaca diplacia	13	10	0	6	0	0	15	2	1	3	8	0	3	0	2	0	63
13	Caloplaca diplacioides	6	0	5	2	0	0	0	0	2	6	0	0	0	0	0	0	21
14	Caloplaca gambiensis	9	0	0	0	4	0	0	12	0	8	0	0	0	0	0	0	33
15	Cryptothecia candida	0	0	0	0	0	0	3	9	0	0	0	2	3	4	0	0	21
16	Cryptothecia punctosorediata	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	7
17	Chrysothrix candelaris	0	0	6	0	0	0	25	9	4	0	5	0	2	2	0	0	53
18	Chrysothrix xanthina	25	28	16	6	3	12	29	30	15	11	9	13	28	9	5	10	249
19	Cladonia fimbriata	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3
20	Cladonia crispate	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3

Table 3.3 Lists of lichen species in the northeast of Thailand in a plot; in = inside municipality and out = outside municipality (Cont.).

NO.	Lichen species	NMA		CPM		KKN		LEI		SKN		NKI		YST		UBN		Total
		out	in	out	in	out	in	in	out	in	out	in	out	in	out	out	in	
21	*Cladonia glauca*	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4
22	*Crocynia pyxinoid*	0	0	0	1	0	0	2	7	0	0	0	0	0	0	0	0	10
23	*Dichosporidium boschianum*	0	1	0	5	0	5	6	0	0	0	0	6	0	7	0	0	30
24	*Dirinaria appleanata*	6	5	3	6	0	0	6	25	10	0	5	13	6	3	5	5	98
25	*Dirinaria confluens*	0	0	0	0	0	0	0	0	0	8	0	0	0	0	6	0	14
26	*Dirinaria pica*	10	8	13	0	0	0	10	5	17	7	8	25	1	0	3	6	113
27	*Graphina sp.*	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
28	*Graphis sp.1*	20	13	7	0	0	0	10	15	16	13	5	7	3	19	13	8	149
29	*Graphis sp.2*	7	15	3	2	0	5	9	25	6	9	7	21	7	0	4	3	123
30	*Graphis sp.3*	4	0	2	3	1	0	13	13	5	5	0	12	0	15	0	0	73
31	*Graphis sp.4*	0	0	12	0	0	0	5	10	0	5	0	7	0	5	0	0	44
32	*Graphis sp.5*	0	0	0	0	0	0	2	0	0	4	0	2	0	0	0	0	8
33	*Graphis sp.6*	0	1	0	3	0	1	3	0	0	0	0	0	0	0	0	0	8
34	*Graphis sp.7*	5	0	0	0	5	0	0	0	0	0	10	0	0	0	0	0	20
35	*Graphis sp.8*	6	0	0	0	0	0	0	0	4	0	0	0	0	0	2	3	15
36	*Graphis sp.9*	0	3	0	0	0	3	0	0	4	5	0	0	2	0	2	0	19
37	*Graphis sp.10*	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	6
38	*Graphis sp.11*	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	5
39	*Graphis sp.12*	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	10
40	*Graphis sp.13*	4	0	5	0	0	0	0	15	0	0	0	0	2	0	0	0	26

Table 3.3 Lists of lichen species in the northeast of Thailand in a plot; in = inside municipality and out = outside municipality (Cont.).

NO.	Lichen species	NMA		CPM		KKN		LEI		SKN		NKI		YST		UBN		Total
		out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	
41	*Graphis* sp.14	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	11
42	*Graphis* sp.15	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	15
43	*Graphis* sp.16	0	4	0	0	0	0	0	0	0	6	0	0	0	0	0	0	10
44	*Graphis* sp.17	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	5
45	*Graphis* sp.18	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	0	12
46	*Graphis* sp.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
47	*Graphis* sp.20	0	0	0	0	0	0	0	0	12	0	0	0	6	0	0	0	18
48	*Graphis* sp.21	0	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	6
49	*Graphis* sp.22	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	6
50	*Graphis* sp.23	0	0	7	0	0	0	0	4	0	0	0	0	0	0	0	0	11
51	*Graphis* sp.24	1	2	0	0	0	0	0	0	0	0	0	0	0	6	5	0	14
52	*Graphis* sp.25	0	0	0	0	0	0	0	0	0	0	0	8	12	0	0	0	20
53	*Graphis* sp.26	0	0	0	0	0	5	0	0	0	0	16	0	0	0	0	0	21
54	*Hyperphyscia adglutinata*	2	35	8	8	8	0	0	5	1	9	0	0	4	4	8	10	94
55	*Hyperphyscia adsendes*	9	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	11
56	*Hyperphyscia flavida*	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	5
57	*Hyperphyscia granulata*	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	6	9
58	*Hypogymnia hypotrypa*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
59	*Hypotrachyna osseoalba*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
60	*Laurera benguelensis*	0	0	0	6	0	1	0	4	13	2	0	0	0	0	0	0	26

Table 3.3 Lists of lichen species in the northeast of Thailand in a plot; in = inside municipality and out = outside municipality (Cont.).

NO.	Lichen species	NMA		CPM		KKN		LEI		SKN		NKI		YST		UBN		Total
		out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	
61	*Laurera megasperma*	3	6	0	5	0	0	0	0	9	0	0	0	0	0	0	0	23
62	*Lecanographa atropunctata*	15	11	0	0	0	0	0	5	0	0	6	0	0	0	0	0	37
63	*Lecanora achrosa*	5	0	0	0	0	0	13	0	9	0	11	3	4	6	0	0	51
64	*Lecanora leprosa*	19	6	0	12	9	15	0	0	17	4	16	19	22	8	4	0	151
65	*Lecanora helva*	11	3	20	0	0	0	0	0	0	0	0	0	5	0	0	0	39
66	*Lecanora tropica*	20	3	6	6	4	1	10	0	6	4	7	0	3	7	7	0	84
67	*Lepraria atrotomentosa*	0	0	1	0	0	0	0	7	0	0	0	0	0	0	0	0	8
68	*Myriotrema microporellum*	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	4
69	*Ocellularia crocea*	0	0	0	0	0	0	4	0	0	0	4	0	4	0	0	0	12
70	*Opegrapha stirtinii*	5	0	0	0	0	0	2	4	0	0	14	0	0	0	0	0	25
71	*Parmotrema praesorediosum*	0	0	0	0	0	0	9	0	0	4	4	0	0	0	0	0	17
72	*Parmotrema tinctorum*	0	0	0	0	0	0	24	0	5	5	6	0	0	0	0	0	40
73	*Phyllopsora sorediata*	0	10	0	0	0	0	0	10	0	0	0	0	0	0	0	0	20
74	*Physcia atrostriata*	5	6	0	0	0	0	0	0	0	0	4	0	4	4	0	0	23
75	*Physcia damidiata*	0	0	0	0	1	0	0	12	1	0	0	2	0	0	0	0	16
76	*Physcia poncinsii*	0	13	0	0	0	0	15	0	4	0	0	6	3	2	0	0	43
77	*Porina eminentior*	0	0	0	0	0	0	7	0	0	0	5	6	4	0	0	0	22
78	*Porina internigrans*	0	0	0	0	0	0	2	5	0	0	0	0	0	0	0	0	7
79	*Pyrenula confinis*	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3
80	*Pyxine cocoes*	58	34	36	20	10	5	24	15	25	34	30	38	27	33	40	67	496

Table 3.3 Lists of lichen species in the northeast of Thailand in a plot; in = inside municipality and out = outside municipality (Cont.).

NO.	Lichen species	NMA		CPM		KKN		LEI		SKN		NKI		YST		UBN		Total
		out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	
81	*Pyxine coccifera*	11	9	6	6	12	0	0	6	5	0	0	0	0	0	27	0	82
82	*Pyxine consocians*	10	21	10	6	0	7	8	0	0	0	0	0	0	0	0	3	65
83	*Pyxine subcinerea*	12	7	6	0	6	0	6	0	0	0	0	0	8	0	0	0	45
84	*Rinodina intrasa*	6	4	0	0	0	0	0	0	0	5	0	0	2	0	0	4	21
85	*Rinodina cinereovirescens*	16	10	17	16	0	2	7	7	0	2	0	4	0	18	31	3	133
86	*Sarcographa* sp.	0	0	0	0	0	0	0	11	12	2	11	0	5	9	0	3	53
87	Sterile crust sp.1	13	4	0	0	0	0	0	0	1	0	4	0	0	0	0	0	22
88	Sterile crust sp.2	7	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	9
89	Sterile crust sp.3	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	4
90	*Trypethelium eluteriae*	10	16	5	0	12	3	0	0	14	0	13	0	11	0	0	0	84
91	*Trypethelium tropicum*	0	0	3	0	4	0	14	4	12	0	11	3	5	0	15	3	74
	Total	380	328	216	144	100	90	442	328	298	225	349	217	282	199	256	203	3513

Table 3.4 Ecological indices.

Study sites	Shannon-Wiener Diversity Index	Species richness (S)	Simpson Diversity Index			Evenness
			D	1-D	1/D	
NMAo	3.3235	40	0.0515	0.9485	19.4175	0.9009
NMAi	3.1380	33	0.0554	0.9446	18.0505	0.8974
CPMo	2.8952	25	0.0732	0.9268	13.6612	0.8994
CPMi	2.7814	20	0.0753	0.9247	13.2802	0.9285
KKNo	2.4094	14	0.1021	0.8979	9.7943	0.9130
KKNi	2.3074	14	0.1248	0.8752	8.0128	0.8743
LEIo	3.4700	42	0.0377	0.9623	26.5252	0.9283
LEIi	3.5112	41	0.0361	0.9639	27.7008	0.9455
NKIo	3.3645	38	0.0416	0.9584	24.0385	0.9249
NKIi	2.9923	28	0.0707	0.9293	14.1443	0.8980
SKNo	3.3029	34	0.0447	0.9553	22.3714	0.9366
SKNi	2.7570	22	0.0908	0.9092	11.0132	0.8919
YSTo	3.0032	28	0.0642	0.9358	15.5763	0.9012
YSTi	2.6910	21	0.0963	0.9037	10.3842	0.8839
UBNo	2.5965	21	0.1050	0.8950	9.5238	0.8528
UBNi	1.9902	15	0.2568	0.7432	3.8941	0.7349

3.4.3.2 Evenness

The Evenness (E) calculated a value of 0.50 for the outside the municipality and 0.45 for inside the municipality areas. For each municipality, Loei was the highest for 0.9455 and among group of inside the municipality showed that the municipality of Chaiyaphum was the highest for 0.9285, whilst Ubon Ratchathani showed the lowest value both inside and outside municipalities for 0.7349 and 0.8528, respectively (Table 3.4). The differentiation of evenness between inside and outside municipalities used one way-ANOVA showed significant difference between inside and outside municipalities (F = 2.005, p<0.05).

3.4.3.3 Species richness

The species richness was higher in outside municipality for 85

and 71 for inside the municipality. For each province, the species richness was the highest in Loei municipality for 83. Outside municipality of Loei showed the highest value for 42 and inside for 41, the lowest found in Khon Kaen municipality for 14 for inside and outside municipalities (Table 3.4). The difference between inside and outside municipalities showed significantly different using one way-ANOVA (F = 0.416, p<0.05).
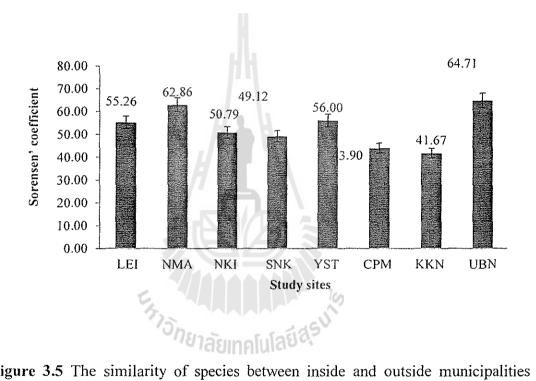
Figure 3.5 The similarity of species between inside and outside municipalities in each province in the Northeast of Thailand (CPM = Chaiyaphum, NMA = Nakhon Ratchasima, UBN = Ubon Ratchathani, KKN = Khon Kaen, NK I= Nong Khai, YST = Yasothon, LEI = Loei, SKN = Sakhon Nakhon).

3.4.3.4 Similarity of Lichen Diversity

The species similarity was compared between inside and outside the municipality areas using the Sørensen's coefficient found lichens species that found inside the municipality areas and outside the municipality areas was high for 85.33%. The similarity was analyses between inside and outside in each province found that Ubon Ratchathani province was the highest (64.71%), but the lowest was in Khon Kaen municipality (41.67%) (Figure 3.5).

3.4.4 Cluster Analysis

The cluster analysis in Figure 3.6 showed four groups. Group A included inside and outside municiapality of Ubon Ratchathani and Nakhon Ratchasima. Group B included inside and outside municiapality of Chaiyaphum and inside municaipality of Nong Khai, Sakhon Nakhon and Yasothon. Group C included inside and outside municipality of Loei, outside municipality of Sakhon Nakhon, Nong Khai and Yasothon. Group D included inside and outside municipality of Khon Kaen. The air quality based on all lichen taxa could be identified the level of air pollution into 4levels depended on the population in each municipality: (i) low population (group C), (ii) low to moderate population (Group B), moderate to high population (Group A), and (iv) high population (Group D).Twenty-one macrolichen species belonged to 9 genera were identified and frequencies recorded on each tree. Cluster analysis of this data showed the separation of five clusters (Figure 3.7). Cluster A included both inside and outside the municipality of KhonKaen. Group B included inside and outside the municipality of Loei. Group C included inside and outside the municipality of Chiyaphyum and Yasothon. Group D included inside and outside municipality of Nakhon Ratchasima and Ubon Ratchathani. Group E included inside

and outside the municipality of Nong Khai and Sakhon Nakhon. This dendrogram could be classified into 4 levels of similarity (Figure 3.8).
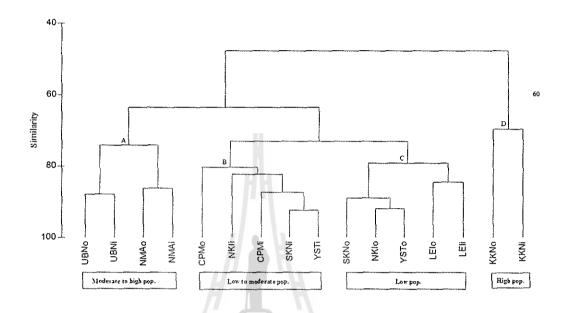
Figure 3.6 Cluster analysis dendrogram, cluster of sites using the Spearman's correlation coefficient based on the total frequency of all lichen taxa.
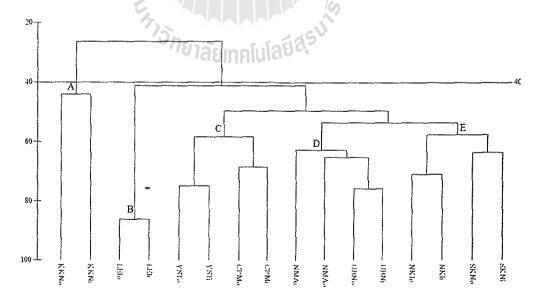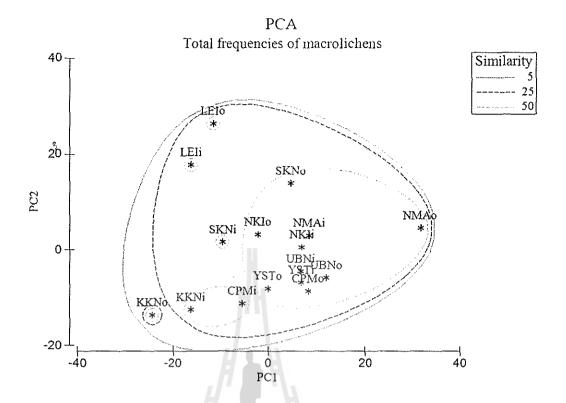
Figure 3.7 Cluster analysis dendrogram, cluster of sites using the Spearman's correlation coefficient based on the total frequencies of macrolichens.

PCA
Total frequencies of macrolichens

Figure 3.8 MDS ordination showing site similarity based on data from Figure 3.7 with sites coded by studied plot.

3.5 Discussions

3.5.1 Lichen Diversity

Both lichen diversity and frequency of occurrence of most species were higher outside municipalities than inside municipalities of the northeast Thailand, consistent with the finding of Saipunkaew et al. (2005) from northern Thailand. Crustose lichens were the most frequently encountered, consistent with Blanco et al. (2006) and Saipunkaew et al. (2007), who mentioned that crustose lichens were prominent in locations 250-400 m above sea level (asl), whilst above 600 m asl, the foliose lichens were more dominant (reported also by Pomphueak (2005)). Crustose lichens were also higher abundant than foliose lichens on Kram and Samaesarn Islands (Buaruang et al. (2005). *Pyxine cocoes* was the most common species both

inside and outside the municipalities of the northeast of Thailand. *Pyxine cocoes*, *Lecanora leprosa*, and *Hyperphyscia adglutinata* were the most widely distributed species in Lumphun and Chiangmai provinces (Subsri and Saipunkaew, 2002; Thanwarat, 2005; Pruksakorn, 2007). The wide distribution and occurrence of *Pyxine cocoes* in both high-density and less-density residential areas in the northeast of Thailand suggests that it is particularly tolerant to air pollution. The stated findings conformed to study results of Pimwong (2002) and Pruksakorn (2007) who investigated lichen distribution and frequency in urban areas of Chiangmai and Lamphun provinces. The results indicated that this lichen species distributed all over the urban and suburban areas, which were not much difference in frequencies. Moreover, Saipunkaew et al. (2005, 2007) indicated that this species well tolerated to the environment of Chiangmai province. *Lecanora leprosa* was found to be the most distributed lichen species. Frequency was high in the high-density residential areas where traffic was heavy and residences were in high density. The high frequencies were found at areas around the Mittapab highway. It indicated that this species tended to tolerate well to pollution. This is consistent with the study of Pruksakorn (2007) which studied lichen distribution and frequency in urban areas in Lamphun province.

3.5.2 Species Composition and Richness

The epiphytic lichen species recorded outside the municipalities showed greater diversity, evenness, and species richness, than inside the municipalities. However, the similarity in species composition between inside and outside municipalities was relatively high. This is consistent with epiphytic lichen frequency not being correlated significantly with the human population number in a municipality, but rather with altitude, annual rainfall, and days of rainfall.

Hyperphyscia adglutinata usually grew on tree trunks near the roads or in nutrient-rich outdoor areas such as where there was a lot of dust, or on walls and rocks with calcium (Purvis, Coppins, Hawksworth, James, and Moore, 1992), consistent with the findings from Chiangmai and Lampun (Subsri, 2002; Thanwarat, 2005; Saipunkaew et al., 2005, 2007; and Pruksakorn, 2007.

3.5.3 Environmental Conditions in the Northeast of Thailand

The northeast of Thailand has been classified as having a relatively low level of epiphytic lichen diversity. The pattern of diversity of epiphytic lichens inside municipalities was driven by various environmental factors. Indeed, the range of parameters within the municipalities that influence epiphytic lichen development has been well recognized (Hauck, 2005; Giordani and Will-Wolf, 2006). Significant parameters that impinge on epiphytic lichens both inside and outside municipalities in the northeast of Thailand include average day of rainfall, annual rainfall, and average year temperature, latitude, traffic effect, and population size. Another important substrate parameter is the acidity of the tree's bark (or pH value), which has a strong influence on epiphytic lichen development. Subsri and Saipunkaew (2002) examined mango bark pH in urban and suburban areas in Chiangmai, and found that bark pH ranged from 5.0-5.3, with bark pH being higher in urban areas than in suburban areas. Moreover, Pomphueak (2005) investigated pH of mango-tree barks in areas of Muang district, Lampang province and found that bark pH was 5.22-5.74. The lowest pH was found in area with the least pollution and the highest one was found in area with the highest pollution. Larsen et al. (2007) reported that high bark pH in central London might be due to calcareous dusts from buildings and demolitions contributing to the alkaline dust effect. Indeed, Purvis et al. (2007) described *Arthonia vinosa* as being

associated with the bark of old trees. *Pyrenula macrospora* was related to the smooth and shaded bark of deciduous trees (Purvis, Seaward, and Loppi, 2008).

However, there was no significant correlation between bark acidity and LDV and lichen frequency in this study ($p < 0.05$). The amount of rainfall and day of rainfall support a level of humidity within the immediate environment and modifies fluctuations in the atmospheric moisture content at the sites (Purvis, Dubbin, Chimonides, Jones, and Read, 2007). Higher levels of humidity also promote the growth of a wider range of other epiphytes, including mosses and liverworts, which all compete with lichens for available resources (Nag, Rai, Upreti, Nayaka, and Gupta, 2012). Traditionally, air quality has been recognized as one of the main factors influencing development of lichen species. The presence of lichen species e.g. *Lecanora tropica*, *Lecanora achrosa*, *Trypethelium tropicum*, and *Parmotrema tincturum* associated with good air quality (Llop, Pinho, Matos, Pereira, and Branquinho, 2012).

3.5.4 Cluster Analysis

Macrolichens found in this work were used of cluster analysis of setting the similarity group. The result was consistent with Saipunkaew et al. (2007) who reported that macrolichens could be used to distinguish geographical variation in species distribution from anthropogenic of environmental conditions.

3.5.5 Management Conservation and Applying

The age and undisturbed characters of mango trees are often positively correlated with greater epiphytic lichen flora. This would suggest that the selected study sites probably represent some of the oldest parts of this region. However, this is not consistent with the relatively low numbers of lichens observed. This in turn may

be related to the current sizes and growth forms of trees within the study site, which suggest a level of human management or interference over time. Indeed, clear evidence exists of coppicing of mango tree within the northeast of Thailand where mango trees have an average girth. This has a direct effect on the age profile of the mango tree trunks in the study sites and consequently the epiphytic lichen maturity and richness. In contrast, mango trees outside municipality did not show evidence of such management practices (average mango tree girth was 87.7).The undisturbed characters of the mango trees outside the municipality area may partly explain the relatively higher richness of epiphytic lichens compare to the north of Thailand (Saipunkaew, Wolseley, and Chimonides, 2005; Aptroot, Saipunkaew, Sipman, Sparrius, and Wolseley, 2007; Saipunkaew et al., 2007). This creates an environmental pressure on the ecosystem, increases disturbance and contributes to the relative low diversity of epiphytic lichens.

3.6 Conclusions

The epiphytic lichens diversity was higher outside than inside municipalities whilst the air quality class was lower. This work observed the lichen taxa that specific in locality, however, two species of *Pyxine cocoes* and *Lecanora leprosa* were found in all study plots. Lichens can be separated the level of population number in each municipality into four levels, whilst the use of macrolichens showed clear result for five clusters. This work concluded that lichens can be used as bioindicator and macro lichens are reliable.

3.7 References

Aptroot, A., Saipunkaew, W., Sipman, H. J. M., Sparrius, L. B., and Wolseley, P. A. (2007). New lichens from Thailand, mainly microlichens from Chiang Mai. **Fungal Diversity.** 24: 75-134.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., and Van Haluwyn, C. (2008). **European guideline for mapping lichen diversity as an indicator of environmental stress.** [On-line]. Available: http://www.thebls.org.uk/content/documents/eumap.pdf.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., Van Haluwyn, C., and Wirth, V. (2002). Mapping lichen diversity as an indicator of environmental quality. In Nimis, P. L., Scheidegger, C., Welseley, P.A. (eds.), **Monitoring with Lichens-Moniitoring Lichens.** UK: Kluwer.

Blanco, O., Crespo, A., Ree, R. H., and Lumbsch, H. T. (2006). Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. **Molecular Phylogenetics and Evolution.** 39(1): 52-69.

Brodekova , L., Gilmer, A., Dowding, P., Fox, H., and Guttov , A. (2006). An assessment of epiphytic lichen diversity and environmental quality in Knocksink Wood Nature Reserve, Ireland. **Natural History.** 223(3): 215-223.

Buaruang, K., Boonpragob, K., Mongkolsuk, P., Homchantara, N., Vongshewarat, K., Sujaritturagan, J., Papong, K., Osathanon, N., and Sanglapcharoenkit, M. (2005). **Some common lichens inhabited Kram Island and Samaesarn**

Island. [Online]. Available: http://joomlas.ru.ac.th/lichen/English/Data/STT/ STT31_Samaesarn.pdf.

Conti, M. E., and Cecchetti, G. (2001). Biological monitoring: Lichens as bioindicators of air pollution assessment--review. **Environmental Pollution.** 114(3):471-492.

Cooper, M., Rajatasereekul, S., Immark, S., Fukai, S., and Basnayake, J. (1999). Rainfed lowland rice breeding strategies for Northeast Thailand: I. Genotypic variation and genotype environment interactions for grain yield. **Field Crops Research.** 64(1-2): 131-151.

Freitas, M., Costa, N. R., Marques, J., and Da Silvamanuela, V. (2011). Lichens as bio indicators of atmospheric pollution Porto, Portugal. **Journal of Biodiversity and Ecological Sciences.** 1(1): 29-39.

Geo-Informatics Centre for the Development of Northeast Thailand. (2008). **Geography of the Northeast Thailand.** [On-line]. Available: http://gecnet.kku.ac.th/ rs_coni_08_Drought_NE_Multi_Temporal.asp.

Giordani, P. (2007). Is the diversity of epiphytic lichens a reliable indicator of air pollution? A case study from Italy. **Environmental Pollution.** 146(2): 317-323.

Giordani, P., and Will-Wolf, S. (2006). Variables influencing the distribution of epiphytic lichens in heterogeneous areas: A case study for Liguria, NW Italy. **Journal of Vegetation Science.** 17(2): 195-206.

Gombert, S., Asta, J., and Seaward, M. R. D. (2004). Assessment of lichen diversity by index of atmospheric purity (IAP), index of human impact (IHI) and other

environmental factors in an urban area (Grenoble, southeast France). **Science of the Total Environment.** 324(1-3): 183-199.

Grangeon, S., Guédron, S., Asta, J., Sarret, G., and Charlet, L. (2012). Lichen and soil as indicators of an atmospheric mercury contamination in the vicinity of a chlor-alkali plant (Grenoble, France). **Ecological Indicators.** 13(1): 178-183.

Hale, M. E. (1969). **How to know the lichens: The pictured key nature series.** (Vol. 5). Dubuque: WC Brown.

Hale, M. E. (1979). **How to know the lichens.** Dubuque: WC Brown.

Hauck, M. (2005). Epiphytic lichen diversity on dead and dying conifers under different levels of atmospheric pollution. **Environmental Pollution.** 135(1): 111-119.

Käffer, M. I., Martins, S. M. d. A., Alves, C., Pereira, V. C., Fachel, J., and Vargas, V. M. F. (2011). Corticolous lichens as environmental indicators in urbanareas in southern Brazil. **Ecological Indicators.** 11(5): 1319-1332.

Kantvilas, G., and James, P. W. (1987). The macrolichens of Tasmanian rainforestkey and notes.**The Lichenologist.** 19(1): 1-28.

Larsen, R. S., Bell, J. N. B., James, P. W., Chimonides, P. J., Rumsey, F. J., Tremper, A., and Purvis, O. W. (2007). Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. **Environmental Pollution.** 146(2): 332-340.

Loppi, S., and Frati, L. (2006). Lichen diversity and lichen transplants as monitors of air pollution in a rural area of central Italy. **Environmental Monitoring and Assessment.** 114(1): 361-375.

Llop, E., Pinho, P., Matos, P., Pereira, M. J., and Branquinho, C. (2012). The use of lichen functional groups as indicators of air quality in a Mediterranean urban environment. **Ecological Indicators.** 13(1): 215-221.

Ludwig, J. A., and Reynolds, J. F. (1988). **Statistical ecology: A primer on methods and computing.** (Vol. 1). Canada: Wiley-Interscience.

Mulligan, L. (2009). **An assessment of epiphytic lichens, lichen diversity and environmental quality in the semi-natural woodlands of Knocksink Wood Nature Reserve, Enniskerry, County Wicklow.** M.S. thesis. Dublin Institute of Technology, Ireland.

Nag, P., Rai, H., Upreti, D. K., Nayaka, S., and Gupta, R. K. (2012). Epiphytic lichens as indicator of land-use pattern and forest harvesting in a community forest in west Nepal. **Journal of Plant Science** .8: 24-32.

Nascimbene, J., Brunialti, G., Ravera, S., Frati, L., and Caniglia, G. (2010). Testing *Lobaria pulmonaria* (L.) Hoffm. as an indicator of lichen conservation importance of Italian forests. **Ecological Indicators.** 10(2): 353-360.

Pinho, P., Augusto, S., Martins-Loução, M. A., Pereira, M. J., Soares, A., Máguas, C., and Branquinho, C. (2008). Causes of change in nitrophytic and oligotrophic lichen species in a Mediterranean climate: Impact of land cover and atmospheric pollutants. **Environmental Pollution.** 154(3): 380-389.

Pimwong, S. (2002). **Distibution and freqeuncy of lichen *Pyxine cocoes* Swartz and *Dirinaria picta* Swartz in Chiang Mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Pooprang, T. (2001). **Systematic study of the lichens family Parmeliaceae in Thailand.** M.S. thesis. Ramkhamhaeng University, Thailand.

Pomphueak, K. (2005). **Use of lichens as bioindicators for air quality monitoring in amphoe Mueang, Lampang.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Pruksakorn, S. (2007). **Used of lichens as bioindicator for air pollution Monitoring in Lamphun province in 2004.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Purvis, O. W., Dubbin, W., Chimonides, P. D. J., Jones, G. C., and Read, H. (2008). The multi-element content of the lichen *Parmelia sulcata*, soil,and oak bark in relation to acidification and climate. **Science of the Total Environment.** 390(2–3): 558-568.

Purvis O. W., Seaward, M. R. D., and Loppi, S. (2007). Lichens in a changing pollution environment: An introduction. **Environmental Pollution.** 146(2): 291-292.

Purvis, O.W., Coppins, B. J., Hawksworth, D. L., James, P. W., and Moore, D. M. (1992). **The lichen flora of Great Britain and Ireland.** UK: Natural History Museum.

Ratanopad, S., and Kainz, W. (2006). Land cover classification and monitoring in northeast Thailand using landsat 5 TM data. **International Achieves of Photogrammistry, Remote Sensing, and Spatial Information Sicences.** 1(2): 137-144.

Saipunkaew, W., Wolseley, P. A., and Chimonides, P. J. (2005). Epiphytic lichens as indicators of environmental health in the vicinity of Chiang Mai city, Thailand. **The Lichenologist.** 37(4): 345-356.

Saipunkaew, W., Wolseley, P. A., Chimonides, P. J., and Boonpragob, K. (2007). Epiphytic macrolichens as indicators of environmental alteration in northern Thailand. **Environmental Pollution.** 146(2): 366-374.

Sipman, H. J. M. (2003). **Key to the lichen genera of Bogor, Cibodas and Singapore.** Berlin: Berlin-DahlemFreie Universität.

Sparrius, L. B., Saipunkaew, W., Wolseley, P. A., and Aptroot, A. (2006). New species of *Bactrospora, Enterographa, Graphidastra*and *Lecanographa* from northern Thailand and Vietnam. **The Lichenologist.** 3 8(1): 27-36.

Svoboda, D. (2007). Evaluation of the European method for mapping lichen diversity (LDV) as an indicator of environmental stress in the Czech Republic. **Biologia.** 62(4): 424-431.

Subsri, P., and Saipankaew, W. (2002). Mango tree bark as bioindicator for air pollution monitoring in Chiang Mai city. **Chiang Mai Journal of Science.** 29(3): 183-188.

Thai Meteorological Department. (2010). **Climate of Northeastern Thailand.** [On-line]. Available: http://www.tmd.go.th/region.php?RegionID=2.

Thanwarat, C. (2005). **The distibution and freqeuncy of lichen** *Hyperphyscia adglutinata* **Florke and** *Lecanora* **cf.** *leprosa* **Fée in Chiang mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Verein Deutscher Ingenieure. (1995). Measurement and evaluation of phytotoxic effects of ambient air pollutants (emissions) with lichens. In **Mapping of lichens for assessment of the air quality,** VDI-Richtlinie 3799, Platt1/part 1: Düsseldorf.

CHAPTER IV

FACTORS INFLUENING EPIPHYTIC LICHEN

DIVERSITYINTHE NORTHEAST OF THAILAND

4.1 Abstract

This work was to define the factors influence on epiphytic lichens on mango trees (*Mangifera indica* L.) in the municipalities based on the Verein Deutscher Ingenierure (VDI) protocol. The physical factors were recorded from 160 selected trees. They were separated into two groups of 80 trees from inside and outside of eight municipalities of eight provinces in the northeast of Thailand. The physical factors included the diameter at breast height (DBH), the areas surrounding, tree bark characteristics, directions of grid frame, distance between selected tree and the road, the level of traffic and bark pH. The lichen thalli were recorded; there were age of lichens and lichen vitality. The different among groups and in groups were analyzed using pair t-test, $p < 0.05$. The correlation between all factors and the lichen frequency and lichen number were analyzed using the Principal Component Analysis (PCA) based on the Spearman's correlation coefficient ($r < 0.01$). The results showed the environmental conditions of selected trees were significantly different between the trees that located inside and outside municipalities ($p < 0.05$), but the tree properties were not significant difference ($p < 0.05$). The correlation analysis showed that the lichen frequency was negatively correlated with the distance from the road

(r = -0.264, p<0.01), and correlated with altitude (r = 0.243, p<0.01), whilst the altitude was the strongest factor influencing the lichen frequency (r = 0.512, p<0.01).

4.2 Introduction

Lichens are symbiotic of fungus (mycobiont) and at least one photosynthetic alga or cyanobacterium (photobiont). They appear in three main forms depending upon their thalli (the vegetative structure); crustose, foliose, and fruticose. Due to their physiology and ability to detect numerous pollutants; lichens are the most widely used as bioindicators of air pollution (Hamada and Miyawaki, 1998; Loppi, 2000). The lichen thallus forms are important to the survival and the tolerance on extreme environment, i.e. crustose is the most tolerant types followed by foliose and fruticose, respectively (Purvis, 2000). The previous researches have been identified the factors influencing on lichen diversity and factors supporting lichen distribution (Ayrault, Clochiatti, Carrot, Daudin, and Bennett, 2007). However, the different areas may also different influencing. Epiphytic lichen diversity has correlation with the flora species. From the appearances of each tree, there are variations in bark properties e.g. rough or smooth, the age of the tree, dead or alive tree, the area condition of standing trees, etc. (Bates, Proctor, Preston, Hodgetts, and Perry, 1997). Some lichen species prefer growing in high NO_2 concentration areas e.g. *Xanthonia* spp. (Gilbert, 1976; Grodzińska, 1977). The correlation of lichens and each pollutant has been reported e.g. SO_2 (Batty, Bates, and Bell, 2003) and nitrogen (van Herk, Mathijssen-Spiekman, and de Zwart, 2003). In addition, the agricultural activity also affected to lichen diversity (Bartók, 1999). In urban areas, some authors admitted that nitrogen deposition mainly comes from emissions of nitrous oxide (NO_x) (Purvis, Seaward, and

Loppi, 2007), whilst Gombert et al. (2003) mentioned that the nitrogen concentration of the nitrophytic *Physcia adscendens* collected near roadsides was related to traffic flow.

In Thailand, lichens were used as bioindicator for air[a] quality (Saipunkaew,Wolseley, and Chimonides, 2005; Saipunkaew, Wolseley, Chimonides, and Boonpragob, 2007) and studied on their diversity. There were some new species added in lichen list of Thailand and world (Sparrius, Saipunkaew, Wolseley, and Aptroot, 2006; Aptroot, Saipunkaew, Sipman, Sparrius, and Wolseley, 2007; Papong, Boonpragob, and Lumbsch, 2011). This research aimed to determine the factors influencing epiphytic lichen diversity on mango trees (*Mangifera indica* L.) in the northeast of Thailand in 2010.

4.3 Materials and Methods

4.3.1 Study Sites and Environmental Variables

The research site is located at the northeast of Thailand (16°0'0"N, 103°0'0"), the largest part of Thailand. It covers an area of about 155,000 km², and average elevation is 200 m. This plateau is divided by Phu Phan Mountains Range (16°46'22"N, 104°21'45"E) into two basins: the northern Sakhon Nakhon Basin, and the southern Korat Basin. The plateau is tilted towards the southeast and drained by the Mun and Chi rivers and tributaries to the Mekong river that forms the northeastern boundary of the area. It is separated from central Thailand by the Phetchabun Mountain Range (16°26'0"N, 101°9'4"E), average elevation 1,571 m and the Dong Phaya Yen mountains (14°19'48"N, 102°3'0"E), average elevation 900 m in the west, the Sankamphaeng Range (14°26'30"N, 101°23'0"E), average elevation 1,351 m in

the southwest and by the Dongrek mountains (14°20′15″N, 103°55′0″E), average elevation 753 m in the south, all of which historically made access to the plateau difficult (Geo-Informatics Centre for the Development of Northeast Thailand, 2008). These together with the Truong Son Range (18°35′30″N, 103°48′0″E) average elevation 2,598 m in the northeast catch a lot of the rainfall, so the southwest monsoon has a much lower intensity than in other regions, the mean annual rainfall in Nakhon Ratchasima is about 1,150 mm, compared with 1,500 mm in the Central of Thailand. There are 20 provinces; the 20th province is BuengKan, which separated from Nong Khai province on March 23, 2011. The population in the year 2010 was 21,305,000, with 40% of the population is concentrated in the provinces of Nakhon Ratchasima, Ubon Ratchathani, Khon Kaen, and Udon Thani. These provinces surround the four major cities of the same names; as of 2010, their populations were 142,169; 137,979; 113,828; and 83,148, respectively. However, as of 2010 only 50% of the region's population lived in municipal areas (National Statistical Office Thailand, 2010). Eight municipalities of eight provinces; Nakhon Ratchasima (NMA), Chaiyaphum CPM), Khon Kaen (KKN), Sakhon Nakhon (SKN), Loei (LEI), Nong Khai (NKI), Yasothon (YST), and Ubon Ratchathani (UBN) were selected based on the human population number in each municipality. Two plots of 1 x 1 km^2 were plotted inside and outside of each municipality, altogether 16 plots were sampled (Chapter III, Figure 3.2). Ten mango trees were selected in a plot followed the suitable trees based on the standard lichen monitoring protocol (Asta et al., 2002). Further criteria were trees with a girth 50-150 cm. All selected trees were geo-referenced using the global positioning system (GPS); therefore, 160 mango trees were selected. In addition, every plot was characterized by the distance from the road,

and mean diameter of sampled trees or diameter at breast height (DBH) (Table 4.1). Epiphytic lichens were recorded as the presence and frequency using a quadrat of 20 x 50 cm^2 comparing 10 small quadrat squares (10 x 10 cm^2), the occurrence of lichen species on the stem up to 1.5 m above ground level on the tree trunk where the lichens were the most presented. The frequency of each lichen species was calculated at each site (Asta et al., 2008). The species list of lichens per study site was compiled, considering the recorded taxa on all sample trees. The unidentified specimens in the field were collected for laboratory identification followed the standard checklists of Hale (1969), Awasthin (1991) and Sipmann (2003).

4.3.2 Lichen Diversity and Selected Trees

The lichen diversity was followed the results of Chapter III, which presented and described that lichen diversity values were higher for outside than inside municipalities.

Table 4.1 The physical factors of study sites; study site as map in Figure 3.2, Chapter III.

No.	Study sites		Physical factors						
			average annual temperature (°C)	average annual rainfall (mm)	average day of rainfall (day)	population number	distance from road (m)	altitude	DBH (cm)
1	CPMi	15°48'35"N, 102°01'13"E	27.22± 0.5	96.12±10.1	8.75±2.0	30,001-50,000	8.30±5.0	1-100	74.10±16.0
2	CPMo	15°48'30"N, 102°01'10"E	27.22 ± 0.7	98.00±10.0	8.75±2.0	30,001-50,000	12.00±5.0	1-100	84.50±16.8
3	KKNi	16°26'05"N, 102°50'0"E	26.72±1.5	100.35±9.0	9.70±2.5	>100,000	15.50±5.0	101-200	77.10±16.8
4	KKNo	16°26'0"N, 102°50'0"E	26.72±2.0	100.50±10.0	9.70±2.5	>100,000	18.00±5.5	101-200	94.40±22.4
5	LEIi	17°29'06"N, 101°43'48"E	26.03±1.5	103.18±6.5	10.75±3.0	<30,000	13.50±5.0	>300	94.00±25.5
6	LEIo	17°29'02"N, 101°43'45"E	26.03±0.8	105.18±5.0	10.75±3.0	<30,000	15.00±5.5	>300	83.90±20.7
7	NKIi	17°52'49"N, 102°44'42"E	26.75±1.5	134.18±4.7	10.50±2.0	30,001-50,000	10.30±5.0	101-200	95.10±26.7
8	NKIo	17°52'40"N, 102°44'45"E	26.00±3.0	135.25±4.5	10.50±2.0	30,001-50,000	20.00±5.0	101-200	87.50±21.0
9	NMAi	14°58'03"N, 102°06'0"E	27.03±2.0	95.00±3.5	9.50±3.0	>100,000	15.00±5.0	201-300	81.80±21.9
10	NMAo	14°58'0"N, 102°06'0"E	28.00±1.0	100.00±3.5	9.50±3.0	>100,000	12.00±5.0	201-300	106.00±33.5
11	SKNi	17°9'20"N, 104°08'02"E	26.15±2.5	117.13±5.5	10.75±2.0	50001-100,000	15.70±5.3	101-200	98.00±18.2
12	SKNo	17°9'15"N, 104°08'02"E	26.20±2.0	117.50±5.0	10.75±2.0	50001-100,000	12.00±5.5	101-200	87.50±10.1
13	UBNi	15°13'44"N, 104°51'15"E	27.18±1.0	136.17±6.0	10.33±2.5	50001-100,000	18.30±5.5	1-100	91.10±25.4
14	UBNo	15°13'40"N, 104°51'15"E	27.50±1.0	136.50±6.5	10.33±2.5	50001-100,000	16.00±5.0	1-100	85.60±24.0
15	YSTi	15°47'41"N, 104°08'26"E	27.18±2.2	117.90±5.0	9.00±2.0	<30,000	8.50±5.0	101-200	82.10±10.6
16	YSTo	15°47'37"N, 104°08'26"E	27.70±2.0	120.00±5.0	9.00±2.0	<30,000	13.70±5.0	101-200	80.20±14.0

Remark: Abbreviation

CPMi = inside municipality of Chaiyaphum, CPMo = outside municipality of Chaiyaphum, NMAi = inside municipality of Nakhon Ratchasima, NMAo = outside municipality of Nakhon Ratchasima, UBNi = inside municipality of Ubon Ratchathani, UBNo = outside municipality of Ubon Ratchathani, KKNi = inside municipality of Khon Kaen, KKNo = outside municipality of Khon Kaen, NKIi = inside municipality of Nong Khai, NKIo = outside municipality of Nong Khai, YSTi = inside municipality of Yasothon, YSTo = outside municipality of Yasothon, LEIi = inside municipality of Loei, LEIo = outside municipality of Loei, SKNi = inside municipality of Sakhon Nakhon, SKNo = outside municipality of Sakhon Nakhon.

4.3.3 Lichen Diversity and Environmental Variables

The environmental conditions around the selected mango trees were expected as factors influencing on lichen diversity. The studied context consisted of area conditions, areas surrounding the sampled trees, traffic effects, tree bark characteristics, directions of lichens found on the trees, distance from study trees to roads, tree circumferences (DBH), and acidity of bark (pH). According to the surveying form in Appendix A, further details were as follows:

4.3.3.1 Trunk Circumference or DBH

Trunk circumference of sampled mango trees or diameter at breast height (DBH) was recorded at 150 cm above the ground. The DBH categories were established as 10 cm intervals starting at 50 cm.

4.3.3.2 Area Conditions

The conditions around the selected mango tree were recorded as building density around the studied trees. They were divided into three groups:

1. High-density residential area: an area with many buildings such as commercial buildings, etc.

2. Low-density residential area: an area with medium density buildings such as houses, temples with spaces, etc.

3. Open area: an area without buildings such as small parks, playground, ground, etc.

4.3.2.3 Areas Surrounding of the Study Trees

The areas surrounding of the selected sampled trees categories were established into three groups of (i) lawn area, (ii) cement area, and (iii) ground area.

4.3.2.4 Tree Bark Characteristics

Tree bark characteristics were classified into three groups by: (i) superficial bark, (ii) moderately deep: not smooth with fairly crack, and (iii) deep-wrinkle bark: characteristics with large and deep cracks.

4.3.2.5 Directions of Grid Frame of the Trees

Due to the record about the directions on survey frame sitting on the tree trunks at the sides where the most lichens or most diversity were found.

4.3.2.6 Distance from Study Trees to Roads

The distance from selected trees to roads could be divided in the ranges of 1-5, >5-10, >10-15, >15-20 and greater than 20 m.

4.3.2.7 Acidity of Bark (pH)

Pieces of bark 2-3 mm thick without lichens were taken from the respective tree trunk at 1-1.50 m above the ground, kept in plastic bags. The bark samples were dried at 80°C for 24 hrs, grounded later. Two g of grounded bark was soaked with 10 ml distilled water and left for 24 hours, pH was determined directly in the solution by pH meter (Staxäng, 1969).

4.3.2.8 Age of Lichens

For the lichen thallus appearances, the age of lichens was recorded as the width of their thalli. As this work, the age of lichen could be classified into 3 groups: (i) young and mature thalli together, (ii) predominantly young thalli and (iii) predominantly mature thalli.

4.3.2.9 Lichen Vitality

For the lichen thallus's healthy, which could be referring to some impact, this work could be classified thalli into 3 categories: (i) predominantly healthy thalli, (ii) healthy and degraded thalli, and (iii) predominantly degraded thalli.

4.3.2.10 Tree Description

According to the surveying form, the mango trees were identified into 2 groups: (i) exposed, 2 sheltered and (ii) 1 unshaded, 2 shaded.

4.3.2.11 Influenced by Traffic

In this work, the traffic was included as the factors with having an impact on lichen diversity in the city habitats and grouped into 3 groups: (i) dirty road, (ii) asphalt road, low traffic and (iii) main road/highway, much traffic.

4.3.4 Data Analyses

The difference of all recorded factors were analyzed between inside and outside municipalities using pair t-test ($p<0.05$).The correlation between all factors and lichen frequency were analyzed using the principal component analysis (PCA) based on the Spearman's correlation coefficient, SPSS V18.

4.4 Results

All physical factors were recorded in each selected tree on surveying form (Appendix A). The results of all factors were illustrated in Appendix B. All data were grouped by calculating into percentage of each parameter as follows:

4.4.1 Grouping of Physical Factors

4.4.1.1 Tree Circumferences (Diameter at Breast Height; DBH)

The 160 selected mango trees, which the diameter at breast height (DBH) at 150 cm were grouped into 10 categories. The average of selected mango tree circumference was 88.14 cm. The DBH of selected trees were almost falling into 80 cm to 90 cm followed by 70- 80 cm and 60-70 cm, respectively (Figure 4.1a). The group was separated between inside and outside municipality areas (Figure 4.1b and 4.1c). There was no significant difference of DBH between inside and outside the municipal areas, but the mean DBH of outside municipalities was higher (89.15 cm) than inside municipalities (86.21 cm).
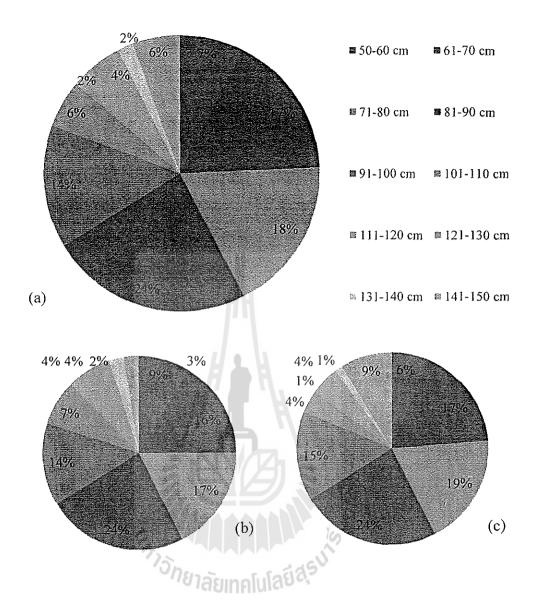
Figure 4.1 Percentage of the DBH of selected trees: (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.2 Area Conditions around the Selected Mango Tree

The area conditions around the selected mango trees were grouped, 62% of the selected mango trees were low-density residential area, 30% of

open area, and only 8% of high-density residential area (Figure 4.2a). The groups were separated between inside and outside the municipalities, the results were different in the inside the municipalities, it showed that the most areas were opened areas followed by the low-density residential areas and the high-density residential areas (Figure 4.2b). The result of area condition around the selected mango trees outside the municipal areas showed the same to the total of the sample tree in the Figure 4.2a (Figure 4.2c). However, there were significant difference between inside and outside the municipalities (df = 159, F = 0.637, p<0.05).
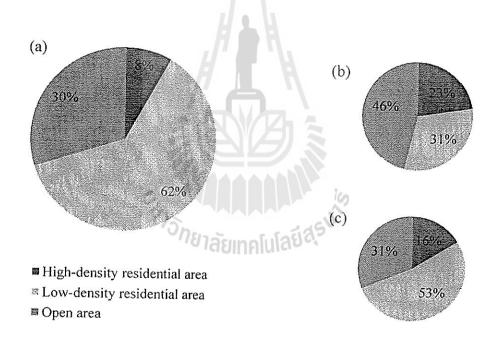
Figure 4.2 Percentage of each study area condition in the northeast of Thailand: (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.3 Areas Surrounding of Selected Mango Tree

The area surrounding selected mango trees were set into three main groups of lawn area, cement area, and ground area. The result of this survey found that 67% of the selected mango trees were ground area, 20% were cement area, and 13% were lawn area (Figure 4.3a). The result was different in the group of inside municipalities, lawn areas were larger than cement areas (Figure 4.3b) but not difference with group of outside municipalities (Figure 4.3c). They were not significant difference between inside and outside municipalities.
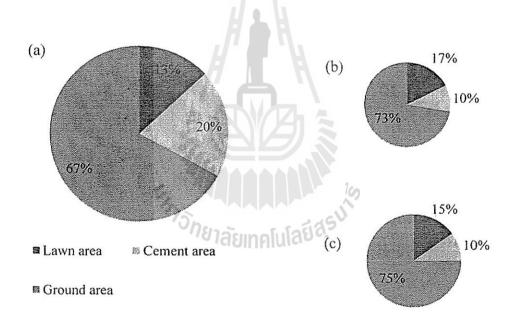
Figure 4.3 Percentage of each area study surrounding the trees: (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.4 Tree Bark Characteristics

The tree bark characteristics were recorded as the importance for lichen growth. For this study, tree bark characteristics (Figure 4.4a) could be classified into 3 groups of smooth, average smooth, and deep- wrinkle. The most selected mango trees were moderately deep of 42%, follow by 40% of superficial bark and the lowest of deep-wrinkle bark of 18%, this bark texture was a little thicker than the smooth bark and cracks. The results were also the same with inside and outside municipalities (Figure 4.4b and 4.4b). The result from pair t-test found that they were not significantly different between inside and outside municipalities.
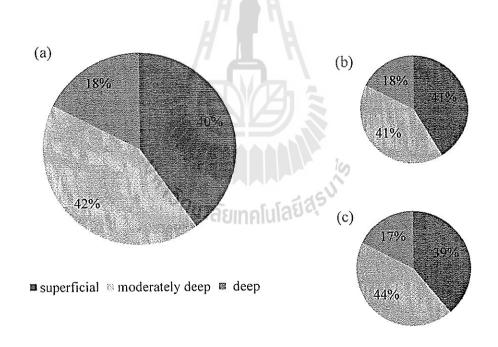
Figure 4.4 Percentage of each study bark characteristic: (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.5 Directions of Survey Grid Frame of the Tree Trunk

The directions of survey grid frame sitting on the tree trunk at the side where the most lichen thalli are the most presented were recorded. The data indicated that the most lichens were found in the northeast and the east of the trunks, respectively (Figure 4.5a). The results were separated both inside and outside municipalities. It showed that very different from total sample trees. For inside municipalities, the most direction was north (38.75%) and northeast (22.50%) followed by the north (13.75%), respectively (Figure 4.5b). For outside municipalities, the most direction that lichens were the most present on the mango trees was the north (27.5%) followed by the northwest (26.25%) and the southwest (13.75%), respectively (Figure 4.5c). They were not significant difference between inside and outside municipalities.

4.4.1.6 Distance of Selected Tree from Road

The distance from the selected tree and the nearest road were recorded and grouped into the percentage. The results showed that 61% of the selected tree were distanced from the nearest road less than 5 m followed by 26% the distance by more than 5 m to 10 m and a few of 3% more than 15 to 20 m (Figure 4.6a). For inside municipalities, it showed that most of selected mango trees were located at distance less than 5 m from the road (49%) followed by distance more than 5 to 10 m (31%) (Figure 4.6b). For outside municipalities, most of selected mango trees included as less than 5 m from the road for 74% followed by 21% more than 5 to 10 m (Figure 4.6c). The result showed significantly different between inside and outside municipalities (df = 159, F = 0.910, p < 0.05).
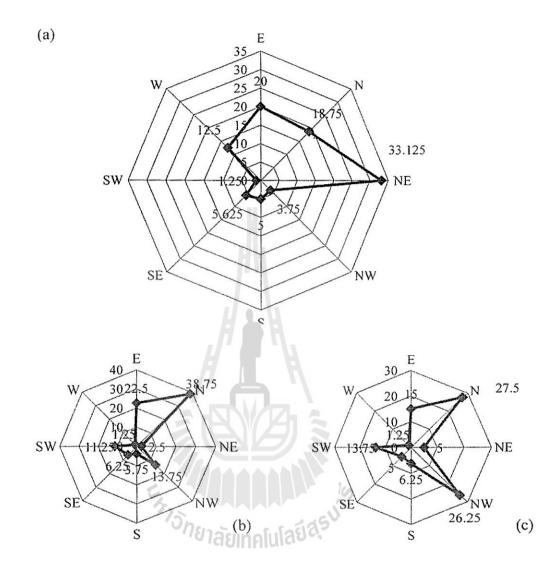
Figure 4.5 Percentage of directions of survey grid frame in the tree: (a) total of selected trees (n = 160) (b) selected trees inside municipalities (n = 80) (c) selected trees outside municipalities (n = 80).

4.4.1.7 Acidity of mango tree barks (pH)

The acidity of mango tree barks ranged from 5.34 to 5.55, the average of all 160 sampled mango trees was 5.4 ± 0.06. The average of pH of 80

sampled trees outside municipalities was significantly higher of 5.43 ± 0.06 than inside municipalities of 5.40 ± 0.07 (p<0.05). The comparison between the two sides of the roadside and the reversed side found that the pH was significant lower in near roadside for 5.39 ± 0.07 and 5.44 ± 0.06 for the reversed side (p<0.05). The Spearman's correlation analysis showed that the number of lichen species and the bark pH was not significantly correlated. The suitable pH for lichens growing on mango trees ranged 5.40-5.50. The number of lichen species was highest at pH of 5.50 and lowest at pH of 5.35. The lichen species number was lower when the pH was higher than 5.50 (Figure 4.7).
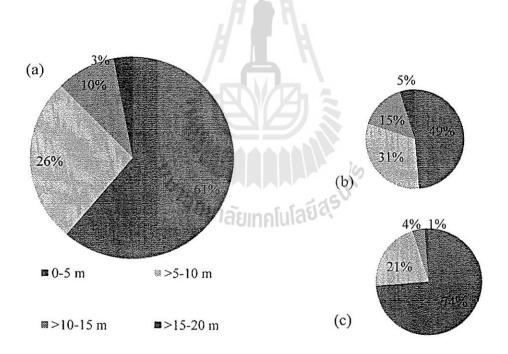
Figure 4.6 Percentage of distance of selected tree from road: (a) total of selected trees (n = 160), (b) selected tree inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).
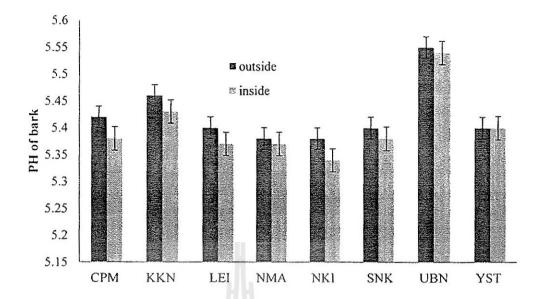
Figure 4.7 The pH of mango tree barks in the northeast of Thailand comparing

between inside and outside municipality in each municipality (CPM=

Chaiyaphum KKN = Khon Kaen, LEI = Loei NMA = Nakhon

Ratchasima, NKI = Nong Khai, SKN = Sakhon Nakhon, UBN = Ubon

Ratchathani, YST = Yasothon.

4.4.1.8 Age of Lichen Thallus

The age of lichen thallus was grouped into 3 groups; most of

thallus were predominantly mature thalli (54%), followed by predominantly young

thalli (26%) and young and mature thalli together (20%) (Figure 4.8a). Most thalli

found in the municipality were result of the total of 54%, 31%, and 15%, respectively

(Figure 4.8b). For outside the municipal areas, the most lichen thallus found

predominantly mature thallus (54%) followed by young and mature thalli together

(25%) and predominantly young thalli (21%) (Figure 4.8c). However, age of lichens

thallus showed no significant difference between inside and outside municipalities.
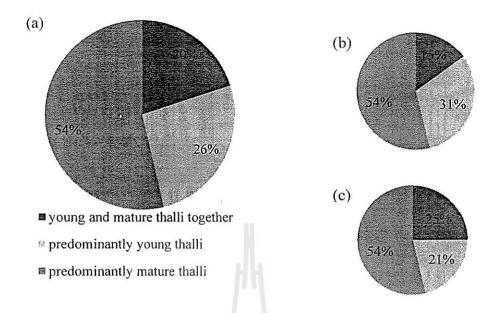
Figure 4.8 Percentage of age of lichens (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.9 Lichen Vitality

The lichen vitality or the health of lichen thallus was observed and grouped into three groups. Most of lichen thallus found in this work was predominantly degraded thallus (39%) followed by healthy and degraded thallus (35%) and predominantly healthy thallus (26%) (Figure 4.9a). The results were separated between inside and outside the municipal areas, the results from in the municipal areas showed the same results of the total sample as shown in the Figure 4.9a about 54%, 34%, and 12%, respectively (Figure 4.9b). For outside the municipality group showed the most of lichen thallus healthy were predominantly healthy thallus (40%) followed by healthy and degraded thallus (35%) and degraded

thallus (25%), respectively (Figure 4.9c). The results of both inside and outside municipalities showed significant difference (df = 159, F = 0. 204, p< 0.05).

(a)

(b) 12%

(c)

■ predominantly healthy thalli

▨ healthy and degraded thalli

▦ predominantly degraded thalli
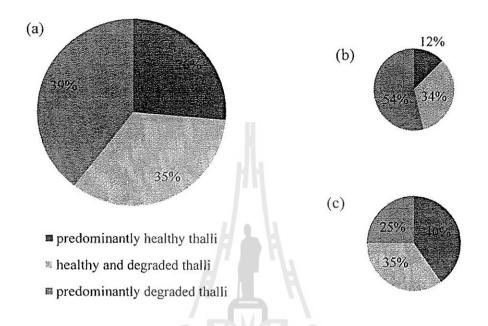
Figure 4.9 Percentage of lichen vitality (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.10 Tree Description

The description of sample trees was recorded as the their shaded, the results showed that the most sampled trees were 1 un-shaded, 2 shaded (53%) and exposed, 2 sheltered (47%) (Figure 4.10a). The results were separated between inside and outside the municipality groups, the results of inside the municipality was the same as a total of all sample trees in Figure 4.10a for 60% and 40%,respectively (Figure 4.10b), but different for outside municipalities with 46%

and 54%, respectively (Figure 4.10c). However, the tree description was not significant difference between both inside and outside municipalities.
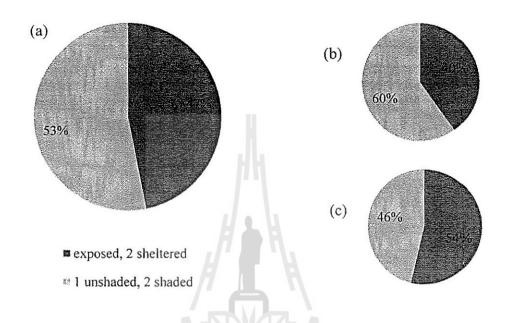
Figure 4.10 Percentage of tree description (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.1.11 Influence by Traffic

The traffic was grouped into 3 groups of road. Most of sample trees were located in main road/ highway, much traffic (54%) followed by asphalt road, low traffic (25%) and dirty road (21%) (Figure 4.11a). The results were separated between inside and outside municipalities. For inside municipalities, the most traffic types were main road/ highway, much traffic (54%) followed by dirty road (29%) and asphalt road, low traffic (17%) (Figure 4.11b). For outside

municipalities, there were main road/ highway, much traffic (55%) followed by asphalt road, low traffic (31%) and dirty road (14%) (Figure 4.11c). However, the traffic was not significantly different between inside and outside municipalities (df = 159, F = 0. 011, p_i<0.05)
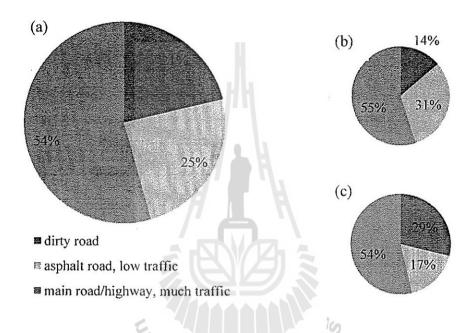

Figure 4.11 Percentage of influence by traffic (a) total of selected trees (n = 160), (b) selected trees inside municipalities (n = 80), (c) selected trees outside municipalities (n = 80).

4.4.2 Correlation of Lichen Diversity and Environmental Factors

Environmental factors around the selected mango trees were classified as a factor influencing lichen growth and lichen diversity. The studied context consisted of area condition around the selected mango tree, the area surrounding of selected mango tree, traffic effects, tree bark characteristic, directions of survey grid frame in

the tree trunk, distance of trees from roads, tree circumferences, and the acidity of bark. These further details were as follows:

4.4.2.1 The Correlation between Species Richness and the Mango Tree Bark's pH

The Spearman's correlation analysis showed that the number of lichen species and the bark pH was significantly correlated ($r = 0.317$, $p<0.05$). The suitable pH for lichens growing on mango trees ranged 5.40-5.50. The highest number of lichen species found at pH of 5.50 and lowest at pH of 5.35. The lichen species number was lower when the pH was higher than 5.50 (Figure 4.12).
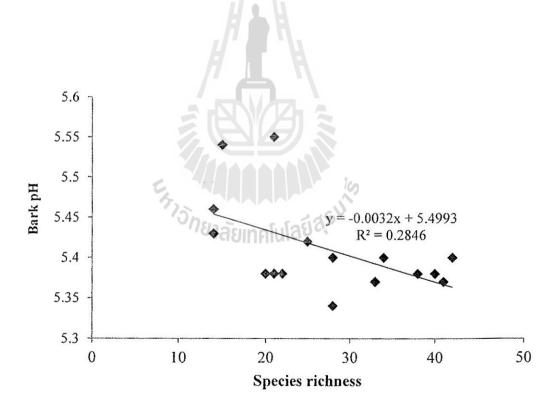
Figure 4.12 The correlation between bark pH and species richness.

4.4.2.2 Correlation between Altitude and Species Richness

The species richness was increased with the altitude height at the altitude about 200-400 m. They showed correlation between altitude and lichen species richness (Figure 4.13).
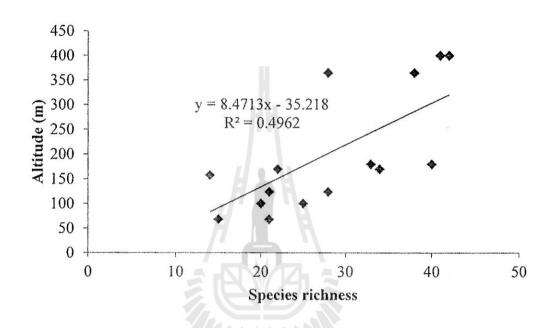
Figure 4.13 The correlation between altitude and species richness.

4.4.2.3 Correlation between Diversity Index Each Province and Population in Each Municipality

The correlation analysis using the Spearman's coefficient showed that there were negative correlation between diversity indices in each province and population in each municipality (r = -0.300, p<0.01). The results showed that the diversity index was increased when the number of population in each municipality decreased (Figure 4.14).

$$y = -5E\text{-}06x + 3.4997$$
$$R^2 = 0.1623$$
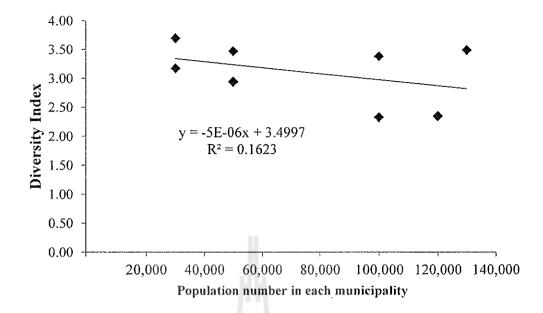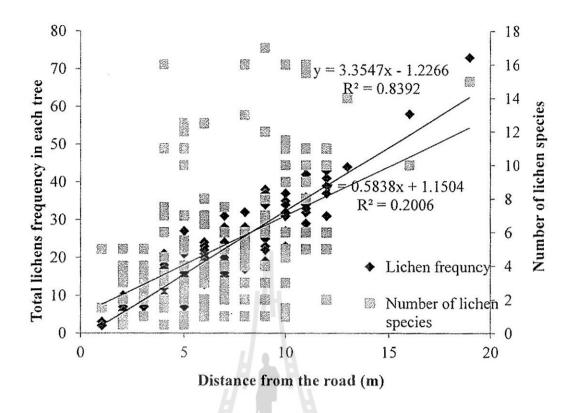
Figure 4.14 The correlation between diversity index and the population number in each municipality.

4.4.2.4 The Correlation between the Lichen Frequency and the Number of Lichen Species and the Distance from the Road

The correlation between the lichen frequency and the distance from the road found that the lichen frequencies were increased when lichen growing far from the road (Figure 4.15). The highest of lichen frequency found on the site where the road distances was highest about 19 m and lowest lichen frequency found at 2 m from the road. They showed significant negative correlation with distance of road ($r = -0.164$, $p<0.01$). The correlation showed significant negative correlation with the distance from the road ($r = -0.200$, $p<0.05$), the highest of number lichens were found at 9 m from the road and lowest at 1 m from the road.

Figure 4.15 The correlation between the lichen frequency and the number of lichen species and the distance from the road.

The PCA based on all parameters separated along first ordination axis, lichen frequency and LDV were positively correlated with areas surrounding, altitude, tree shade, DBH, and area conditions, but negatively with annual rainfall, bark pH, and human population number. From the PCA, it indicated that various factors influence air quality in this region including the population, traffic effect, and distance from the road. Moreover, the correlation of lichen vitality to the area surrounding and annual rainfall was found (Figure 4.16). The results showed that the lichen diversity was negatively correlated with air quality class. The values of similarity were calculated based on the Spearman's correlations (Table 4.3).
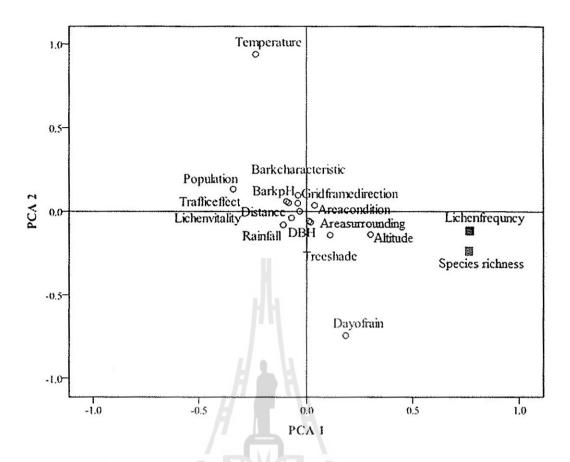
Figure 4.16 The PCA analysis based on lichen frequency and all variable factors.

4.4.3 Correlation of Lichens Frequency and Physical Factors

The Spearman's correlation coefficients among lichen frequency and species richness and physiological parameters showed that the both frequency and richness were highest correlated with the altitude followed by average day of rainfall and negatively correlated with population in inside the municipality and average of days of rainfall around the year. The average day of rainfall in this region was high significance level with average rainfall and average temperature around the year. The latitude showed high significant correlation with rainfall, average temperature, and traffic effect, and other correlation of population in the municipality and average of days of rainfall (Table 4.3).

Table 4.2 Spearman's correlation coefficients among lichens frequency, lichens diversity value, and physiological parameters. only significant value is presented.

	D	AS	BA	P	AT	AR	AD
Lichens frequency (F)	-0.264**				0.243**		
Species Richness (SR)				-0.329**	0.512**	-0.210**	0.284**
Tree circumference (TC)			-0.195*		-0.220**		
Distance from road (D)		-0.271**					0.197*
Bark characteristic (BC)					0.198*	-0.167*	-0.170*
Area surrounding (AS)				-0.170*			0.169*
Bark's acidity (BA)						0.298**	0.304**
Traffic effect (TE)				-0.192*	0.267**	-0.251**	-0.289**
Average of temperature around year (AT)							-0.800**
Average of rainfall (AR)				-0.342**	-0.395**		
Average day of rainfall (AD)						0.358**	
Altitude (AT)							0.333**

* Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

4.5 Discussions

The factors influencing on lichen diversity were identified by previous study from various countries (Gustafsson and Eriksson, 1995; Fujiwara, Gómez, Dawidowski, Perelman, and Faggi, 2011). The factor impact on lichens might be influencing living organisms as well as human. The main variable involved are similar to those reported in much larger tropical areas of Southeast Asia, especially Thailand (Wolseley, Moncrieff, and Aguirre-Hudson, 1994; Saipunkaew, Wolseley, Chimonides, and Boonpragob, 2007). Lichen diversity had correlation with tree shade, the area surrounding, DBH, and altitude. The altitude was determined as influencing on lichen diversity in this work, but the result was not clear that altitude was considered as important factors because the average of altitude was not significant difference between study sites. However, Saipunkaew et al. (2005) reported that lichen diversity was increased with increasing of the altitude, but decreased with higher human population number. The distance from the road had correlated with total lichen frequencies and number of lichen species in each plot (Giordani and Incerti, 2008). In several cases, the relative importance and co-variation between variables was different. Tree size and tree species influenced lichen species presence; large, old trees have been exposed to colonization for longer, increasing the probability of propagule arrival, then the dispersing propagules are more likely to be caught in the cracks and crevices of larger trees with rough bark than on young trees with smooth bark (Uliczka and Angelstam, 1999). In addition, larger and older trees may have properties that make them more suitable for lichen establishment, such as different bark chemistry (Gustafsson and Eriksson, 1995). These factors may also vary between tree species and, for epiphytic lichens, the importance of tree age, tree

species and bark chemistry is often stressed (Gauslaa and Solhaug, 1998). The result observed that the bark pH was lower for inside municipality than outside municipality, this result is similar to van Herk (2001) which, reported that the pollution in urban reduced the pH of bark and traffic had an impact on lichens (Roorda-Knape et al., 1998). This result indicated that the bark pH was dependent on type of pollutants (Marmor, Tõrra, and Randlane, 2010). Transport- related pollutants and bark acidity influence the distribution and abundance of lichens and bryophyte (Larsen et al., 2007). The age of the tree is another important factor affecting lichen communities, as many lichen species are associated with older trees (Ranius, Johansson, Berg, and Niklasson, 2008; Fritz, 2009). Substrate quality change and a longer period are available for colonization when trees grow older; age related changes also occur vertically in forest canopies being one reason for vertical changes in lichen communities. Young substrate age is probably the main reason for the lower species richness in the treetops where lichen communities have not had enough time to develop. Higher wind speed could be an additional reason, presumably hindering the attachment of pendulous lichen thalli, and causing drought. This result confirmed many previous studies that lichen diversity clearly affected by air pollution in urban and suburban areas (Frati and Brunialti, 2006; Larsen et al., 2007). A similar pattern was achieved when focusing only on macrolichens (Uliczka and Angelstam, 1999; Pinho, Augusto et al., 2004; Marmor et al., 2010). The local land-use traffic clearly showed the lowest lichen richness and LDV evidencing to be the worst location in a small city in term of air quality. This is not new information since it was reported by several other authors (Gombert, Asta, and Seaward, 2004; Bräuniger, Knapp, Kühn, and Klotz, 2010). The latter information together with the fact that mango tree bark

pH is higher in traffic areas may suggest the existence of pollutants such as alkaline dust particles or atmospheric NH_3 (Frati and Brunialti, 2006). A high correlation between the accumulation of nitrogen in lichens and the proximity to areas with a dense traffic has been established in urban areas (Gombert, Asta, and Seaward, 2003; 2006). It is reported that an increase of lichen diversity as it move away from the city center to suburban or non-urban areas (Loppi, Frati, Benedettini, Pirintsos, and Leonzio, 2002). However, there is not a regular distribution or gradient of the lichen diversity or abundance like in big cities. Nevertheless, in all the cases, traffic is the main factor for the declining in lichen diversity and abundance. The deposition of particles, heavy metals, and gaseous pollutants is higher close to roadsides, decreasing with distance (Frati and Brunialti, 2006). Domestic activity was evaluated with the houses land-use, may have some impact, but it can be almost negligible when compared to traffic. With functional diversity, it was not only able to distinguish between green and houses land-use areas but also has an insight into the factors that might drive lichen community changes. Apparently, green areas are the best location in terms of lichen diversity and LDV in a small city. Thus, it seems that the general air quality in green areas is in fact better than houses land-use areas, maybe because the latter, although with less traffic areas, still has some vehicle circulation. However, houses land-cover, which was close to car parking, was also lack of vegetation, but did not show a higher proportion of xerophytic lichens. The mixture of pollutants dispersed by roads may cause shifts in the lichen communities towards a more xerophytic community (LeBlanc and De Sloover, 1970; Brodo, 1973). Further studies should elucidate whether traits in this species are responding specifically to a specific environmental change or whether they are responding to a large number of factors.

Nevertheless, more importantly, we have observed large changes in lichen variables in trees located at short distances from each other. This is due to the changes in the local land-use, causing a change in lichen variables even if trees are located at a few meters apart. Thus, as in other studies that aimed in using ecological indicators to monitor the effects of factors that change at a short-distance, such as atmospheric ammonia (Pinho et al., 2004), when sampling in cities, each tree should be considered as representative of the site but this fact should be compensated by a denser sampling network.

This work recorded various factors e.g. area condition around the selected mango tree, three categories, not high-density residential area, open area, and high-density residential area. The most of condition was included as not high-density residential area and this work showed no significant correlation with lichen diversity in this area. This result of areas surrounding the selected trees was not influencing on lichen diversity (Ayrault et al., 2007; Fujiwara, Gómez, Dawidowski, Perelman, and Fuji, 2011), and the study of lichen in urban habitats which reported that the condition of tree hosts was not impacted on lichen (Käffer et al., 2011). However, the results of the condition surrounding inside the municipal areas showed a difference, the most condition found in the municipality areas were open areas and the results showed no a significant correlation with lichen diversity inside and outside the municipal areas. Area surrounding the selected trees consisted of three main groups of lawn area, cement area, and ground area. The results showed that most area was ground area and this result was the same in both inside and outside the municipal area groups, so this would be a good sampled area surrounding of selected trees with non-biases. This

factor showed no significant correlation with lichen diversity explained by Käffer et al. (2011) which reported the factors influencing on lichen diversity in Brazil.

For directions of the survey grid frame of the tree trunk, the grid frame was used to calculate the lichen diversity by putting on the tree trunk where the most abundant of lichens. The result showed that more lichens grew in the northeast of the tree than other sides. The result showed highest for both inside and outside the municipal area groups at the north direction. The results explained that the direction of light in the morning was a good supporting factor for lichen growth. Beside this reason, lichens needed humidity for the growth and the fog in every morning was suitable for it photosynthesis so this would be a reason that why lichens were observed in the northeast and north directions.

In the distance of selected tree from the road, the traffic has been a main some of air pollution and was included as factors influencing on phototoxic so it might influence lichen diversity in this work. The influence was not identified as traffic pollutants but the distances from the road and selected trees were recorded. The result showed most of sampled trees located less than 5 m from the road; this result was found both inside, and outside municipalities, thus these sampled trees were a good sample of this work. For the correlation with lichen diversity and road distances showed the significant correlation. This result supported Roorda-Knape et al. (1998), who reported that most pollutants from traffic near the motorway were negative correlation with the distance and could be damaging lichen thallus (Frati et al., 2006). In comparing between inside and outside municipalities, found no significant difference, this suggested that the sample trees were not any biases for researching and completely useful as a good host sampled trees for this work.

The lichen thallus characteristics were considered e.g. age of lichen thalli and their vitality. As lichens compacted by symbiotic organisms and their thalii were broken down by pollution so this investigation might be observed some important factors. The lichen thallus healthy found that thalli outside the municipality were healthier than inside the municipality this results supported Palee (2002) who reported that lichens in the town showed higher diversity than urban areas. Anyway, lichen found inside municipalities showed younger thalli than that outside municipalities. The result supported that the propagules can be develop in the city than that outside.

The northeast of Thailand has been noted with a relatively low level of epiphytic lichen diversity. Differences in species richness, evenness, and composition between inside and outside municipalities related lichen species number was observed. The pattern of diversity of epiphytic lichens at inside municipalities was driven by various environmental factors. Indeed, the range of parameters inside municipalities influencing the epiphytic lichen development has been well recognized (Hauck, 2005; Giordani and Will-Wolf, 2006). Some of the most significant parameters, which influence epiphytic lichens inside and outside the municipalities in the northeast of Thailand, include average day of rainfall, average rainfall, and average temperature around the year, altitude, traffic effect, population in the municipality. Another important substrate parameter is the acidity of tree bark, the pH of bark has a strong influence on epiphytic lichen development (Lamit et al., 2011). However, the correlation between the acidity of barks and lichens frequency showed no significance. The rainfall and average day of rainfall support a level of humidity within the immediate environment and modifies fluctuations in the atmospheric moisture content at the sites.

4.6 Conclusions

There were various factors influencing on lichen diversity in the northeast of Thailand. The population in each municipality was strongly influenced on lichen diversity followed by the altitude and the distance from the road. From this work could be concluded that human and traffic has affected lichen in the city habitats in the northeast of Thailand. The effect of environmental factors on lichen diversity results from their power and from the degree of dependence between the variables. It may be very difficult to determinate an influence of a particular factor on lichens (living organism). The factors impact on lichens are may the same such as air pollution, temperature, humidity, light intensity, but the parameter with stronger correlations with lichens could sometimes override the effect of other environmental parameter. Therefore, the factors influencing lichen diversity in the northeast of Thailand were identified; altitudes, annual rainfall, human population, bark properties.

4.7 References

Aptroot, A., Saipunkaew, W., Sipman, H. J. M., Sparrius, L. B., and Wolseley, P. A. (2007). New lichens from Thailand, mainly microlichens from Chiang Mai. **Fungal Diversity.** 24: 75-134.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., and Van Haluwyn, C. (2008). **European Guideline for mapping lichen diversity as an indicator of environmental stress.** UK: British Lichen Society.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P. L., Purvis, O. W., Pirintsos, S., Scheidegger, C., Van Haluwyn, C., and Wirth, V. (2002). Mapping lichen diversity as an indicator of environmental quality. In Nimis, P. L., Scheidegger, C. and Welseley, P. A. (eds.). **Monitoring with Lichens-Monitoring Lichens**. UK: Kluwer.

Awasthi, D. D. (1991). **A key to the microlichens of India, Nepal and Sri Lanka.** USA: Cramer.

Ayrault, S., Clochiatti, R., Carrot, F., Daudin, L., and Bennett, J. P. (2007). Factors to consider for trace element deposition biomonitoring surveys with lichen transplants. **Science of the Total Environment.** 372(2-3): 717-727.

Bartók, K. (1999). Pesticide usage and epiphytic lichen diversity in Romanian orchards. **The Lichenologist.** 31(01): 21-25.

Bates, J. W., Proctor, M. C. F., Preston, C. D., Hodgetts, N. G., and Perry, A. R. (1997). Occurrence of epiphytic bryophytes in a tetrad transect across southern Britain: Geographical trends in abundance and evidence of recent change. **Journal of Bryology.** 19(4): 685-714.

Batty, K., Bates, J. W., and Bell, J. N. B. (2003). A transplant experiment on the factors preventing lichen colonization of oak bark in southeast England under declining SO_2 pollution. **Canadian Journal of Botany.** 81(5): 439-451.

Bräuniger, C., Knapp, S., Kühn, I., and Klotz, S. (2010). Testing taxonomic and landscape surrogates for biodiversity in an urban setting. **Landscape and Urban Planning.** 97(4): 283-295.

Brodo, I. M. (1973). **Substrate ecology.** (Vol. 401). New York: Academic Press.

Frati, L., Caprasecca, E., Santoni, S., Gaggi, C., Guttova, A., Gaudino, S., Pati, A., Rosamilia, S., Pirintsos, S., and Loppi, S. (2006). Effects of NO_2 and NH_3 from road traffic on epiphytic lichens. **Environmental Pollution.** 142(1): 58-64.

Fritz, Ö. (2009). Vertical distribution of epiphytic bryophytes and lichens emphasizes the importance of old beeches in conservation. **Biodiversity and Conservation.** 18(2): 289-304.

Fujiwara, F. G., Gómez, D. R., Dawidowski, L., Perelman, P., and Faggi, A. (2011). Metals associated with airborne particulate matter in road dust and tree bark collected in a megacity (Buenos Aires, Argentina). **Ecological Indicators.** 11(2): 240-247.

Gauslaa, Y., and Solhaug, K. A. (1998). The significance of thallus size for the water economy of the cyanobacterial old-forest lichen *Degelia plumbea*. **Oecologia.** 116(1): 76-84.

Geo-Informatics Centre for the Development of Northeast Thailand. (2008). **Geography of the Northeast Thailand.** [On-line]. Avilable: http://gecnet.kku.ac.th/ rs_coni_08_Drought_NE_Multi_Temporal.asp.

Gilbert, O. L. (1976). An alkaline dust effect on epiphytic lichens. **The Lichenologist.** 8(2): 173-178.

Giordani, P., and Incerti, G. (2008). The influence of climate on the distribution of lichens: A case study in a borderline area (Liguria, NW Italy). **Plant Ecology.** 195(2): 257-272.

Giordani, P., and Will-Wolf, S. (2006). Variables influencing the distribution of epiphytic lichens in heterogeneous areas: A case study for Liguria, NW Italy. **Journal of Vegetation Science.** 17(2): 195-206.

Gombert, S., Asta, J., and Seaward, M. R. D. (2003). Correlation between the nitrogen concentration of two epiphytic lichens and the traffic density in an urban area. **Environmental Pollution.** 123(2): 281-290.

Gombert, S., Asta, J., and Seaward, M. R. D. (2004). Assessment of lichen diversity by index of atmospheric purity (IAP), index of human impact (IHI) and other environmental factors in an urban area (Grenoble, southeast France). **Science of the Total Environment.** 324(1-3): 183-199.

Gombert, S., Asta, J., and Seaward, M. R. D. (2006). Lichens and tobacco plants as complementary biomonitors of air pollution in the Grenoble area (Isère, southeast France). **Ecological Indicators.** 6(2): 429-443.

Grodzińska, K. (1977). Acidity of tree bark as a bioindicator of forest pollution in southern Poland. **Water, Air, & Soil Pollution.** 8(1): 3-7.

Gustafsson, L., and Eriksson, I. (1995). Factors of importance for the epiphytic vegetation of aspen *Populus tremula* with special emphasis on bark chemistry and soil chemistry. **Journal of Applied Ecology.** 32: 412-424.

Hale, M. E. (1969). **How to know the lichens: The pictured key nature series.** (Vol. 5). Dubuque: Brown.

Hamada, N., and Miyawaki, H. (1998). Lichens as bioindicators of air pollution. **Japanese Journal of Ecology.** 48(1): 49-60.

Hauck, M. (2005). Epiphytic lichen diversity on dead and dying conifers under different levels of atmospheric pollution. **Environmental Pollution.** 135(1): 111-119.

Johnsen, I., and Søchting, U. (1973). Influence of air pollution on the epiphytic lichen vegetation and bark properties of deciduous trees in the Copenhagen area. **Oikos.** 24:344-351.

Käffer, M. I., Martins, S. M. d. A., Alves, C., Pereira, V. C., Fachel, J., and Vargas, V. M. F. (2011). Corticolous lichens as environmental indicators in urban areas in southern Brazil. **Ecological Indicators.** 11(5): 1319-1332.

Lamit, L. J., Bowker, M. A., Holeski, L. M., Næsborg, R. R., Wooley, S. C., Zinkgraf, M., Lindroth, R. L., Whitham, T. G., and Gehring, C. A. (2011). Genetically-based trait variation within a foundation tree species influences a dominant bark lichen. **Fungal Ecology.** 4(1): 103-109.

Larsen, R. S., Bell, J. N. B., James, P. W., Chimonides, P. J., Rumsey, F. J., Tremper, A., and Purvis, O. W. (2007). Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. **Environmental Pollution.** 146(2): 332-340.

LeBlanc, F., and De Sloover, J. (1970). Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. **Canadian Journal of Botany.** 48: 1485-1496.

Loppi, S. (2000). Lichen biomonitoring as a tool for assessing air quality in geothermalareas. In **Proceeding of the World Geothermal Congress 2000,** Kyushu-Tohoku, Japan.

Loppi, S., Frati, L., Benedettini, G., Pirintsos, S. A. R. G., and Leonzio, C. (2002). Biodiversity of epiphytic lichens as indicator of air pollution in the geothermal area of Lardello (Tuscany, Central Italy). **Israel Journal of Plant Sciences.** 50(2): 119-126.

Marmor, L., Tõrra, T., and Randlane, T. (2010). The vertical gradient of bark pH and epiphytic macrolichen biota in relation to alkaline air pollution. **Ecological Indicators.** 10(6): 1137-1143.

McCune, B., and Mefford, M. J. (1999). **PC-ORD: multivariate analysis of ecological data; Version 4 for Windows; [User's Guide].** MjM Software Design.

National Statistical Office Thailand. (2010). **Key statistics of Thailand 2010.** [On-line]. Avilable: http://web.nso.go.th/en/survey/keystat/keystat10.pdf.

Palee, T. (2002). **Used of lichens as bioindicator for air pollution monitoring in town and urban of the Chiang Mai province in 2001.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Papong, K., Boonpragob, K., and Lumbsch, H. T. (2011). A new species and new records of *Lecanora* (Lecanoraceae, Ascomycota) from south–east Asia. **The Lichenologist.** 43(1): 47-50.

Pinho, P., Augusto, S., Branquinho, C., Bio, A., Pereira, M. J., Soares, A., and Catarino, F. (2004). Mapping lichen diversity as a first step for air quality assessment. **Journal of Atmospheric Chemistry.** 49(1-3): 377-389.

Purvis, O. W., Seaward, M. R. D., and Loppi, S. (2007). Lichens in a changing pollutionenvironment: An introduction. **Environmental Pollution.** 146 (2): 291-292.

Purvis, O.W. (2000). **Lichens.** UK: Natural History Museum.

Ranius, T., Johansson, P., Berg, N., and Niklasson, M. (2008). The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. **Journal of Vegetation Science.** 19(5): 653-662.

Roorda-Knape, M. C., Janssen, N. A. H., De Hartog, J. J., Van Vliet, P. H. N., Harssema, H., and Brunekreef, B. (1998). Air pollution from traffic in city districts near major motorways. **Atmospheric Environment.** 32(11): 1921-1930.

Saipunkaew, W., Wolseley, P. A., and Chimonides, P. J. (2005). Epiphytic lichens as indicators of environmental health in the vicinity of Chiang Mai city, Thailand. **The Lichenologist.** 37(4): 345-356.

Saipunkaew, W., Wolseley, P. A., Chimonides, P. J., and Boonpragob, K. (2007). Epiphytic macrolichens as indicators of environmental alteration in northern Thailand. **Environmental Pollution.** 146(2): 366-374.

Sipman, H. J. M. (2003). **Key to the lichen genera of Bogor, Cibodas and Singapore.** Berlin: Berlin-Dahlem Freie Universität.

Sparrius, L. B., Saipunkaew, W., Wolseley, P. A., and Aptroot, A. (2006). New species of *Bactrospora, Enterographa, Graphidastra* and *Lecanographa* from northernThailand and Vietnam. **The Lichenologist.** 38(1): 27-36.

Staxäng, B. (1969). Acidification of bark of some deciduous trees. **Oikos.** 20: 224-230.

Uliczka, H., and Angelstam, P. (1999). Occurrence of epiphytic macrolichens in relation to tree species and age in managed boreal forest. **Ecography.** 22(4): 396-405.

van Herk, C. M., Mathijssen-Spiekman, E. A. M., and de Zwart, D. (2003). Long

distance nitrogen air pollution effects on lichens in Europe. **TheLichenologist.**

35(4): 347-359.

Verein Deutscher Ingenieure. (1995). Measurement and evaluation of phytotoxic

effects of ambient air pollutants (emissions) with lichens. In **Mapping of**

lichens for assessment of the air quality, VDI-Richtlinie 3799, Platt1/part 1:

Düsseldorf.

Wolseley, P., Moncrieff, C., and Aguirre-Hudson, B. (1994). Lichens as indicators of

environmental stability and change in the tropical forests of Thailand. **Global**

Ecology and Biogeography Letters. 4(4): 116-123.

CHAPTER V

GENETIC RELATIONSHIP IN *PYXINE COCOES* (SW.)

NYL. (FAMILY PHYSIACEAE)

5.1 Abstract

Pyxine cocoes (Sw.) Nyl. (Physciaceae), the foliose lichens are found throughout tropical to pantropical areas. This species is recorded as a good indicator for air pollution. This work aimed to study the genetic variation of *P. cocoes* growing in differences of two pollution levels (inside and outside municipal areas) using the Randomly Amplified Polymorphic DNA (RAPD) method. The specimens were taken from 6 study sites include inside and nearby Nakhon Ratchasima municipality; 4 aspects (N, E, W, and S of the municipality), in municipal areas and control site was selected at Suranaree University of Technology. Three healthy thalli of *P. cocoes* at each site were taken from mango tree (*Mangifera indica* L.), totally eighteen thalli. Eight RAPD primers producing 170 bands suggested that the genetic high variability within *P. cocoes* that were located in different locations. The distance of similarity in genetic of *P. cocoes* ranged from 51% to 93% and average was 71% for RAPD markers. Although a high number of sample specific bands (31) were observed, but no specific species band was presented. The RAPD-based dendrogram clustered the samples of *P. cocoes* into two main groups. The first main group included 12 samples, which were collected from outside the municipality and another consisted of three

samples that were collected in the municipal area where the pollution is higher than four aspects outside the municipal area. Moreover, the group of outside the municipality of Nakhon Ratchasima showed four subgroups depending on the location. This work concluded that the genetic variation among species was based on their location origins and can be predicted that air pollution in the city had an impact on the genetic variation.

5.2 Introduction

Lichens are accepted as an important role in the ecosystem, especially tropical rain forest. They are useful as a good indicator of environmental monitoring (Loppi, 1996). The lichens Physciaceae was reported as dominant family in the northeast of Thailand (Dathong, Thanee, and Saipunkaew, 2012). The habitat of this family was observed in open situations, such as on roadside trees, in thorn bush forests, dry dipterocarp forests, coastal vegetation, or dry evergreen forests (Cáceres, Lücking, and Rambold, 2007, 2008). There are 27 genera included in this family and the *Pyxine* Fr., one member of the family Physciaceae, is widespread distribution in pantropical to subtropical regions and contains about 65 species (Kirk and Ainsworth, 2008; Elix, 2009). Morover, several species extend into temperate or oceanic regions (Kalb et al., 1987; Kalb, 1994) and 15 species of *Pyxine* (Physciaceae, lichenized ascomycetes) are recorded in Australia (Rogers, 1986). In addition, 12 species of *Pyxine* were observed and described in Thailand (Mongkolsuk, Meesim, Poengsungnoen, and Kalb, 2012), the first three species identified were *P. asiatica* and *P. schmidtii*, both from Trat province, Koh Chang Island in the Gulf of Thailand (Vainio 1907) and *P. retirugella* var. laevior (= P. *retirugella*) from nearby Lem Ngob (Vainio, 1909).

Wolseley et al. (2002) added *P. coccifera*, *P. consocians* (= *P. retirugella*) and *P. coralligera* from Chiang Mai province, bringing the number of species to five. Subsequently, Aptroot et al. (2007) recorded *P. cylindrica*, *P. daedalea*, *P. farinosa*, *P. obscurascens*, *P. petricola*, *P. sorediata* and *P. subcinerea* from Chiang Mai province.

The species *P. cocoes* was studied in various countries on diversity, taxonomy, using as bioindicator and bioaccumulation because this species was wide rages habitats and tolerance to pollution (Elix, 2009; Pabroa, Quirit, Hernandez, Balansay, and Gruezo, 2009). The *P. cocoes* was described as a good bioindicator of air pollution, some of the data presented that the pollutants such as arsenic influenced the thallus physiology of this species (Bajpai et al., 2012). Other works determined that some heavy metals had effect on *P. cocoes* thalli (Shukla and Upreti, 2008).

Environmental pollution is a major concern for public health (Waisberg, Joseph, Hale, and Beyersmann, 2003) as well as Jimi et al. (2004) reported that the heavy metal ions induce several stress respond damage to different cell components such as membranes, proteins and DNA.

There are various works studied on genotoxic effects of metals on plants (Steinkellner, Kassie, and Knasmüller, 1999; Angelis, McGuffie, Menke, and Schubert, 2000). Lichens are the species considered as one of the best bioindicator for the determination of air pollution and numerous studies have been conducted on their ability to accumulate the pollutant such as heavy metals (Aras, Kanlıtepe, Cansaran-Duman, Halıcı, and Beyaztaş, 2010), arsenic (As) and fluoride (F) (Bajpai, Upreti, and Nayaka, 2010). The RAPD can be used for comparative study in genetic variation from polluted and non-polluted samples at the same time and the process of this

method was short (Qi, Li, Liu, and Xie, 2006). The result of lichen diversity and lichen taxa in eight municipalities of the northeast of Thailand in the Chapter III found that *P. cocoes* was the highest in both lichen frequency and lichen distribution. Moreover, this species was found at all study plots (Table 3.2 Chapter III). This lichen species was used as bioindicator for air quality and included into the species that was tolerant to air pollution (Kanjoem, 2009; Pabroa et al., 2009; Bajpai et al., 2012). This work was to determine the genetic variation in *P. cocoes* comparing between inside and outside of Nakhon Ratchasima municipal area.

5.2.1 Taxonomy of the *Pyxine cocoes*

Pyxine can be separated from other genera of Physciaceae by testing the epithecium becoming purple in potassium hydroxide solution (PD). The presence of a dark pigmented hypothecium and the *Dirinaria*-type ascospores and the occurrence of apothecia with carbonized margins in the major part of the species (Imshaug 1957; Swinscow and Krog 1975; Awasthi 1980; Rogers 1986; Kalb 1987). The internal part of the stipe of the apothecia in *Pyxine* is also taxonomically useful, since it can vary in color and chemistry, and maculae and pruina are typical features of some species (Imshaug, 1957; Swinscow and Krog, 1975; Awasthi, Dao, and Saneto, 1980). Maculae could be absent or have typical forms in different degrees of development and be restricted to specific areas of the thallus. Some examples are *P. coccifera* (Fée) Nylander, with red, mostly marginal maculae, or *P. fallax* (Zahlbruckner) Kalb, with sub- reticulate white maculae throughout the upper surface. Subapical pruina in small amounts could be found generally in all the species and may be dependent of habitat conditions, but in some species, as *P. cocoes*, the pruina usually forms white dense laminal plaques.

It is frequent in *Pyxine* species with the upper part of the medulla pigmented from a very pale yellow to a sulphur yellow, dark orange or ochre color (Kalb et al., 1987). Some species have an upper medulla with constant color, but *P. daedalea* Krog & R. Santesson has a cream upper medulla in distal parts of the thallus that is generally pale salmon in the central ones. Generally, a white and thin layer of the medulla is present below the pigmented one (Kalb et al., 1987). This layer does not react with the usual color tests (spot tests) used in lichenology.

The range of medullar color denotes the chemistry of the genus. The array of triterpenes obtained through thin layer chromatographic is also very useful (Swinscow and Krog, 1975). About half of the species of *Pyxine* has lichexanthone in upper cortex, rendering the thallus bright yellow under UV light (Sammy, 1988; Hu and Chen, 2003). The complex genus *Dirinaria* is morphologically similar to *Pyxine*, and also has dark hypothecium and, of course, *Dirinaria*-type ascospores, but the epithecium is K– instead of K+ purple as in *Pyxine*, and the margins of apothecia never become carbonized. Additionally, lichexanthone does not occur in *Dirinaria* and the medulla is generally white, exceptionally of some different color in some delimited regions, but never forming distinct layers. Moreover, the absent of rhizines in *Dirinaria* is a practical feature to distinguish it from *Pyxine*. Isidia, polysidiangia and soredia are the vegetative propagules found in *Pyxine*. Polysidiangia is the name that Kalb et al. (1987) gave to the projections of the upper cortex from which additional verruciform structures (polysidia) are produced. These polysidia break exposing the medulla. In the sequence, the remaining scars of the broken tissue regenerate and grow again, giving a shapeless to coralloid appearance to the resulting structure, *P. coralligera* Malme is an example. Sometimes, these structures

disintegrate into soredia (Kalb et al., 1987). The development of this structure is not yet understood in all the species where these shapeless masses occur. It is possible that this name has been applied to somewhat similar structures that grow through different processes of ontogeny. In the literature, they received the following denominations: pustulate isidia with soredia-like granules (Swinscow and Krog, 1975), pustules, crateriform isidia that produce granular soredia (Awasthi, 1980), schizidia (Brodo, Sharnoff, and Sharnoff, 2001) or dactyls (Elix, 2009).

In a different way, isidia are solid, cylindrical, base-constricted structures that do not burst nor release granules, while pustules usually do not branch and are always hollow, frequently bursting at the apices to produce soredia, granules or fragments. True isidia are present in the Asiatic *P. cilindrica* while pustules in the sense used here are not usually found in *Pyxine*. Soralia vary in color, form, and localization and constitute a good character to distinguish species (Swinscow and Krog, 1975). Soralia can be laminal or marginal, orbicular, maculiform or ellipsoid. For example, *Pyxine daedalea* Kalb has orbicular to semi-spherical laminal soralia, while *P. subcinerea* Stirton has marginal, orbicular, becoming crescent-shaped soralia.

Pyxine cocoes (Sw.) Nyl. is belonged to family Physciaceae Zahlbr, the family which currently accepted circumscription, comprises 27 genera of lichenized ascomycetes of various growth forms (Eriksson, Baral, Currah, Hansen, Kurtzman, Rambold, and Laessøe, 2003) i.e. crustose, foliose, and fruticose. Zahlbruckner placed these taxa into two separate families, the Buelliaceae Zahlbr (1907) and the Physciaceae Zahlbr (1898), but Poelt (1973) unified both families into the Physciaceae, and this concept essentially has remained unchanged until now (Rogers,

1986; Jungbluth, Marcelli, and Kalb, 2011).

5.2.2 Researching in *Pyxine cocoes*

Pyxine is a mainly pantropical to subtropical genus, with several species extending into temperate or oceanic regions; currently considered to comprise c. 65 species, 26 of which occur in Australia. Only *P. sorediata* (Ach.) Mont and *P. subcinerea* Stirt. have a relatively expanded distribution, occurring also in temperate Europe (Moberg, 1983). This foliose genus is characterized by the pigmented hypothecium, the epihymenium K+ purple, two to rarely three celled brown *Dirinaria*-type ascospores, and bacillary to sublageniform conidia, from 3 to 4 μm long. The pictures of *P. cocoes* were shown in figure 5.1.

Important characters to distinguish the species in *Pyxine* are the chemistry specially the thin layer chromatographic profile, the presence, localization, and development of vegetative propagules and maculae, the color of the medullar layers, the characterization of the different parts of apothecia, as the carbonization process of the margin and the pigmentation and degree of development of the internal stipe. Some publications of *Pyxine* with an interesting point of views and discussions of morphology, anatomy, and/or chemistry are Imshaug (1957), Swinscow and Krog (1975), Kalb et al. (1987) and Scutari (1995) and are essential for the comprehension of this genus. Twenty-nine species and two varieties of *Pyxine* are recorded from Brazil and little was added to the knowledge of this genus in this country since Kalb's monograph (1987). Six of these species have atranorine in upper cortex and do not produce vegetative propagules.
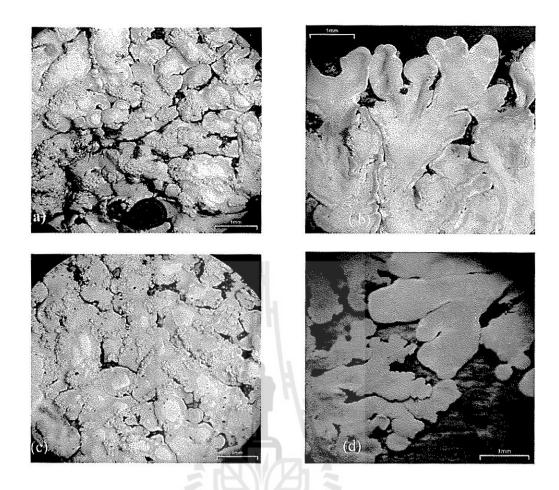
Figure 5.1 *Pyxine cocoes* characteristics; (a) with a apothecia, (b) methallic, (c) soridia, (d) UV+ (next to UV- *Physcia* for comparison) Photo by: Jason Hollinger.

The nrSSU and/or nrITS sequence data of as listed in Eriksson et al. (2003) could be determined and proved particularly useful for the understanding of phylogenetic relationships among these genera as well as for testing the phylogenetic significance of various morphological characters. In the last three decades, a number of studies have underlined the relevance of using lichens as biomonitors of air quality (Loppi, Pirintsos, and De Dominicis, 1997; Garty, Tomer, Levin, and Lehr, 2003; Loppi and Frati, 2006; Jeran et al., 2007). The lichens are a symbiotic association of a

heterotrophic fungus (mycobiont) and a photosynthetic partner (photobiont). The lichen symbiosis typically involves a close physiological interaction. The dominant mycobiont derives its carbon nutrition from the photobiont. The heteromorphy of lichen thallus consists of the external cortex and the internal medulla made up of fungal tissue. A photobiont layer is sandwiched between the cortex and medulla and its cells are enveloped by fungal hyphae. The photobiont layer, which contributes only 10% of the biomass and thallus structure, is the most important part of lichens (Nash, 1996). High sensitivity of lichens to harmful pollutants is related to their stress biology (Gries, 1996). Free diffusibility of lichen thalli due to lack of cuticle enables quick penetration of toxic compounds from the atmosphere to the photobiont layer. Therefore, the response of lichens to the environmental pollution is more sensitive than in higher plants.

Among all the lichens that were used for biomonitoring studies in Thailand, *P. cocoes* is found to be more toxic tolerant and suitable for monitoring different metals (Shukla and Upreti, 2008; Bajpai et al., 2012). *P. cocoes* is a foliose lichens often found abundantly growing on trees and rocks in the tropical areas of Thailand (Pimwong, 2002; Thanomsap, 2006).

5.3 Materials and Methods

5.3.1 Lichen Materials and DNA Isolation

The specimens were collected in five different aspects (N, E, S, W, and center) of the municipality of Nakhon Ratchasima, the control samples were taken at Suranaree University of Technology (SUT), Mueang district, Nakhon Ratchasima province, northeast of Thailand. The location of sampling plots was recorded and

listed in Table 5.1. Anatomical studies were made by examinations of sections under stereomicroscope and free hand cut with a razor blade. Apothecia whose thalline margins become excluded are named *cocoes*-type, while apothecia without thalline margins in any stage of development are called *obscurascens*-type, following the nomenclature proposed by Kalb et al. (1987). Except for the types, more than five apothecia in different states of maturity were cut in the middle from each thallus in order to understand the development and pigmentation process of the internal stipes. The chemistry of the thalli was determined through traditional color reactions (K, C, K, C, and P) and thin layer chromatography (TLC) in solvent A and C, following Orange et al. (2001). Types were tested when there was sufficient material to allow such studies. The descriptions presented below refer to all studied specimens of the taxa, including the types. The specimens were identified following the several identification keys e.g., Sammy (1988) and Hu and Chen (2003). Eighteen *P. cocoes* growing on mango tree trunk (*Mangifera indica* L.) were collected aspect from the municipality of Nakhon Ratchasima province (26°28′16.50″ N and 81°05′30.15″ E, average elevation 120 m). The species was chosen due to the most abundant and widely spread in both of inside and outside municipalities of eight provinces (Dathong et al., 2012) and previously used in various biomonitoring studies (Pitakpong, Thanee, and Saipankaew, 2010). The trees were selected randomly but the factors around of the samples were the same (Table 5.1). Lichen specimens were removed from tree bark wrapped in a brown paper packet, and taken for laboratory. The lichen thalli were rinsed with deionized water to remove dust and debris.

5.3.2 RAPD Procedure

Eight decamer primers were used (Table 5.2), which had yielded good results in previous studies on *Xanthoria* species (Honegger, Zippler, Gansner, and Scherrer, 2004; Honegger, Zippler, Scherrer, and Dyer, 2004). PCR amplifications were carried out with 1 µl of template DNA in 25 µl reaction volumes, consisting of 19·75 µl sterile ddH$_2$O, ×1 reaction buffer for DyNAzyme, 0·2 mM dNTP, 0·2 µM RAPD primer and 0·5U of DyNAzyme DNA polymerase (Finnzymes, Espoo). The following cycles were run on a PTC-220 DNA Engine Dyad™ Peltier Thermal Cycler (MJ Research, Waltham, MA): initial denaturation for 3 min at 94°C, 40 cycles of 30 s at 93°C, 40 s at 37°C, 1min 20 s at 72°C, 30 s at 93°C and a final extension of 5 min at 72°C. Fifteen µl of each amplification product were separated in 1·5% agarose gels in ×1 TBE buffer at 40 V for 5 h, stained with ethidium bromide and photographed under UV light. Constant reaction conditions and thermal profile during amplification were ensured (Meunier, 1992).

5.3.2.1 Polymerase Chain Reaction (PCR)

All PCR thermal cycles were performed in a PCR Express Thermal Cycler (Thermo Hybaid, Middlesex, UK) or Gene Amp 2400 (PerkinElmer, Waltham, MA). DNA amplifications were carried out using *Taq* polymerase (Invitrogen, Calrlsbad, CA) with the buffer supplied or alternatively, Go*Taq* Green Master Mix (Promega, Madison, WI). For amplification of PCR products longer than 3 kb, an enzyme cocktail of *Taq* DNA polymerase and HotStar HiFidelity DNA polymerase (QIAGEN) was used following the protocol for long PCR products as described by the manufacturer. Nuclease-free water (Promega) was used in all PCR reactions, which was ordered from commercial oligonucleotide synthesis services

from either Gene Works (Thebarton, SA, Australia) or Micromon Services (Clayton, VIC, Australia). The primer stocks were made up to a concentration of 25 μM for non-degenerate primers and 50 μM for degenerate primers with nuclease-free water (Promega). PCR amplification, 0.5 μl of each forward and reverse primer was used in a 25 μl PCR reaction (final concentration of 0.5 μM for non-degenerate primers, and 1 μM for degenerate primers). Equal concentrations of forward and reverse primers were used when both primers varied in the degree of degeneracy.

The PCR was followed the standard protocol. The optimal annealing temperature (Ta) range for the touchdown cycles for each primer pair was calculated using the "Primer Test" function embedded in Fast PCR 3.6.6 by Kalendar (www.biocenter.helsinki.fi/bi/programs/fastpcr.htm). The formula used for the calculation of Ta in Fast PCR 3.66 was modified and applied to degenerate primers as well. Touchdown PCR cycles started at the highest annealing temperature based on the calculated Ta range and decreased 1°C per cycle for 6 cycles, followed by an additional 29-34 cycles at the lowest annealing temperature of the calculated Ta range Initial denaturation was at 95°C for 3 mins and denaturation steps during the cycle were at 94°C for 30 seconds each. Extension steps during the cycles were at 72°C for 1 minute in most standard cycles, with a final extension step for 7-10 minutes.

Table 5.1 Locations of *Pyxine cocoes* materials used in RAPD analysis; specimens were taken on 12 January 2011, the control were taken on 14 January 2011.

NO.	Code	Description	Land used
1	C1	The control samples were taken at SUT, Suranaree subdistric, Maung district, Nakhon	University
2	C2	Ratchasima province. The specimens were taken from the mongo tree trunk at	area
3	C3	14° 52′ 22.5″ N, 102° 1′ 25.32″ E. The average altitude is 183 m above sea level.	
4	E1	The specimens were taken at the eastern of the Nakhon Ratchasima municipality. The three	Housing
5	E2	thalli were taken from three mango trees at 14° 58′ 30″ N, 102° 18′ 36.4″ E. The average	&shopping
6	E3	altitude is 160 m above sea level.	mall
7	N1	The specimens were taken at the northern of the Nakhon Ratchasima municipality. The three	Industrial&
8	N2	thalli were taken from three mango trees at 14° 59′ 47.5″ N, 102° 6′ 0″ E The average altitude	commercial
9	N3	is 163 m above sea level.	
10	W1	The specimens were taken at the western side of the Nakhon Ratchasima municipality. The	Hotel,
11	W2	three thalli were taken from three mango trees at 14° 58′ 50.3″ N, 102° 4′ 35.95″ E. The	hospital,
12	W3	average altitude is 174 m above sea level.	school
13	S1	The specimens were taken at the southern of the Nakhon Ratchasima municipality. The three	Housing,
14	S2	thalli were taken from three mango trees at 14° 47′ 37″ N, 102° 6′ 0″ E. The average altitude	school &bus
15	S3	is 169 m above sea level.	stop
16	I1	The specimens were taken at the inside of the Nakhon Ratchasima municipality. The three	Bus stop,
17	I2	thalli were taken from three mango trees at 4° 58′ 30″ N, 102° 6′ 0″ E. The average altitude is	commercial
18	I3	160 m above sea level.	& indusial

Table 5.2 RAPD primers used in PCR.

No.	Code	5' to 3'	No.	Code	5' to 3'
1	OPA-09	GGGTAACGCC	5	OPD-03	GTCGCCGTCA
2	OPA-10	GTGATCGCAG	6	OPD-13	GGGGTGACGA
3	OPB-10	CTGCTGGGAC	7	OPD-16	AGGGCGTAAG
4	OPB-17	AGGGAACGAG	8	OPD-20	ACCCGGTCAC

5.3.2.2 Gel Electrophoresis (GE)

DNA was electrophoresed with a Sub Cell GT gel electrophoresis apparatus (BioRad, Hercules, CA) using agarose gel (AppliChem, Darmstadt, Germany).

5.3.3 Data Analysis

Presence (1) or absence (0) of 150 polymorphic RAPD bands (as achieved with eight primers) were visually scored and recorded in a binary data matrix. Bands of equal fragment size were assumed homologous. The computer program GenAlEx 6 (Peakall and Smouse, 2005) was used to calculate the statistics of genetic variation based on individual haplotypes for each population. To assess the distribution of the total genetic variation per population we calculated the percentage of polymorphic bands (P) and Nei's standard genetic distances between pairs of populations (Nei, 1972; 1978). A dendrogram based on the distance matrix was produced using the unweighted pair-group method with arithmetic averages (UPGMA) under the NJ subprogram in the PHYLIP software package version 3.6a3 (Felsenstein, 2002). Genetic distances between pairs of isolates were calculated with

the Nei coefficient (Nei, 1972). Genetic distances were spatially visualized with the Principal Coordinate Analysis (PCO) and plotted in a chart. This analysis shows the relationships between isolates and between populations, based on the first two principal coordinates of each distance matrix. The partitioning of genetic variance within and among populations and groups of populations was assessed by an Analysis of Molecular Variance (AMOVA). The significance test was performed with 999 permutations. This analysis also calculates fixation indices (ϕ statistics), which are indices of population subdivision analogous to the F-statistics (Weir, 1996).

5.4 Results

5.4.1 RAPD Fingerprinting of *P. cocoes*

The eight selected primers amplified a total of 170 DNA bands, all of which were polymorphic bands ranged from 100-2000 bp in length and from 12 (OPA-09 and OPD-03) to 30 (OPB-10) in number. The average was 23.7 loci per primer (Table 5.3). The fingerprint analyses of epiphytic *P. cocoes* was a high level of polymorphism was found in all populations. The average (P) in the total sample was 73·5%. It is not surprising that the phenotypically heterogenous population from the West of the municipality was genetically highly diverse, but the phenotypically homogenous population reached even slightly higher values (Table 5.1). The eight primers used this work were chosen for further DNA amplification of the lichen sample used.

Figure 5.2 illustrates an example of RAPD profile produced by primer OPD-12. When the RAPD dataset was partitioned within and between populations, most of the genetic variation resided within populations (90%; Figure 5.3), relative to

the amount of variation among regions (3.9%) or among populations within regions (6.4%; Table 5.4). AMOVA produced significantly (p< 0.001) fixation indices (Table 5.5).

Table 5.3 Total number of bands and polymorphic bands based on characteristic of bands.

Primer	Number of total bands	Primer	Number of total bands
OPA-09	12	OPD-03	12
OPA-10	20	OPD-13	28
OPB-10	30	OPD-16	24
OPB-17	29	OPD-20	15
		Total = 170 bands	
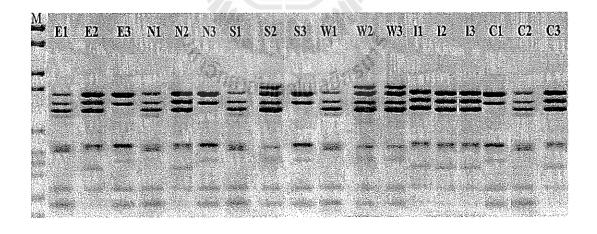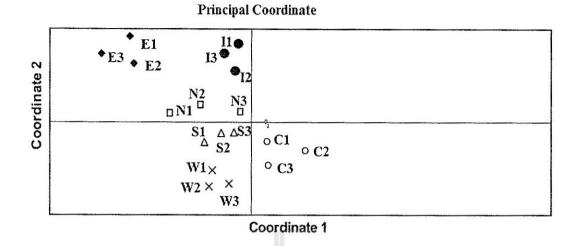
Figure 5.2 RAPD-PCR (OPB-10) of sterile cultured multispore isolates of samples of the phenotypically diverse population of *Pyxine cocoes* from different areas (M=size marker, and label of each column refer to the specimens in Table 5.1).

Principal Coordinate

Figure 5.3 Grouping of *Pyxine cocoes* populations with Principal Coordinates

Analysis (the signs were labeled in Table 5.1).

5.4.2 Genetic Relationship

Genetic relationships among the eighteen *Pyxine cocoes* found in different locations in the Mueang district, Nakhon Ratchasima province were indicated in the UPGMA tree (Figure 5.4). The topology of the UPGMA tree shows the expected groupings with the relation of location of the specimens. Based on the UPGMA, excluded the control specimens sampling at SUT (C1-C3), the *P. cocoes* samples were divided into two main groups. The first subgroup consisted of two subgroups of (i) consisted of I1-I3 and E1-E3, (ii) consisted of N1-N3 while the second comprised S1-S3 and W1-W3. Within the second subgroups, the W1 makes a separate group with other (Figure 5.4.)
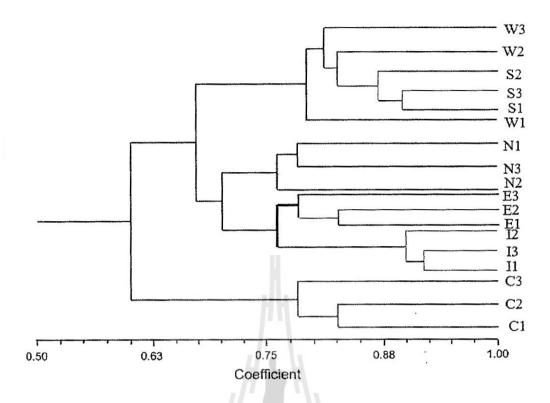
Figure 5.4 Dendrogram based on UPGMA analysis of genetic distance diversity obtained from RAPD data, showing relationship of the *Pyxine cocoes* specimens (C = control, I = inside municipality, E = East, N = North, W = West, S = South).

5.4.3 Genetic Distances

Genetic distances for RAPD analysis were generated from the Dice similarity coefficients obtained from the RAPD data (Table 5.4). Pairwise genetic distance between the samples of *P. cocoes* for RAPD analysis ranged from 33 to 91%, the average was 62.5%.

144

Table 5.4 Pairwise population matrix genetic distances between pairs of populations

of *Pyxine cocoes* from RAPD markers (as percentages); 1-18 refer to the

codes in Table 5.1.

No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	_																	
2	52	_																
3	53	33	_															
4	51	55	56	_														
5	62	53	53	57	_													
6	62	55	48	65	54	_												
7	69	75	68	74	80	64	_											
8	54	60	65	61	71	69	58	_										
9	65	69	65	76	74	70	62	61	_									
10	76	73	71	78	74	65	76	71	58	_								
11	72	73	70	80	85	73	70	63	71	70	_							
12	83	93	88	91	89	86	81	85	84	78	84	_						
13	73	84	87	72	82	79	62	63	61	72	70	77	_					
14	76	84	82	68	83	82	63	66	70	72	68	81	51	_				
15	77	86	86	88	87	86	81	85	74	73	77	68	76	70	_			
16	65	73	66	76	87	76	80	74	80	71	75	79	84	87	78	_		
17	77	85	84	65	89	86	81	85	54	60	65	61	79	84	76	70	_	
18	75	70	70	87	86	81	85	74	73	77	68	76	70	86	81	85	74	_

Table 5.5 Analysis of molecular variance (AMOVA) in *Pyxine cocoes* from different

sampling sites.

Source of variation	Variance component				
	Df	SSD	Absolute	%	*P*
Among regions	5	187·13	0·700	4	<0·001
Among populations within regions	2	129·75	1·263	6	<0·001
Within populations	17	2264·98	17·695	90	<0·001

5.4 Discussions

RAPD marker has been widely used in the analysis of genetic relationships and genetic diversity in a number of plant taxa because of simplicity, speed and relatively low cost, compared to other DNA-based markers (Esselman et al., 2000; Rout, 2006). However, the limitation relating to the dominant nature of RAPD bands can be component to some degree by examining a large number (more than 30) of RAPD loci (Gillies et al., 1999). The genetic variation within populations of *P. cocoes* as observed in the present study was depended on their habitats. The group of inside municipality (I1-I3) shared with E1-E2, the areas in the east of municipal areas. This result can be explained that the pollution in the city was dispersed in the east of the city. The lichen thalli were active when wet, therefore, when the rainy season the thalli could be absorbed the pollutants, and damaged their gene. As an ascomycete *P. cocoes* has to form a new dikaryon on the haploid mycelium prior to the formation of every fruiting body, but it is not known how this proceeds. Pycnidia as potential donors of spermatia are present and ascogonia are easy to find within and slightly below the algal layer of submarginal thallus areas (Nayaka, Bajpai, and Upreti, 2010), but no trichogynes have so far been detected with light or electron microscopy techniques. Therefore, the spermatization is a common and widespread mode of dikaryon formation in lichenforming ascomycetes, is not the mode of dikaryotization in *Pyxine cocoes*. In a homothallic species, facultative gene flow might occur via parasexual events, for example via DNA transfer through hyphal anastomosis between different haplotypes. Such phenomena have not yet been detected among lichen-forming fungi, but cannot be excluded. Murtagh et al. (2000) found uniform fingerprints among sporelings from the same apothecium, but polymorphisms among

sporelings from different apothecia on the same thallus of *Ochrolechia parella* or from different thalli of *Graphis scripta*; thus they concluded the patterns were the result of facultative homothallism or opportunistic outbreeding, respectively.

Accumulation of mutations and intense genotype rather than gene (allele) flow might be the main causes of the high genetic diversity in *P. cocoes*. High genotype flow is typically found in fungal species that disperse primarily by means of vegetative propagules (Honegger, 1984). Thousands of soredia are asexually produced on a single infection of powdery mildew, and hundreds of uredospores detach from a rust pustule. Both can be transported by wind over hundreds of kilometers and germinate immediately upon landing on a suitable host (McDonald and Linde, 2002). This is caused by the accumulation of mutations (Park et al., 2008), efficient dispersal via asexually produced propagules (conidia) and partly also due to parasexual exchange of DNA via hyphal anastomoses, as observed in sterile co-cultures in the laboratory (Zeigler et al., 1997). As shown in field experiments free-living photobiont cells are more common than previously assumed (Sanders, 2005). However, vegetative dispersal via thallus fragments was also documented in the field (Honegger, 1998). The high regenerative capacity of *P. cocoes* facilitates the establishment of new thalli from larger or smaller fragments under natural or experimental conditions (Honegger, 2006). Upon being glued to adequate substrata, thallus fragments of growing very well within short periods, that is 3 mm in 10 months (Elix, 2009). Short distance dispersal of minute fragments by grazing invertebrates might be more common than previously assumed. The fecal pellets of the ever present lichenivorous mites (acari) were shown to contain viable ascospores of *X. parietina* and viable cells of its unicellular green algal photobiont, *Trebouxia*

arboricola (Meier, Scherrer, and Honegger, 2008). The frequency of vegetative dispersal in *P. cocoes* is not comparable to dispersal via asexually produced propagules of non-lichenized fungi (e.g. conidia, uredospores, etc.), but precise data are missing. Wind dispersal over long distances is documented for vegetative symbiotic propagules (soredia) of lichens (Marshall, 1996; Tormo, Recio, Silva, and Munoz, 2001; Muñoz, Felicísimo, Cabezas, Burgaz, and Martínez, 2004; Seaward, 2008). Vertebrates, especially birds, are active and passive vectors of lichen fragments and propagules (Seaward, 2008). The importance of resident and migratory birds as passive transporters of either ascospores, tiny thallus fragments of *P. cocoes*, of lichenivorous mites on lichens and/or their fecal pellets over short or long distances remains to be explored, but has certainly been underestimated. *Pyxine cocoes* grows abundantly at sites where birds land and rest (Honegger, 2006) and nutrient input via droppings facilitate the growth of this nitrophilous species at the resting places. However, passive genotype transfer from adjacent populations might provide the starter material for establishing new populations and later for increasing the genetic diversity of existing ones. Theoretically, only one viable photobiont cell and one or a few cells of the fungal partner (cells of the haustorial complex or germ tubes of ascospores) as found on bark substrata (Bubrick, Galun, and Frensdorff, 1984; Sanders, 2005), might be sufficient to start a new thallus. Taking homothallism and the different options for vegetative (symbiotic or aposymbiotic) dispersal into account, a clonal structure was expected at microsites, that is among vicinal thalli, which might have derived from one "founder thallus" in phenotypically homogenous populations, as investigated in the present study. However, no major differences were evident, probably because all sites are embedded within the main area of distribution

of *P. cocoes* and genotype flow proceeded in a similar manner throughout.

5.6 Conclusions

The use of the RAPD method indicated that the genetic relationship in *P. cocoes* was high. It was separated the samples that collected inside municipality and outside municipalities. The result suggests that pollution inside the city may have an impact on genetic of lichen *P. cocoes*, this species was indicated that the tolerant species to pollution, therefore, it was suitable for studying genetic changes. Because of this species presented data of the high value of both percentage frequency and percentage of species presence at both inside and outside the municipality. The data obtained on various parameters in the present study clearly showed that *P. cocoes* is a suitable lichen material for using as bioindicator and it would be interesting to know which pollutants influence genetic variation of this species.

5.7 References

Angelis, K. J., McGuffie, M., Menke, M., and Schubert, I. (2000). Adaptation to alkylation damage in DNA measured by the comet assay. **Environmental and Molecular Mutagenesis.** 36(2): 146-150.

Aptroot, A., Saipunkaew, W., Sipman, H. J. M., Sparrius, L. B., and Wolseley, P. A. (2007). New lichens from Thailand, mainly microlichens from Chiang Mai. **Fungal Diversity.** 24: 75-134.

Aras, S., Kanlıtepe, Ç., Cansaran-Duman, D., Halıcı, M. G., and Beyaztaş, T. (2010). Assessment of air pollution genotoxicity by molecular markers in the exposed

samples of *Pseudevernia furfuracea* (L.) Zopf in the province of Kayseri (Central Anatolia). **Journal of Environmental Monitoring.** 12(2): 536-543.

Awasthi, D. D. (1980). *Pyxine* in India. **Phytomorphology.** 30: 359-379.

Awasthi, Y. C., Dao, D., and Saneto, R. P. (1980). Interrelationship between anionic and cationic forms of glutathione S-transferases of human liver. **Biochemical Journal.** 191(1): 1.

Bajpai, R., Pandey, A. K., Deeba, F., Upreti, D. K., Nayaka, S., and Pandey, V. (2012). Physiological effects of arsenate on transplant thalli of the lichen *Pyxine cocoes* (Sw.) Nyl. **Environmental Science and Pollution Research.** 19(5): 1494-1502.

Bajpai, R., Upreti, D. K., and Nayaka, S. (2010). Accumulation of arsenic and fluoride in lichen *Pyxine cocoes* (Sw.) Nyl., growing in the vicinity of coal-based thermal power plant at Raebareli, India. **Journal of Experimental Sciences.** 1(4): 37-40.

Brodo, I. M., Sharnoff, S. D., and Sharnoff, S. (2001). **Lichens of North America.** USA: Yale University.

Bubrick, P., Galun, M., and Frensdorff, A. (1984). Observations on free-living *Trebouxia* de *puymaly* and *Pseudotrebouxia archibald*, and evidence that both symbionts from *Xanthoria parientina* (L.) can be found free-living in nature. **New Phytologist.** 97: 455-462.

Cáceres, M. E. S., Lücking, R., and Rambold, G. (2007). Phorophyte specificity and environmental parameters versus stochasticity as determinants for species composition of corticolous crustose lichen communities in the Atlantic rain forest of northeastern Brazil. **Mycological Progress.** 6(3): 117-136.

Cáceres, M. E. S., Lücking, R., and Rambold, G. (2008). Corticolous microlichens in northeastern Brazil: Habitat differentiation between coastal Mata Atlântica, Caatinga and Brejos de Altitude. **The Bryologist.** 111(1): 98-117.

Dathong, W., Thanee, N., and Saipunkaew, W. (2012). The investigation of epiphytic lichens diversity in the Northeast of Thailand. In **Proceeding of the 7th International Association for Lichenologist**, Bangkok, Thailand.

Elix, J. A. (2009). *Pyxine*. **Flora of Australia** 57: 517-533.

Eriksson, O. E., Baral, H.-O., Currah, R. S., Hansen, K., Kurtzman, C. P., Rambold, G., and Laessøe, T. (eds.). (2003). **Outline of ascomycota - 2003** (Vol. 9): Myconet.

Esselman, E. J., Crawford, D. J., Brauner, S., Stuessy, T. F., Anderson, G. J., and Silva, M. (2000). RAPD marker diversity within and divergence among species of *Dendroseris* (Asteraceae: Lactuceae). **American Journal of Botany.** 87(4): 591-596.

Felsenstein, J. (2002). **PHYLIP:Phylogeny Inference Package** version 3.6 a3.

Garty, J., Tomer, S., Levin, T., and Lehr, H. (2003). Lichens as biomonitors around a coal-fired power station in Israel. **Environmental Research.** 91(3): 186-198.

Gillies, A. C. M., Navarro, C., Lowe, A. J., Newton, A. C., Hernandez, M., Wilson, J., and Cornelius, J. P. (1999). Genetic diversity in Mesoamerican populations of mahogany (*Swietenia macrophylla*), assessed using RAPDs. **Heredity.** 83(6): 722-732.

Gries, C. (1996). Lichens as indicators of air pollution. In Nash, T. H. (ed.). **Lichen biology** (Vol. 1). UK: Cambridge University press.

Honegger, R. (1984). Scanning electron microscopy of the contact site of conidia and trichogynes in *Cladonia furcata*. **The Lichenologist.** 16(1): 11-19.

Honegger, R. (1998). The lichen symbiosis: What is so spectacular about it? **The Lichenologist.** 30(3): 193-212.

Honegger, R. (2006). Experimental studies of growth and regenerative capacity in the foliose lichen *Xanthoria parietina*. **New Phytologist.** 133(4): 573-581.

Honegger, R., Zippler, U., Gansner, H., and Scherrer, S. (2004). Mating systems in the genus *Xanthoria* (lichen-forming ascomycetes). **Mycological Research.** 108(5): 480-488.

Honegger, R., Zippler, U., Scherrer, S., and Dyer, P. S. (2004). Genetic diversity in *Xanthoria parietina* (L.) Th. Fr.(lichen-forming ascomycete) from worldwide locations. **The Lichenologist.** 36(6): 381-390.

Hu, G. R., and Chen, J. B. (2003). The lichen family Physciaceae (Ascomycota) in China. VI. The genus *Pyxine*. **Mycotaxon.** 86: 445.

Imshaug, H. A. (1957). Alpine lichens of western United States and adjacent Canada I. The macrolichens. **The Bryologist.** 60(3):177-272.

Jeran, Z., Mrak, T., Jacimovic, R., Batic, F., Kastelec, D., Mavsar, R., and Simoncic, P. (2007). Epiphytic lichens as biomonitors of atmospheric pollution in Slovenian forests. **Environmental Pollution.** 146(2): 324-331.

Jimi, S., Uchiyama, M., Takaki, A., Suzumiya, J., and Hara, S. (2004). Mechanisms of cell death induced by cadmium and arsenic. **Annals of the New York Academy of Sciences.** 1011(1): 325-331.

Jungbluth, P., Marcelli, M. P., and Kalb, K. (2011). A new species and a new record of *Pyxine* (Physciaceae) with norstictic acid from São Paulo State, Brazil. **Mycotaxon.** 115(1): 435-442.

Kalb, R. G. (1994). Regulation of motor neuron dendrite growth by NMDA receptor activation. **Development.** 120(11): 3063-3071.

Kalb, V. F., Woods, C. W., Turi, T. G., Dey, C. R., Sutter, T. R., and Loper, J. C. (1987). Primary structure of the P_{450} lanosterol demethylase gene from *Saccharomyces cerevisiae*. **DNA.** 6(6): 529-537.

Kanjoem, R. (2009). **Lichen diversity and monitoring of sulphur dioxide around Mae Moh power plant area, Mae Moh District, Lampang Province.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Kirk, P. M., and Ainsworth, G. C. (2008). Ainsworth and Bisby's dictionary of the fungi. Wallingford: Cabi.

Loppi, S. (1996). Lichens as bioindicators of geothermal air pollution in central Italy. **The Bryologist.** 41-48.

Loppi, S., and Frati, L. (2006). Lichen diversity and lichen transplants as monitors of air pollution in a rural area of central Italy. **Environmental Monitoring and Assessment.** 114(1): 361-375.

Loppi, S., Pirintsos, S. A., and De Dominicis, V. (1997). Analysis of the distribution of epiphytic lichens on *Quercus pubescens* along an altitudinal gradient in a mediterranean area (Tuscany, central Italy). **Israel Journal of Plant Sciences.** 45(1): 53-58.

Marshall, W. A. (1996). Aerial dispersal of lichen soredia in the maritime Antarctic. **New Phytologist.** 134: 523-530.

McDonald, B. A., and Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. **Annual Review of Phytopathology.** 40(1): 349-379.

Meier, F. A., Scherrer, S., and Honegger, R. (2008). Faecal pellets of lichenivorous mites contain viable cells of the lichen forming ascomycete *Xanthoria parietina* and its green algal photobiont, *Trebouxia arboricola*. **Biological Journal of the Linnean Society.** 76(2): 259-268.

Meunier, B. (1992). Metalloporphyrins as versatile catalysts for oxidation reactions and oxidative DNA cleavage. **Chemical Reviews.** 92(6): 1411-1456. .

Muñoz, J., Felicísimo, Á. M., Cabezas, F., Burgaz, A. R., and Martínez, I. (2004). Wind as a long-distance dispersal vehicle in the Southern Hemisphere. **Science.** 304(5674): 1144-1147.

Murtagh, G., Dyer, P., and Crittenden, P. (2000). Reproductive systems: Sex and the single lichen. **Nature.** 404(6778): 564-564.

Nash, T. H. (1996). **Lichen biology.** United Kingdom: Cambridge University Press.

Nayaka, S., Bajpai, R., and Upreti, D. K. (2010). Accumulation of arsenic and fluoride in lichen *Pyxine cocoes* (Sw.) Nyl., growing in the vicinity of coal-based thermal power plant at Raebareli, India. **Journal of Experimental Sciences.** 1(4).

Nei, M. (1972). Genetic distance between populations. **American Naturalist.** 106: 283-292.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics.** 89(3): 583-590.

Orange, A., James, P. W., and White, F. (2001). **Microchemical methods for the identification of lichens.** UK: Twayne.

Park, S. Y., Milgroom, M. G., Han, S. S., Kang, S., and Lee, Y. H. (2008). Genetic differentiation of *Magnaporthe oryzae* populations from scouting plots and commercial rice fields in Korea. **Phytopathology.** 98(4): 436-442.

Peakall, R. O. D., and Smouse, P. E. (2005). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. **Molecular Ecology Notes.** 6(1): 288-295.

Pimwong, S. (2002). **Distibution and freqeuncy of lichen *Pyxine cocoes* Swartz and *Dirinaria picta* Swartz in Chiang Mai city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Pitakpong, A., Thanee, N., and Saipankaew, W. (2010). Use of lichens as bioindicators for air quality monitoring in Nakhon Ratchasima municipality area. In **Proceeding of the 3rd Technology and Innovation for Sustainable Development International Conference (TISD2010)**, Thailand.

Qi, X., Li, P., Liu, W., and Xie, L. (2006). Multiple biomarkers response in maize (*Zea mays* L.) during exposure to copper. **Journal of Environmental Sciences.** 18(6): 1182-1188.

Rogers, R. (1986). The genus *Pyxine* (Physciaceae, lichenized ascomycetes) in Australia. **Australian Journal of Botany.** 34(2): 131-154.

Rout, G. R. (2006). Evaluation of genetic relationship in *Typhonium* species through random amplified polymorphic DNA markers. **Biologia Plantarum.** 50(1): 127-130.

Sammy, N. (1988). The genus *Pyxine* (Physciaceae. Lichenes) in Western Australia. **Nuytsia.** 6(3): 279-284.

Sanders, W. B. (2005). Observing microscopic phases of lichen life cycles on transparent substrata placed *in situ*. **The Lichenologist.** 37(5): 373-382.

Scutari, N. C. (1995). Los macrolíquenes de Buenos Aires, I: *Dirinaria, Heterodermia, Hyperphyscia* (Physciaceae, Ascomycotina). **Darwiniana.** 33(1-4): 149-176.

Seaward, M. R. D. (2008). Environmental role of lichens. In Nash, T.H.(ed) **Lichen biology** (2 ed., pp. 274-298). UK: Cambridge University Press.

Shukla, V., and Upreti, D. K. (2008). Effect of metallic pollutants on the physiology of lichen, *Pyxine subcinerea* Stirton in Garhwal Himalayas. **Environmental Monitoring and Assessment.** 141(1): 237-243.

Steinkellner, H., Kassie, F., and Knasmüller, S. (1999). *Tradescantia* micronucleus assay for the assessment of the clastogenicity of Austrian water. **Mutation Research.** 426(2): 113-116.

Swinscow, T. D. V., and Krog, H. (1975). The genus *Pyxine* in East Africa. **Norwegian Journal of Botany.** 22: 43-68.

Thanomsap, K. (2006). **Distibution and freqeuncy of lichen *Pyxine cocoes* Swartz and *Lecanora* cf. *leprosa* Fée in Lampun city.** M.S. thesis. Chiang Mai University, Chiang Mai, Thailand.

Tormo, R., Recio, D., Silva, I., and Munoz, A. (2001). A quantitative investigation of airborne algae and lichen soredia obtained from pollen traps in south-west Spain. **European Journal of Phycology.** 36(4): 385-390.

Vainio, E. A. (1909). **Lichenes.** Stockholm: Almqvist & Wicksell.

Waisberg, M., Joseph, P., Hale, B., and Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. **Toxicology.** 192(2): 95-117.

Weir, B. S. (1996). **Genetic data analysis II: Methods for discrete population genetic data.** USA: University of Washington.

Wolseley, P. A., Aguirre-Hudson, B., and Mc Carthy, P. M. (2002). Catalogue of the lichens of Thailand. **Bulletin of the Natural History Museum: Botany.** 32(1): 13-59.

Zeigler, R., Scott, R., Leung, H., Bordeos, A., Kumar, J., and Nelson, R. (1997). Evidence of parasexual exchange of DNA in the rice blast fungus challenges its exclusive clonality. **Phytopathology.** 87(3): 284-294.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The thesis aimed to assess the epiphytic lichen diversity and their distribution in the city habitats of the northeast of Thailand and to evaluate the air quality using lichens as bioindicators. The study focused on four different levels of population number in each municipality. The research also addressed the different distance from the road that could be used to test, whether the traffic had an influence on lichen diversity. This work also investigated how air pollution influences on the genetic variation of *Pyxine cocoes*. The frequency of occurrence of lichen species on a defined portion of mango tree (*Mangifera indica* L.) was used in estimation of diversity and to evaluate the degree of environmental stress on the sensitive lichen community. The epiphytic lichens were investigated on 160 mango trees, 80 mango trees were located inside municipal area, where higher influence from the pollution inside the city than other 80 mango trees that located outside the municipality areas. These objectives formed the research basis:

1) Surveying on epiphytic lichen diversity in the northeast of Thailand.

2) Using epiphytic lichens to assess air quality in the northeast of Thailand.

3) Evaluating environmental parameters influencing epiphytic lichen diversity in the northeast of Thailand.

4) Investigating of genetic relationship in *Pyxine cocoes*.

6.1 Surveying on Epiphytic Lichen Diversity in the Northeast of Thailand

The accomplished investigation and identification of lichens using the Verein Deutscher Ingenieure (VDI) method and the European guideline for mapping lichen diversity as an indicator of environmental stress protocol found 91 species belonged to 34 genera and 18 families on 160 mango tree trunks in the northeast of Thailand in 2010. The ecological indices calculated that the lichen diversity inside municipalities were significantly lower than outside municipalities (F = 1.365, p<0.05). *Pyxine cocoes* was the highest of frequency and presence followed by *Chrysothrix xanthina*, *Lecanora leprosa* and *Graphis* sp.1. Six lichen species, i.e., *Graphis* sp. 6, *Graphis* sp. 12, *Graphis* sp.14, *Graphis* sp.16, *Graphis* sp.19 and *Phyllopsora sorediata* were found only in municipalities, and 20 lichen species e.g., *Amandinea punctata*, *Athena elegance*, *Buellia erubescens*, and *Cladonia fimbriata* were found only outside municipalities. The result could be applied lichens as bioindicator of air quality in this region.

6.2 Using Epiphytic Lichens to Assess Air Quality in the Northeast of Thailand

The using of all lichen taxa can be separated air quality in this region into four groups, depending on population number in each municipality. The use of macrolichens showed clear result for five groups. This work concluded that epiphytic lichens can be used as bioindicator of air pollution in the municipality of the northeast of Thailand.

6.3 Evaluating Environmental Parameters Influencing Epiphytic Lichen Diversity in the Northeast of Thailand
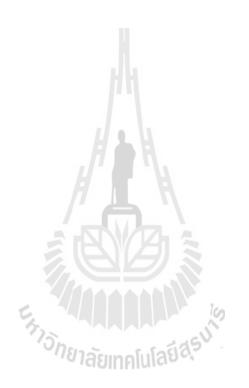
The environmental conditions of selected mango trees were significantly different between the trees that located inside and outside municipalities ($p<0.05$), but the tree properties were not significantly different ($p<0.05$). The factors influencing lichen diversity in the northeast of Thailand were population numbers in each municipality, and altitude, whilst the distance between selected trees and the road and the air quality class had negative correlation ($p<0.01$). The air quality in the northeast of Thailand was classified as moderate; it was correlated with the population in each municipality.
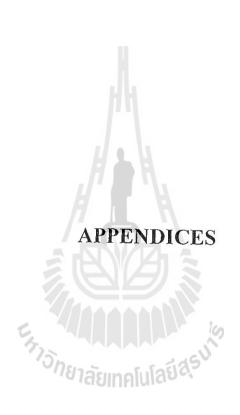
6.4 The Genetic Relationship in *Pyxine cocoes*

The genetic variation of *Pyxine cocoes*, the species was selected based on the highest in frequency and presence in both inside and outside the municipal areas of the northeast of Thailand were studied using the RAPD analysis. The RAPD-based dendrogram clustered the samples of *P. cocoes* into two main groups. The first main group included 12 samples, which collected from outside the municipality and another consisted of three samples that collected in the municipal area where the pollution is higher than four aspects outside the municipal area. Moreover, the group of outside the municipality of Nakhon Ratchasima showed four subgroups depended on the location.

6.5 Contribution to Knowledge and Recommendations

For further study, other trees and other substrates e.g. royal palm, pine, rock, cement will be considered. The other levels on the trees will also be interested e.g. canopy and twigs. The air pollutants will be measured as influencing lichen diversity. In addition, other lichen species will be used to study on the genetic properties.

APPENDICES

APENDIX A

LICHEN SURVEYING FORM

Date:_____   Direction of grid frame

Surveyor:_____

Mapping unit number: _____

Tree number:_____

Topographical map number:_____

General Data

_____Easting   _____altitude above sea level (m)

_____Northing   _____GPS coordinates

_____Main aspect of the community i.e. principally foliose, fruticose, crustose

Tree species Mango tree (*Mangifera indica* L.)

_____Circumference of tree trunk at 150 cm above ground level (cm)

_____**Age of lichens**

    1 young and mature thalli together   2 predominantly young thalli

    3 predominantly mature thalli

_____**Lichen vitality**

    1 predominantly healthy thalli   2 healthy and degraded thalli

    3 predominantly degraded thalli**Tree description**

    ☐1 exposed, 2 sheltered   ☐1 unshaded, 2 shaded

_____**Bark cracks**   1 superficial   2 moderately deep   3 deep

_____**Influence by traffic**   _____**Distance to the road**

    1 dirty road   1 0-5 m   2 >5-10 m

    2 asphalt road, low traffic   3 > 10-15 m   4 > 15-20 m

    3 main road/highway, much traffic   5>20 m

_____Area conditions

1High-density residential area   2 Not high-density residential area

3 Open area

_____Area around the mango's tree

1Lawn area   2 Cement area   3 Ground area

_____Influence by traffic

1 dirty road   2 asphalt road, low traffic   3 main road/highway, much traffic

Tree number	Lichen frequency				
	Crustose	Foliose	Fruticose	Squamulose	Total
Total					

Note:_____

_____

_____

_____

_____

_____

_____

APPENDIX B

CONTENT INFORMATION AROUND MANGO TREES

AND NUMBER OF LICHEN SPECIES AND

FREQUENCY

Table B.1 Content information around mango trees and number of lichen species and frequency of Nakhon Ratchasima (NMAi = inside municipality of Nakhon Ratchasima, NMAo = outside municipality of Nakhon Ratchasima).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
NMAi1	55	1	W	3	2	C	1	5.35	10	34
NMAi2	81	1	NE	1	1	D	1	5.39	6	19
NMAi3	131	1	NE	1	1	B	1	5.35	7	28
NMAi4	68	1	NE	3	1	C	1	5.43	8	45
NMAi5	76	1	NE	1	1	C	3	5.38	9	29
NMAi6	65	1	NE	2	2	C	2	5.34	6	24
NMAi7	71	1	N	1	2	C	2	5.34	8	38
NMAi8	113	1	NE	2	2	C	3	5.37	7	27
NMAi9	83	3	NW	2	3	A	1	5.33	10	44
NRM10	75	1	E	1	1	C	3	5.45	11	32
NMAo1	68	1	SW	2	3	A	2	5.38	10	36
NMAo2	69	1	SE	2	3	A	2	5.37	8	34
NMAo3	86	1	E	2	3	A	2	5.3	7	31
NMAo4	142	1	N	3	1	C	2	5.45	9	44
NMAo5	150	1	W	3	1	C	2	5.38	9	42
NMAo6	79	2	NE	3	2	A	1	5.36	6	24
NMAo7	143	2	NE	2	3	A	1	5.38	10	45
NMAo8	87	1	E	2	3	A	2	5.39	8	36
NMAo9	86	1	N	2	3	A	2	5.33	7	29
NMAo10	150	1	E	1	1	C	2	5.45	13	55

Table B.2 Content information around mango trees and number of lichen species and
frequency of Chaiyaphum (CPMi = inside municipality of Chaiyaphum,
CPMo = outside municipality of Chaiyaphum).

| Tree | Variable in each tree | | | | | | | | Lichens | |
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
CPMi1	62	1	NE	2	3	C	5	5.42	8	21
CPMi2	112	1	N	2	2	A	5	5.43	7	16
CPMi3	60	1	N	2	2	A	5	5.33	6	17
CPMi4	75	1	NE	1	3	C	2	5.43	5	10
CPMi5	88	1	NE	2	2	C	2	5.4	4	12
CPMi6	64	1	E	2	2	C	2	5.33	4	7
CPMi7	80	4	NE	2	2	B	5	5.39	5	10
CPMi8	54	1	E	2	3	C	3	5.38	5	10
CPMi9	69	2	SE	2	2	B	4	5.34	5	12
CPMi10	77	4	W	2	1	B	5	5.38	6	13
CPMo1	112	1	NE	2	2	C	4	5.4	9	29
CPMo2	98	1	S	2	2	C	4	5.45	6	18
CPMo3	68	1	W	2	2	B	3	5.43	4	13
CPMo4	58	2	NE	1	2	B	3	5.45	5	25
CPMo5	69	1	E	1	2	C	4	5.32	4	17
CPMo6	106	1	NE	1	2	B	4	5.45	4	15
CPMo7	95	1	SE	2	3	C	4	5.46	4	17
CPMo8	83	1	N	2	3	C	4	5.48	5	26
CPMo9	76	1	NE	1	2	C	5	5.41	5	21
CPMo10	80	1	E	1	2	C	4	5.4	6	23

Table B.3 Content information around mango trees and number of lichen species and frequency of Khon Kaen (KKNi = inside municipality of Khon Kaen, KKNo = outside municipality of Khon Kaen).

| Tree | Variable in each tree | | | | | | | | Lichens | |
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
KKNi1	55	1	N	1	3	C	5	5.43	4	11
KKNi2	60	1	W	1	3	C	4	5.43	3	7
KKNi3	85	2	NE	2	2	A	5	5.45	2	4
KKNi4	96	2	S	2	2	A	5	5.4	2	5
KKNi5	101	2	E	1	2	C	1	5.54	2	6
KKNi6	98	2	N	2	2	B	2	5.42	2	4
KKNi7	65	2	W	2	2	C	2	5.43	3	6
KKNi8	58	1	NE	1	3	C	2	5.46	3	12
KKNi9	83	1	NE	1	3	C	2	5.4	2	6
KKNi10	70	1	E	3	3	B	1	5.37	3	5
KKNo1	79	1	E	1	2	C	2	5.45	3	9
KKNo2	90	1	N	2	1	C	2	5.47	3	10
KKNo3	76	1	W	2	1	C	2	5.45	5	14
KKNo4	81	1	NE	1	2	B	2	5.46	2	5
KKNo5	121	1	NE	2	2	A	2	5.47	2	7
KKNo6	150	1	E	1	1	C	2	5.46	2	3
KKNo7	86	2	W	3	3	C	2	5.49	3	10
KKNo8	86	1	E	2	1	C	2	5.43	3	8
KKNo9	77	1	E	1	1	C	2	5.45	4	7
KKNo10	98	2	NE	3	3	C	2	5.44	3	7

Table B.4 Content information around mango trees and number of lichen species and frequency of Loei (LEIi = inside municipality of Loei, LEIo = outside municipality of Loei).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
LEIi1	61	1	NE	3	2	C	2	5.39	15	53
LEi2	143	1	NE	3	2	C	2	5.36	7	23
LEIi3	89	2	N	1	3	C	2	5.37	8	30
LEIi4	106	1	N	1	3	C	1	5.37	8	26
LEIi5	90	1	W	1	2	C	1	5.4	10	31
LEIi6	65	2	W	1	1	C	1	5.38	9	26
LEIi7	131	2	N	1	3	C	1	5.36	7	20
LEIi8	87	2	SE	2	3	C	1	5.38	8	22
LEIi9	70	1	E	2	3	C	2	5.36	9	31
LEIi10	98	1	S	1	2	C	2	5.37	11	34
LEIo1	53	1	NE	1	2	C	2	5.5	19	75
LEo2	56	1	E	1	2	C	2	5.4	11	41
LEIo3	67	1	N	1	3	C	1	5.42	11	40
LEIo4	98	1	W	1	3	C	1	5.51	11	38
LEIo5	90	1	NE	1	2	C	1	5.5	11	34
LEIo6	83	1	N	3	1	C	1	5.42	12	41
LEIo7	76	1	E	3	2	C	4	5.53	13	41
LEIo8	94	1	NE	2	2	C	4	5.44	9	28
LEIo9	125	1	NW	2	2	C	4	5.37	11	27
LEIo10	97	1	N	1	1	C	3	5.4	12	49

Table B.5 Content information around mango trees and number of lichen species and frequency of Nong Khai (NKIi = inside municipality of Nong Khai, NKIo = outside municipality of Nong Khai).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
NKIi1	104	1	N	1	1	C	3	5.37	8	28
NKIi2	100	1	S	3	2	C	4	5.31	5	21
NKIi3	75	1	E	2	2	C	4	5.3	5	18
NKIi4	70	1	NE	2	2	C	2	5.34	5	11
NKIi5	53	1	NE	2	2	C	2	5.36	4	14
NKIi6	95	1	SE	1	3	C	3	5.34	11	32
NKIi7	91	1	E	1	2	C	3	5.33	7	16
NKIi8	150	1	N	2	2	B	2	5.37	5	7
NKIi9	85	1	NE	2	3	C	2	5.35	6	14
NKIi10	128	1	NE	1	3	C	3	5.36	7	24
NKIo1	98	1	N	2	3	C	3	5.34	8	28
NKIo2	79	1	W	3	2	C	4	5.36	7	27
NKIo3	57	1	E	1	2	C	3	5.42	8	32
NKIo4	103	1	NE	2	3	C	1	5.37	5	18
NKIo5	69	1	NW	2	3	C	1	5.4	5	19
NKIo6	78	1	W	1	3	C	2	5.35	10	42
NKIo7	84	1	S	1	3	C	2	5.38	10	48
NKIo8	135	1	S	1	2	C	3	5.4	7	24
NKIo9	73	1	E	2	2	C	3	5.37	8	35
NKIo10	99	1	N	3	2	C	2	5.36	11	32

Table B.6 Content information around mango trees and number of lichen species and frequency of Sakhon Nakhon (SKNi = inside municipality of Sakhon Nakhon, SKNo = outside municipality of Sakhon Nakhon).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
SKNi1	95	1	N	1	1	C	2	5.38	4	14
SKNi2	89	1	W	2	3	A	2	5.38	8	21
SKNi3	83	1	NE	2	3	A	2	5.41	6	19
SKNi4	76	1	N	1	2	C	3	5.42	6	18
SKNi5	81	1	SE	1	2	C	3	5.4	5	15
SKNi6	101	1	N	1	1	C	1	5.42	8	24
SKNi7	84	1	NE	1	2	C	1	5.33	7	19
SKNi8	129	1	E	3	1	C	2	5.34	6	19
SKNi9	123	1	W	3	1	C	1	5.39	7	21
SKNi10	119	3	E	1	2	A	1	5.37	5	15
SKNo1	89	1	N	1	2	A	1	5.38	12	35
SKNo2	85	1	NE	1	2	C	1	5.45	8	24
SKNo3	93	1	E	1	2	C	2	5.39	11	39
SKNo4	105	1	W	2	2	B	4	5.42	8	24
SKNo5	74	1	E	1	2	C	4	5.4	8	25
SKNo6	93	1	E	3	1	C	4	5.37	11	27
SKNo7	98	1	NW	2	3	B	2	5.38	10	23
SKNo8	87	1	NW	1	3	C	2	5.36	8	18
SKNo9	70	1	E	1	2	C	3	5.44	7	19
SKNo10	81	1	NE	1	2	C	2	5.39	11	28

Table B.7 Content information around mango trees and number of lichen species and frequency of Ubon Ratchathani (UBNi = inside municipality of Ubon Ratchathani UBNo = outside municipality of Ubon Ratchathani).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
UBNi1	145	1	NE	3	2	C	4	5.55	5	20
UBNi2	130	1	SW	3	2	C	4	5.54	4	14
UBNi3	76	1	N	1	2	C	2	5.53	3	17
UBNi4	65	1	N	3	2	A	2	5.54	2	10
UBNi5	84	1	N	3	2	C	2	5.53	3	10
UBNi6	75	1	N	3	2	C	4	5.57	7	20
UBNi7	99	2	SE	2	3	A	4	5.55	3	10
UBNi8	86	1	NE	2	3	C	4	5.56	4	15
UBNi9	87	1	NE	2	2	C	1	5.53	3	8
UBNi10	64	1	W	2	2	A	1	5.57	4	15
UBNo1	76	1	SE	2	3	C	2	5.54	6	30
UBNo2	70	1	S	3	3	C	2	5.55	5	18
UBNo3	149	1	N	1	2	B	3	5.52	3	15
UBNo4	68	1	NE	1	2	C	3	5.54	3	10
UBNo5	97	1	E	1	3	C	2	5.53	3	17
UBNo6	78	3	W	1	3	C	2	5.55	7	23
UBNo7	57	1	W	2	2	A	3	5.55	5	20
UBNo8	93	1	W	3	2	A	2	5.55	5	19
UBNo9	87	1	NE	2	3	C	2	5.56	5	17
UBNo10	81	1	NE	2	1	C	2	5.55	7	27

Table B.8 Content information around mango trees and number of lichen species and

      frequency of Yasothon (YSTi = inside municipality of Yasothon, YSTo =

      outside municipality of Yasothon).

Tree	Variable in each tree								Lichens	
	DBH (cm)	RD	GD	Bc	Ac	As	Te	BA	No.	F
YSTi1	108	1	NE	2	3	C	2	5.38	6	16
YSTi2	92	1	NE	2	3	C	4	5.37	6	17
YSTi3	75	1	NE	1	1	A	4	5.41	6	19
YSTi4	69	1	NE	1	1	A	4	5.37	5	12
YSTi5	86	1	NE	2	1	C	2	5.39	5	14
YSTi6	79	1	NW	2	3	B	2	5.41	4	15
YSTi7	73	1	NE	2	1	C	2	5.35	5	11
YSTi8	80	2	N	2	3	A	2	5.4	4	14
YSTi9	81	1	E	3	1	C	2	5.39	5	12
YSTi10	78	3	NE	1	1	C	2	5.37	5	13
YSTo1	68	1	S	3	3	C	2	5.51	6	30
YSTo2	74	3	SE	3	2	C	2	5.53	5	18
YSTo3	87	1	N	2	2	B	2	5.53	3	15
YSTo4	83	1	E	2	2	C	2	5.54	3	10
YSTo5	65	1	E	1	1	A	4	5.53	3	17
YSTo6	107	1	E	1	2	C	4	5.55	7	23
YSTo7	98	1	NE	2	2	C	4	5.58	5	20
YSTo8	68	1	NE	2	2	C	4	5.54	5	19
YSTo9	64	1	NE	2	2	C	4	5.56	5	17
YSTo10	88	1	NE	2	2	C	2	5.53	7	27

Note: Ac = Area conditions (1 = High-density residential area, 2 = Low high-density residential area and 3 = Open area)

As = Area surrounding (A = Lawn area, B = Cement area and C = Ground area)

Bc = Bark characteristic (1 = Smooth, 2 = Average smooth and 3 = Deep-wrinkle)

D = Distance from trees to road (1 = 0-5 m, 2 = >5-10 m, 3 = >10-15 m, 4 = >15-20 m and 5 = > 20 m)

DBH = Diameter at breast height (cm)

Te = Traffic effects (1 = Highway, 2 = Main road; high traffic, 3 = Main road; low traffic, 4 = Small road; high traffic, 5 = Small road car; low traffic and 6 = Country road)

APPENDIX C

LICHEN SPECIES AND FREQUENCY IN EACH PLOT

Table C.1 Lichen frequency in Nakhon Ratchasima (F = frequency, NMAi = inside municipality of Nakhon Ratchasima and NMAo = outside municipality of Nakhon Ratchasima, 1-10 refer to number of selected tree in each plot).

	Lichens			Lichens	
Plot	**Species**	**F**	**Plot**	**Species**	**F**
NMAi1			**NMAo1**		
1	*Arthonia catenatuta*	1	1	*Amandinea punctata*	2
2	*Bacidia pallidocarnea*	3	2	*Chrysothrix xanthina*	3
3	*Chrysothrix xanthina*	3	3	*Dirinaria appleanata*	3
4	*Dirinaria pica*	2	4	*Dirinaria pica*	3
5	*Graphis* sp.1	3	5	*Graphis* sp.1	5
6	*Graphis* sp.2	2	6	*Hyperphyscia adsendes*	4
7	*Hyperphyscia adglutinata*	5	7	*Hyperphyscia granulata*	1
8	*Lecanographa atropunctata*	6	8	*Laurera megasperma*	3
9	*Lecanora tropica*	3	9	*Lecanora leprosa*	6
10	*Phyllopsora sorediata*	6	10	*Pyxine coccifera*	6
	Total frequency	**34**		**Total frequency**	**36**
NMAi2			**NMAo2**		
1	*Arthonia tumidula*	2	1	*Chrysothrix xanthina*	4
2	*Bacidia pallidocarnea*	3	2	*Dirinaria pica*	4
3	*Dirinaria pica*	2	3	*Graphis* sp.1	3
4	*Graphis* sp.2	1	4	*Lecanographa atropunctata*	5
5	*Hyperphyscia adglutinata*	6	5	*Lecanora leprosa*	6
6	*Lecanographa atropunctata*	5	6	*Arthonia tumidula*	2
	Total frequency	**19**	7	*Pyxine coccifera*	5
			8	*Pyxine consocians*	5
				Total frequency	**34**

Table C.1 Lichen frequency of Nakhon Ratchasima (F = frequency, NMAi = inside municipality of Nakhon Ratchasima and NMAo = outside municipality of Nakhon Ratchasima, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens Species	F	Plot	Lichens Species	F
NMAi3			**NMAo3**		
1	Arthonia catenatuta	1	1	Amandinea punctata	4
2	Arthonia tumidula	2	2	Chrysothrix xanthina	5
3	Graphis sp.1	2	3	Graphis sp.1	5
4	Lecanora leprosa	6	4	Lecanographa atropunctata	5
5	Lecanora helva	3	5	Lecanora leprosa	5
6	Phyllopsora sorediata	4	6	Pyxine cocoes	2
7	Pyxine cocoes	10	7	Pyxine consocians	5
	Total frequency	28		**Total frequency**	31
NMAi4			**NMAo4**		
1	Arthonia tumidula	4	1	Caloplaca diplacioides	2
2	Caloplaca diplacia	3	2	Dirinaria pica	3
3	Graphis sp.1	3	3	Graphis sp.1	7
4	Graphis sp.2	5	4	Lecanographa atropunctata	5
5	Hyperphyscia adglutinata	4	5	Lecanora achrosa	5
6	Physcia atrostriata	6	6	Pyxine cocoes	2
7	Physcia poncinsii	10	7	Pyxine subcinerea	6
8	Pyxine cocoes	10	8	Rinodina cinereovirescens	8
	Total frequency	45	9	Trypethelium eluteriae	6
				Total frequency	44
NMAi5			**NMAo5**		
1	Arthonia catenatuta	2	1	Caloplaca diplacioides	4
2	Caloplaca diplacia	3	2	Caloplaca gambiensis	3
3	Chrysothrix xanthina	5	3	Graphina sp.	4
4	Dirinaria pica	2	4	Lecanora leprosa	2
5	Graphis sp.1	3	5	Opegrapha stirtinii	2
6	Graphis sp.24	2	6	Physcia atrostriata	5
7	Hyperphyscia adglutinata	6	7	Pyxine cocoes	10
8	Laurera megasperma	3	8	Pyxine subcinerea	6
9	Physcia poncinsii	3	9	Rinodina intrasa	6
	Total frequency	29		**Total frequency**	42

Table C.1 Lichen frequency of Nakhon Ratchasima (F = frequency, NMAi = inside municipality of Nakhon Ratchasima and NMAo = outside municipality of Nakhon Ratchasima, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens Species	F	Plot	Lichens Species	F
NMAi6			**NMAo6**		
1	*Arthonia tumidula*	4	1	*Caloplaca diplacia*	4
2	*Chrysothrix xanthina*	4	2	*Caloplaca gambiensis*	3
3	*Graphis* sp.1	2	3	*Chrysothrix xanthina*	3
4	*Graphis* sp.21	1	4	*Lecanora helva*	5
5	*Laurera megasperma*	3	5	*Pyxine cocoes*	7
6	*Pyxine cocoes*	10	6	Sterile crust sp.2	2
	Total frequency	**24**		**Total frequency**	**24**
NMAi7			**NMAo7**		
1	*Caloplaca diplacia*	4	1	*Anthracothecium prasinum*	2
2	*Chrysothrix xanthina*	5	2	*Caloplaca diplacia*	4
3	*Hyperphyscia adglutinata*	4	3	*Graphis* sp.3	4
4	*Pyxine cocoes*	4	4	*Graphis* sp.7	5
5	*Pyxine coccifera*	5	5	*Lecanora helva*	6
6	*Pyxine subcinerea*	7	6	*Opegrapha stirtinii*	3
7	*Rinodina intrasa*	4	7	*Pyxine cocoes*	10
8	*Rinodina cinereovirescens*	5	8	*Rinodina cinereovirescens*	4
	Total frequency	**38**	9	Sterile crust sp.2	3
			10	*Trypethelium eluteriae*	4
				Total frequency	**45**
NMAi8			**NMAo8**		
1	*Arthonia catenatuta*	1	1	*Caloplaca gambiensis*	3
2	Chrysothrix xanthina	5	2	*Chrysothrix xanthina*	5
3	*Dirinaria appleanata*	1	3	*Graphis* sp.2	2
4	*Graphis* sp.2	4	4	*Graphis* sp.8	3
5	*Hyperphyscia adglutinata*	5	5	*Lecanora tropica*	5
6	*Pyxine coccifera*	4	6	*Pyxine cocoes*	7
7	*Pyxine consocians*	7	7	*Rinodina cinereovirescens*	4
	Total frequency	**27**	8	Sterile crust sp.1	7
				Total frequency	**36**

Table C.1 Lichen frequency of Nakhon Ratchasima (F = frequency, NMAi = inside municipality of Nakhon Ratchasima and NMAo = outside municipality of Nakhon Ratchasima, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Species	F	Plot	Species	F
NMAi9			**NMAo9**		
1	*Arthonia cytodes*	1	1	*Caloplaca diplacia*	2
2	*Arthonia tumidula*	3	2	*Dirinaria appleanata*	3
3	*Chrysothrix xanthina*	2	3	*Lecanora tropica*	5
4	*Dirinaria appleanata*	2	4	*Pyxine cocoes*	10
5	*Graphis* sp.16	2	5	Sterile crust sp.1	4
6	*Hyperphyscia adglutinata*	2	6	Sterile crust sp.2	2
7	*Pyxine consocians*	7	7	*Arthonia incospicua*	3
8	*Rinodina cinereovirescens*	5		**Total Frequency**	29
9	Sterile crust sp.1	4			
10	*Trypethelium eluteriae*	16			
	Total frequency	44			
NMAi10			**NMAo10**		
1	*Arthonia tumidula*	4	1	*Arthonia cytodes*	2
2	*Chrysothrix xanthina*	4	2	*Bacidia pallidocarnea*	3
3	*Dichosporidium boschianum*	1	3	*Caloplaca diplacia*	3
4	*Dirinaria appleanata*	2	4	*Chrysothrix xanthina*	5
5	*Dirinaria pica*	2	5	*Graphis* sp.2	5
6	*Graphis* sp.2	3	6	*Graphis* sp.8	3
7	*Graphis* sp.6	1	7	*Graphis* sp.13	4
8	*Graphis* sp.9	3	8	*Graphis* sp.24	1
9	*Graphis* sp.16	2	9	*Hyperphyscia adglutinata*	2
10	*Hyperphyscia adglutinata*	3	10	*Hyperphyscia adsendes*	5
11	*Pyxine consocians*	7	11	*Lecanora tropica*	10
	Total frequency	32	12	*Pyxine cocoes*	10
			13	Sterile crust sp.1	2
				Total frequency	55

Table C.2 Lichen frequency of Chaiyaphum (F = frequency, CPMi = inside municipality of Chaiyaphum and CPMo = outside municipality of Chaiyaphum, 1-10 refer to number of selected tree in each plot).

Plot	Lichens Species	F	Plot	Lichens Species	F
CPMi1			**CPMo1**		
1	*Arthonia cinnabarina*	1	1	*Anthracothecium prasinum*	3
2	*Caloplaca diplacia*	2	2	*Arthonia cinnabarina*	2
3	*Chrysothrix xanthina*	1	3	*Chrysothrix xanthina*	4
4	*Dirinaria appleanata*	2	4	*Dirinaria appleanata*	2
5	*Graphis* sp.9	1	5	*Dirinaria pica*	3
6	*Hyperphyscia adglutinata*	2	6	*Graphis* sp.4	4
7	*Lecanora leprosa*	4	7	*Pyxine cocoes*	6
8	*Pyxine cocoes*	8	8	*Trypethelium eluteriae*	2
	Total frequency	21	9	*Trypethelium tropicum*	3
				Total frequency	29
CPMi2			**CPMo2**		
1	*Caloplaca diplacioides*	1	1	*Buellia erubescens*	1
2	*Graphis* sp.6	1	2	*Dirinaria appleanata*	1
3	*Graphis* sp.9	2	3	*Dirinaria pica*	2
4	*Laurera megasperma*	3	4	*Graphis* sp.4	4
5	*Lecanora tropica*	3	5	*Pyxine cocoes*	4
6	*Rinodina* sp.	3	6	*Rinodina cinereovirescens*	6
7	*Pyxine cocoes*	3		**Total frequency**	18
	Total frequency	16			
CPMi3			**CPMo3**		
1	*Chrysothrix xanthina*	2	1	*Buellia erubescens*	3
2	*Graphis* sp.6	2	2	*Dirinaria pica*	3
3	*Laurera megasperma*	2	3	*Rinodina cinereovirescens*	4
4	*Lecanora tropica*	3	4	*Trypethelium eluteriae*	3
5	*Rinodina* sp.	4		**Total frequency**	13
6	*Pyxine cocoes*	4			
	Total frequency	17			

Table C.2 Lichen frequency of Chaiyaphum (F = frequency, CPMi = inside municipality of Chaiyaphum and CPMo = outside municipality of Chaiyaphum, 1-10 refer to number of selected tree in each plot) (Cont.).

	Lichens			Lichens	
Plot	Species	F	Plot	Species	F
CPMi4			**CPMo4**		
1	*Arthonia cinnabarina*	1	1	*Caloplaca diplacioides*	3
2	*Caloplaca diplacia*	1	2	*Lecanora helva*	5
3	*Caloplaca diplacioides*	1	3	*Lecanora tropica*	6
4	*Lecanora leprosa*	4	4	*Lepraria atrotomentosa*	1
5	*Pyxine cocoes*	3	5	*Pyxine cocoes*	10
	Total frequency	10		**Total frequency**	25
CPMi5			**CPMo5**		
1	*Dichosporidium* sp.	3	1	*Caloplaca diplacioides*	2
2	*Graphis* sp.9	2	2	*Graphis* sp.4	4
3	*Hyperphyscia adglutinata*	2	3	*Graphis* sp.13	5
4	*Pyxine coccifera*	5	4	*Graphis* sp.18	6
	Total frequency	12		**Total frequency**	17
CPMi6			**CPMo6**		
1	*Caloplaca diplacia*	1	1	*Chrysothrix xanthina*	4
2	*Dichosporidium* sp.	2	2	*Dirinaria pica*	2
3	*Dirinaria appleanata*	2	3	*Graphis* sp.3	2
4	*Pyxine cocoes*	2	4	*Graphis* sp.23	7
	Total frequency	7		**Total frequency**	15
CPMi7			**CPMo7**		
1	*Arthonia cinnabarina*	2	1	*Chrysothrix candelaris*	3
2	*Graphis* sp.2	1	2	*Lecanora helva*	5
3	*Laurera benguelensis*	3	3	*Pyxine cocoes*	4
4	*Lecanora leprosa*	3	4	*Pyxine consocians*	5
5	*Pyxine coccifera*	1		**Total frequency**	17
	Total frequency	10			
CPMi8			**CPMo8**		
1	*Crocynia pyxinoid*	1	1	*Chrysothrix xanthina*	4
2	*Graphis* sp.3	2	2	*Dirinaria pica*	3
3	*Hyperphyscia adglutinata*	4	3	*Pyxine cocoes*	6
4	*Lecanora leprosa*	1	4	*Pyxine subcinerea*	6
5	*Pyxine consocians*	2	5	*Rinodina cinereovirescens*	7
	Total frequency	10		**Total frequency**	26

Table C.2 Lichen frequency of Chaiyaphum (F = frequency, CPMi = inside municipality of Chaiyaphum and CPMo = outside municipality of Chaiyaphum, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens Species	F	Plot	Lichens Species	F
CPMi9			**CPMo9**		
1	*Chrysothrix xanthina*	3	1	*Chrysothrix candelaris*	3
2	*Dirinaria appleanata*	2	2	*Graphis* sp.1	4
3	*Laurera benguelensis*	1	3	*Lecanora helva*	6
4	*Rinodina cinereovirescens*	4	4	*Pyxine cocoes*	3
5	*Pyxine consocians*	2	5	*Pyxine consocians*	5
	Total frequency	**12**		**Total frequency**	**21**
CPMi10			**CPMo10**		
1	*Caloplaca diplacia*	2	1	*Chrysothrix xanthina*	4
2	*Graphis* sp.2	1	2	*Graphis* sp.1	3
3	*Graphis* sp.3	1	3	*Graphis* sp.2	3
4	*Laurera benguelensis*	2	4	*Lecanora helva*	4
5	*Rinodina cinereovirescens*	5	5	*Pyxine cocoes*	3
6	*Pyxine consocians*	2	6	*Pyxine coccifera*	6
	Total frequency	**13**		**Total frequency**	**23**

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of
Loei and LEIo = outside municipality of Loei, 1-10 refer to number of
selected tree in each plot).

| Plot | Lichens | | Plot | Lichens | |
	Species	F		Species	F
LEIi1			**LEIo1**		
1	*Caloplaca diplacia*	2	1	Sterile crust sp.3	2
2	*Lecanographa atropunctata*	2	2	*Bacidia pallidocarnea*	3
3	*Arthonia catenatuta*	4	3	*Arthonia cytodes*	4
4	*Lepraria atrotomentosa*	5	4	*Crocynia pyxinoid*	5
5	*Amandinea extunata*	3	5	*Parmotrema praesorediosum*	9
6	*Graphis* sp.2	5	6	*Lecanora tropica*	3
7	*Chrysothrix xanthina*	4	7	*Lecanora achrosa*	3
8	*Physcia damidiata*	5	8	*Pyxine consocians*	8
9	*Caloplaca gambiensis*	5	9	*Parmotrema tinctorum*	4
10	*Anthracothecium prasinum*	3	10	*Chrysothrix xanthina*	7
11	*Graphis* sp.12	1	11	*Graphis* sp.2	3
12	*Graphis* sp.14	5	12	*Dirinaria appleanata*	4
13	*Sarcographa* sp.	3	13	*Pyxine cocoes*	4
14	*Graphis* sp.1	3	14	*Chrysothrix candelaris*	2
15	*Dirinaria pica*	3	15	*Graphis* sp.1	3
	Total frequency	53	16	*Caloplaca diplacia*	4
			17	*Graphis* sp.15	2
			18	*Physcia poncinsii*	3
			19	*Trypethelium tropicum*	2
				Total frequency	75

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of Loei and LEIo = outside municipality of Loei, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens Species	F	Plot	Lichens Species	F
LEIi2			**LEIo2**		
1	*Chrysothrix xanthina*	6	1	*Chrysothrix xanthina*	3
2	*Pyxine cocoes*	5	2	*Graphis* sp.2	7
3	*Caloplaca gambiensis*	2	3	*Dirinaria appleanata*	5
4	*Graphis* sp.12	2	4	*Pyxine cocoes*	3
5	*Arthonia incospicua*	2	5	*Parmotrema tinctorum*	10
6	*Amandinea extunata*	4	6	*Caloplaca diplacia*	1
7	*Bacidia pallidocarnea*	2	7	*Pyxine subcinerea*	1
	Total frequency	23	8	*Crocynia pyxinoid*	1
			9	*Ocellularia crocea*	4
			10	*Arthonia catenatuta*	6
				Total frequency	41
LEIi3			**LEIo3**		
1	*Chrysothrix xanthina*	4	1	*Anthracothecium prasinum*	2
2	*Graphis* sp.3	6	2	*Chrysothrix xanthina*	8
3	*Arthonia incospicua*	3	3	*Graphis* sp.2	8
4	*Phyllopsora sorediata*	5	4	*Pyxine cocoes*	3
5	*Graphis* sp.4	5	5	*Chrysothrix candelaris*	4
6	*Trypethelium tropicum*	2	6	*Caloplaca diplacia*	1
7	*Graphis* sp.23	4	7	*Graphis* sp.15	2
8	*Myriotrema microporellum*	1	8	*Physcia poncinsii*	4
	Total frequency	30	9	*Trypethelium tropicum*	2
			10	*Rinodina cinereovirescens*	5
			11	*Crocynia pyxinoid*	1
				Total frequency	40

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of Loei and LEIo = outside municipality of Loei, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
LEIi4			**LEIo4**		
1	*Chrysothrix xanthina*	3	1	*Chrysothrix xanthina*	3
2	*Pyxine cocoes*	3	2	*Pyxine cocoes*	5
3	*Anthracothecium prasinum*	3	3	*Chrysothrix candelaris*	3
4	*Graphis* sp.1	7	4	*Graphis* sp.13	6
5	*Chrysothrix candelaris*	3	5	*Graphis* sp.15	5
6	*Dirinaria appleanata*	2	6	*Physcia poncinsii*	2
7	*Graphis* sp.6	3	7	*Graphis* sp.4	5
8	Sterile crust sp.2	2	8	*Lecanora tropica*	2
	Total frequency	**26**	9	*Arthonia cytodes*	1
			10	*Amandinea punctata*	2
			11	*Arthonia elegans*	4
				Total frequency	**38**
LEIi5			**LEIo5**		
1	*Chrysothrix xanthina*	5	1	*Chrysothrix xanthina*	2
2	*Pyxine coccifera*	2	2	*Graphis* sp.2	4
3	*Physcia damidiata*	1	3	*Pyxine cocoes*	2
4	*Graphis* sp.12	4	4	*Parmotrema tinctorum*	10
5	*Dirinaria pica*	4	5	*Graphis* sp.13	3
6	*Dirinaria appleanata*	4	6	*Pyxine subcinerea*	2
7	*Porina internigrans*	5	7	*Lecanora tropica*	2
8	*Arthonia tumidula*	2	8	*Arthonia catenatuta*	4
9	*Opegrapha stirtinii*	2	9	*Dirinaria pica*	2
10	*Graphis* sp.5	2	10	*Amandinea punctata*	1
	Total frequency	**31**	11	*Arthonia tumidula*	2
				Total frequency	**34**

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of Loei and LEIo = outside municipality of Loei, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
---	Species	F	---	Species	F
LEIi6			LEIo6		
1	*Chrysothrix xanthina*	2	1	*Chrysothrix xanthina*	3
2	*Pyxine coccifera*	1	2	*Pyxine cocoes*	3
3	*Graphis* sp.3	4	3	*Chrysothrix candelaris*	3
4	*Arthonia incospicua*	3	4	*Graphis* sp.13	4
5	*Chrysothrix candelaris*	3	5	*Trypethelium tropicum*	2
6	*Lepraria atrotomentosa*	2	6	*Graphis* sp.3	7
7	*Arthonia catenatula*	2	7	*Graphis* sp.4	5
8	*Sarcographa* sp.	5	8	*Lecanora tropica*	3
9	*Laurera benguelensis*	4	9	*Arthonia cinnabarina*	2
	Total frequency	**26**	10	*Dirinaria pica*	1
			11	*Porina internigrans*	2
			12	*Graphis* sp.15	6
				Total frequency	**41**
LEIi7			LEIo7		
1	*Pyxine coccifera*	1	1	*Chrysothrix candelaris*	2
2	*Physcia damidiata*	6	2	*Dirinaria appleanata*	5
3	*Caloplaca gambiensis*	3	3	*Pyxine cocoes*	2
4	*Graphis* sp.3	3	4	*Graphis* sp.1	1
5	*Arthonia incospicua*	3	5	*Caloplaca diplacia*	4
6	*Cryptothecia candida*	2	6	*Graphis* sp.3	6
7	*Opegrapha stirtinii*	2	7	*Cladonia fimbriata*	3
	Total frequency	**20**	8	*Arthonia elegans*	3
			9	*Dirinaria pica*	2
			10	*Opegrapha stirtinii*	2
			11	*Cladonia crispate*	3
			12	*Cladonia glauca*	4
			13	*Amandinea punctata*	4
				Total frequency	**41**

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of Loei and LEIo = outside municipality of Loei, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
LEIi8			**LEIo8**		
1	*Pyxine coccifera*	2	1	*Chrysothrix xanthina*	3
2	*Caloplaca gambiensis*	2	2	*Dirinaria appleanata*	7
3	*Graphis* sp.2	4	3	*Pyxine cocoes*	1
4	*Chrysothrix candelaris*	3	4	*Graphis* sp.1	2
5	*Cryptothecia candida*	3	5	*Caloplaca diplacia*	3
6	*Lecanographa atropunctata*	3	6	*Trypethelium tropicum*	4
7	*Rinodina cinereovirescens*	3	7	*Rinodina cinereovirescens*	2
8	*Crocynia pyxinoid*	2	8	*Arthonia catenatuta*	2
	Total frequency	**22**	9	*Arthonia cinnabarina*	4
				Total frequency	**28**
LEIi9			**LEIo9**		
1	*Chrysothrix xanthina*	2	1	*Chrysothrix candelaris*	1
2	*Pyxine cocoes*	4	2	*Graphis* sp.2	3
3	*Graphis* sp.14	3	3	*Pyxine cocoes*	1
4	*Phyllopsora sorediata*	5	4	*Graphis* sp.1	2
5	*Cryptothecia candida*	2	5	*Caloplaca diplacia*	2
6	*Dichosporidium boschianum*	6	6	*Physcia poncinsii*	2
7	*Arthonia cytodes*	2	7	*Lecanora achrosa*	3
8	*Cryptothecia punctosorediata*	3	8	*Porina eminentior*	2
9	*Rinodina cinereovirescens*	4	9	*Amandinea extunata*	4
	Total frequency	**31**	10	*Pyrenula confinis*	3
			11	*Cryptothecia punctosorediata*	4
				Total frequency	**27**

Table C.3 Lichen frequency of Loei (F = frequency, LEIi = inside municipality of Loei and LEIo = outside municipality of Loei, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
LEIi10			LEIo10		
1	*Arthonia tumidula*	2	1	*Anthracothecium prasinum*	2
2	*Trypethelium tropicum*	2	2	*Porina eminentior*	5
3	*Chrysothrix xanthina*	4	3	*Dirinaria appleanata*	4
4	*Pyxine cocoes*	3	4	*Chrysothrix candelaris*	6
5	*Graphis* sp.12	3	5	*Graphis* sp.1	7
6	*Graphis* sp.14	3	6	*Graphis* sp.13	2
7	*Sarcographa* sp.	3	7	*Physcia poncinsii*	4
8	*Dirinaria pica*	3	8	*Trypethelium tropicum*	4
9	*Cryptothecia candida*	2	9	*Pyxine subcinerea*	3
10	*Bacidia pallidocarnea*	4	10	*Lecanora achrosa*	7
11	*Hyperphyscia adglutinata*	5	11	*Amandinea extunata*	2
	Total frequency	34	12	*Cryptothecia candida*	3
				Total frequency	49

Table C.4 Lichen frequency of Khon Kaen (F = frequency, KKNi = inside municipality of Khon Kaen and KKNo = outside municipality of Khon Kaen, 1-10 refer to number of selected tree in each plot).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
KKNi1			**KKNo1**		
1	*Lecanora leprosa*	3	1	*Lecanora leprosa*	4
2	*Chrysothrix xanthina*	4	2	*Hyperphyscia adglutinata*	2
3	*Graphis* sp.9	3	3	*Pyxine coccifera*	3
4	*Arthonia cytodes*	1		**Total frequency**	9
	Total frequency	11			
KKNi2			**KKNo2**		
1	*Lecanora leprosa*	3	1	*Graphis* sp.7	3
2	*Chrysothrix xanthina*	3	2	*Lecanora tropica*	4
3	*Lecanora tropica*	1	3	*Trypethelium eluteriae*	3
	Total frequency	7		**Total frequency**	10
KKNi3			**KKNo3**		
1	*Dichosporidium boschianum*	3	1	*Lecanora leprosa*	3
2	*Graphis* sp.6	1	2	*Hyperphyscia adglutinata*	2
	Total frequency	4	3	*Graphis* sp.7	2
			4	*Trypethelium eluteriae*	3
			5	*Pyxine coccifera*	4
				Total frequency	14
KKNi4			**KKNo4**		
1	*Lecanora leprosa*	3	1	*Pyxine subcinerea*	3
2	*Graphis* sp.2	2	2	*Caloplaca gambiensis*	2
	Total frequency	5		**Total frequency**	5
KKNi5			**KKNo5**		
1	*Lecanora leprosa*	4	1	*Hyperphyscia adglutinata*	2
2	*Graphis* sp.26	2	2	*Caloplaca gambiensis*	2
	Total frequency	6		*Pyxine coccifera*	3
				Total frequency	7

Table C.4 Lichen frequency of Khon Kaen (F = frequency, KKNi = inside municipality of Khon Kaen and KKNo = outside municipality of Khon Kaen, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
KKNi6			**KKNo6**		
1	*Pyxine cocoes*	2	1	*Hyperphyscia adglutinata*	2
2	*Dichosporidium boschianum*	2	2	*Physcia damidiata*	1
	Total frequency	**4**		**Total frequency**	**3**
KKNi7			**KKNo7**		
1	*Pyxine cocoes*	3	1	*Pyxine subcinerea*	3
2	*Graphis* sp.2	1	2	*Pyxine cocoes*	3
3	*Rinodina* sp.	2	3	*Trypethelium eluteriae*	4
	Total frequency	**6**		**Total frequency**	**10**
KKNi8			**KKNo8**		
1	*Pyxine consocians*	7	1	*Pyxine cocoes*	3
2	*Graphis* sp.2	2	2	*Chrysothrix xanthina*	3
3	*Graphis* sp.26	3	3	*Pyxine coccifera*	2
	Total frequency	**12**		**Total frequency**	**8**
KKNi9			**KKNo9**		
1	*Chrysothrix xanthina*	3	1	*Pyxine cocoes*	2
2	*Trypethelium eluteriae*	3	2	*Arthonia tumidula*	1
	Total frequency	**6**	3	*Pyxine cocoes*	2
			4	*Trypethelium eluteriae*	2
				Total frequency	**7**
KKNi10			**KKNo10**		
1	*Lecanora leprosa*	2	1	*Lecanora leprosa*	2
2	*Chrysothrix xanthina*	2	2	*Graphis* sp.3	1
3	*Laurera benguelensis*	1	3	*Trypethelium tropicum*	4
	Total frequency	**5**		**Total frequency**	**7**

Table C.5 Lichen frequency of Nong Khai (F = frequency, NKIi = inside municipality of Nong Khai and NKIo = outside municipality of Nong Khai, 1-10 refer to number of selected tree in each plot).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
NKIi1			**NKIo1**		
1	*Pyxine cocoes*	5	1	*Pyxine cocoes*	3
2	*Chrysothrix xanthina*	3	2	*Lecanora leprosa*	2
3	*Cryptothecia candida*	2	3	*Graphis* sp.2	5
4	*Lecanora leprosa*	4	4	*Dirinaria pica*	4
	Total frequency	14	5	*Graphis* sp.26	6
			6	*Physcia atrostriata*	4
			7	*Graphis* sp.22	2
			8	*Caloplaca diplacia*	2
				Total frequency	28
NKIi2			**NKIo2**		
1	*Pyxine cocoes*	5	1	*Pyxine cocoes*	2
2	*Dirinaria pica*	1	2	*Lecanora leprosa*	2
3	*Chrysothrix xanthina*	7	3	*Graphis* sp.1	2
4	*Graphis* sp.7	5	4	*Graphis* sp.2	5
5	*Rinodina cinereovirescens*	2	5	*Caloplaca diplacia*	6
6	*Arthonia tumidula*	3	6	*Chrysothrix candelaris*	5
	Total frequency	23	7	*Graphis* sp.26	5
				Total frequency	27
NKIi3			**NKIo3**		
1	*Pyxine cocoes*	7	1	*Pyxine cocoes*	6
2	*Graphis* sp.1	5	2	*Chrysothrix xanthina*	3
3	*Dirinaria appleanata*	1	3	*Arthonia catenatuta*	1
4	*Lecanora leprosa*	2	4	*Dirinaria appleanata*	4
5	*Trypethelium tropicum*	3	5	*Dichosporidium boschianum*	3
6	*Arthonia cinnabarina*	1	6	*Lecanographa atropunctata*	6
7	*Porina eminentior*	3	7	*Graphis* sp.3	4
	Total frequency	22	8	*Graphis* sp.26	5
				Total frequency	32

Table C.5 Lichen frequency of Nong Khai (F = frequency, NKIi = inside municipality of Nong Khai and NKIo = outside municipality of Nong Khai, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
NKIi4			**NKIo4**		
1	*Pyxine cocoes*	4	1	*Pyxine cocoes*	3
2	*Dirinaria appleanata*	2	2	*Chrysothrix xanthina*	3
3	*Lecanora leprosa*	2	3	*Graphis* sp.22	4
4	*Arthonia cinnabarina*	1	4	*Trypethelium eluteriae*	4
5	*Chrysothrix xanthina*	2	5	*Graphis* sp.3	4
	Total frequency	11		**Total frequency**	18
NKIi5			**NKIo5**		
1	*Pyxine cocoes*	6	1	*Pyxine cocoes*	4
2	*Dirinaria pica*	3	2	*Lecanora tropica*	7
3	*Arthonia cinnabarina*	1	3	*Arthonia cytodes*	2
4	*Lecanora achrosa*	3	4	*Porina eminentior*	3
5	*Physcia poncinsii*	3	5	*Trypethelium tropicum*	3
	Total frequency	16		**Total frequency**	19
NKIi6			**NKIo6**		
1	*Pyxine cocoes*	3	1	*Sarcographa* sp.	6
2	*Dirinaria pica*	2	2	*Lecanora leprosa*	7
3	*Graphis* sp.2	3	3	*Graphis* sp.1	4
4	*Graphis* sp.7	5	4	*Lecanora achrosa*	3
5	*Arthonia tumidula*	2	5	*Graphis* sp.21	5
6	*Rinodina cinereovirescens*	2	6	*Arthonia cinnabarina*	3
7	*Lecanora leprosa*	3	7	*Dirinaria pica*	5
8	*Physcia damidiata*	2	8	Graphis sp.2	3
9	*Physcia poncinsii*	3	9	*Graphis* sp.3	4
	Total frequency	25	10	*Graphis* sp.5	2
				Total frequency	42

Table C.5 Lichen frequency of Nong Khai (F = frequency, NKIi = inside municipality of Nong Khai and NKIo = outside municipality of Nong Khai, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
NKIi7			**NKIo7**		
1	*Pyxine cocoes*	2	1	*Sarcographa* sp.	5
2	*Chrysothrix xanthina*	1	2	*Lecanora leprosa*	2
3	*Graphis* sp.2	2	3	*Dirinaria appleanata*	5
4	*Arthonia tumidula*	2	4	Sterile crust sp.1	2
5	*Graphis* sp.25	4	5	*Opegrapha stirtinii*	10
6	*Anthracothecium prasinum*	3	6	*Dirinaria pica*	5
7	*Porina eminentior*	3	7	*Graphis* sp.2	5
	Total frequency	17	8	*Lecanora achrosa*	4
			9	*Parmotrema praesorediosum*	4
			10	*Parmotrema tinctorum*	6
				Total frequency	48
NKIi8			**NKIo8**		
1	*Pyxine cocoes*	1	1	*Pyxine cocoes*	3
2	*Graphis* sp.2	2	2	*Chrysothrix xanthina*	3
3	*Arthonia incospicua*	1	3	*Dirinaria pica*	4
4	*Arthonia catenatuta*	2	4	*Dirinaria appleanata*	4
5	*Lecanora leprosa*	4	5	Sterile crust sp.1	2
	Total frequency	10	6	*Lecanora achrosa*	4
			7	*Trypethelium eluteriae*	4
				Total frequency	24
NKIi9			**NKIo9**		
1	*Pyxine cocoes*	3	1	*Pyxine cocoes*	5
2	*Dirinaria appleanata*	2	2	*Graphis* sp.2	3
3	*Anthracothecium prasinum*	2	3	*Trypethelium eluteriae*	5
4	*Graphis* sp.25	4	4	*Anthracothecium prasinum*	2
5	*Arthonia incospicua*	1	5	*Dirinaria pica*	5
6	*Lecanora leprosa*	4	6	*Dichosporidium boschianum*	3
7	*Arthonia cytodes*	4	7	*Graphis* sp.4	7
	Total frequency	20	8	*Trypethelium tropicum*	5
				Total frequency	35

Table C.5 Lichen frequency of Nong Khai (F = frequency, NKIi = inside municipality of Nong Khai and NKIo = outside municipality of Nong Khai, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
NKIi10			**NKIo10**		
1	*Pyxine cocoes*	2	1	*Dirinaria pica*	2
2	*Dirinaria pica*	2	2	*Pyxine cocoes*	4
3	*Anthracothecium prasinum*	4	3	*Lecanora leprosa*	3
4	*Arthonia incospicua*	1	4	*Graphis* sp.1	1
5	*Arthonia catenatuta*	2	5	*Trypethelium tropicum*	3
	Total frequency	11	6	*Anthracothecium prasinum*	2
			7	*Arthonia catenatuta*	4
			8	*Porina eminentior*	2
			9	*Myriotrema microporellum*	3
			10	*Ocellularia crocea*	4
			11	*Opegrapha stirtinii*	4
				Total frequency	32

Table C.6 Lichen frequency of Sakhon Nakhon (F = frequency, SKNi = inside municipality of Sakhon Nakhon and SKNo = outside municipality of Sakhon Nakhon, 1-10 refer to number of selected tree in each plot).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
SKNi1			**SKNo1**		
1	*Pyxine cocoes*	3	1	*Pyxine cocoes*	3
2	*Chrysothrix xanthina*	5	2	*Dirinaria pica*	2
3	*Dirinaria pica*	2	3	*Graphis* sp.2	1
4	*Lecanora tropica*	4	4	*Graphis* sp.20	4
	Total frequency	14	6	*Trypethelium eluteriae*	5
			7	*Graphis* sp.3	1
			8	*Trypethelium tropicum*	3
			9	*Sarcographa* sp.	2
			10	*Chrysothrix xanthina*	5

Table C.6 Lichen frequency of Sakhon Nakhon (F = frequency, SKNi = inside municipality of Sakhon Nakhon and SKNo = outside municipality of Sakhon Nakhon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
			SKNo1		
			11	*Laurera benguelensis*	6
			12	*Laurera megasperma*	3
				Total frequency	35
SKNi2			**SKNo2**		
1	*Pyxine cocoes*	2	1	*Pyxine cocoes*	4
2	*Lecanora leprosa*	2	2	*Graphis* sp.2	2
3	*Chrysothrix xanthina*	3	3	*Graphis* sp.20	4
4	*Anthracothecium prasinum*	1	4	*Graphis* sp.3	2
5	*Caloplaca diplacia*	3	5	*Trypethelium tropicum*	4
6	*Caloplaca diplacioides*	3	6	*Sarcographa* sp.	3
7	*Caloplaca gambiensis*	4	7	*Graphis* sp.5	2
8	*Dirinaria pica*	3	8	*Laurera megasperma*	3
	Total frequency	21		Total frequency	24
SKNi3			**SKNo3**		
1	*Pyxine cocoes*	4	1	*Physcia poncinsii*	4
2	*Lecanora leprosa*	2	2	*Pyxine cocoes*	4
3	*Caloplaca diplacioides*	3	3	*Dirinaria pica*	1
4	*Caloplaca gambiensis*	4	4	*Lecanora leprosa*	5
5	*Dirinaria appleanata*	2	5	*Dirinaria appleanata*	1
6	*Dirinaria pica*	4	6	*Lecanora achrosa*	6
	Total frequency	19	7	*Parmotrema tinctorum*	5
			9	*Arthonia elegans*	1
			10	*Chrysothrix xanthina*	5
				Laurera benguelensis	7
				Total frequency	39
SKNi4			**SKNo4**		
1	*Pyxine cocoes*	3	1	*Pyxine cocoes*	4
2	*Dirinaria pica*	2	2	*Graphis* sp.2	2
3	*Graphis* sp.16	4	3	*Graphis* sp.20	4
4	*Graphis* sp.1	4	4	*Trypethelium eluteriae*	3
5	*Graphis* sp.2	2	5	*Graphis* sp.1	2

Table C.6 Lichen frequency of Sakhon Nakhon (F = frequency, SKNi = inside municipality of Sakhon Nakhon and SKNo = outside municipality of Sakhon Nakhon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens Species	F	Plot	Lichens Species	F
SKNi4			**SKNo4**		
6	Dirinaria appleanata	3	6	Lecanora tropica	4
	Total frequency	18	7	Arthonia elegans	1
			8	Dirinaria confluens	4
				Total frequency	24
SKNi5			**SKNo5**		
1	Pyxine cocoes	4	1	Pyxine cocoes	5
2	Dirinaria pica	2	2	Dirinaria pica	2
3	Graphis sp.16	2	3	Lecanora leprosa	4
4	Dirinaria appleanata	3	4	Graphis sp.3	2
5	Graphis sp.2	4	5	Lecanora achrosa	3
	Total frequency	15	6	Graphis sp.1	3
			7	Graphis sp.4	2
			8	Chrysothrix candelaris	4
				Total frequency	25
SKNi6			**SKNo6**		
1	Pyxine cocoes	2	1	Pyxine cocoes	2
2	Dirinaria pica	4	2	Dirinaria pica	2
3	Dirinaria appleanata	2	3	Dirinaria appleanata	3
4	Hyperphyscia adglutinata	3	4	Graphis sp.1	2
5	Hyperphyscia adsendes	2	5	Graphis sp.4	1
6	Laurera benguelensis	2	6	Arthonia elegans	1
7	Parmotrema praesorediosum	4	7	Caloplaca diplacioides	2
8	Parmotrema tinctorum	5	8	Graphis sp.10	3
	Total frequency	24	9	Graphis sp.11	5
			10	Graphis sp.17	2
			11	Graphis sp.18	4
				Total frequency	27

Table C.6 Lichen frequency of Sakhon Nakhon (F = frequency, SKNi = inside municipality of Sakhon Nakhon and SKNo = outside municipality of Sakhon Nakhon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
SKNi7			SKNo7		
1	Pyxine cocoes	4	1	Pyxine coccifera	4
2	Chrysothrix xanthina	3	2	Lecanora leprosa	4
3	Graphis sp.1	4	3	Dirinaria appleanata	1
4	Rinodina intrasa	1	4	Sarcographa sp.	2
5	Arthonia cinnabarina	1	5	Caloplaca diplacia	1
6	Arthonia incospicua	3	6	Graphis sp.4	1
7	Hyperphyscia adglutinata	3	7	Arthonia elegans	2
	Total frequency	**19**	8	Sterile crust sp.1	1
			9	Graphis sp.10	3
			10	Dirinaria confluens	4
				Total frequency	**23**
SKNi8			SKNo8	Hypotrachyna osseoalba	1
1	Pyxine cocoes	3	1	Pyxine cocoes	2
2	Graphis sp.8	4	2	Lecanora leprosa	2
3	Graphis sp.1	5	3	Graphis sp.4	1
4	Rinodina intrasa	1	4	Graphis sp.1	3
5	Graphis sp.9	4	5	Laurera megasperma	3
6	Rinodina cinereovirescens	2	6	Graphis sp.17	3
	Total frequency	**19**	7	Hyperphyscia granulata	2
			8	Hypogymnia hypotrypa	1
				Total frequency	**18**
SKNi9			SKNo9		
1	Pyxine cocoes	3	1	Pyxine cocoes	1
2	Graphis sp.3	5	2	Graphis sp.2	4
3	Anthracothecium prasinum	2	3	Trypethelium eluteriae	6
4	Graphis sp.1	3	4	Trypethelium tropicum	2
5	Arthonia tumidula	4	5	Sarcographa sp.	3
6	Rinodina intrasa	1	6	Lecanora tropica	2
7	Hyperphyscia adglutinata	3	7	Physcia damidiata	1
	Total frequency	**21**		**Total frequency**	**19**

Table C.6 Lichen frequency of Sakhon Nakhon (F = frequency, SKNi = inside
municipality of Sakhon Nakhon and SKNo = outside municipality of
Sakhon Nakhon, 1-10 refer to number of selected tree in each plot)
(Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
SKNi10			SKNo10		
1	*Pyxine cocoes*	6	1	*Arthonia tumidula*	2
2	*Anthracothecium prasinum*	3	2	*Pyxine coccifera*	1
3	*Rinodina intrasa*	2	3	*Lecanora leprosa*	2
4	*Sarcographa* sp.	2	4	*Trypethelium tropicum*	3
5	Sterile crust sp.3	2	5	*Sarcographa* sp.	2
	Total frequency	**15**	6	*Chrysothrix xanthina*	5
			7	*Graphis* sp.1	3
			8	*Graphis* sp.5	2
			9	*Graphis* sp.18	2
			10	*Hyperphyscia adglutinata*	1
			11	*Hyperphyscia flavida*	5
				Total frequency	**28**

Table C.7 Lichen frequency of Ubon Ratchathani (F= frequency, UBNi = inside
municipality of Ubon Ratchathani and UBNo = outside municipality of
Ubon Ratchathani, 1-10 refer to number of selected tree in each plot).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
UBNi1			UBNo1		
1	*Pyxine cocoes*	9	1	*Pyxine cocoes*	10
2	*Hyperphyscia adglutinata*	1	2	*Rinodina cinereovirescens*	10
3	*Chrysothrix xanthina*	3	3	*Trypethelium tropicum*	3
4	*Rinodina intrasa*	4	4	*Graphis* sp.1	3
5	*Trypethelium tropicum*	3	5	*Hyperphyscia adglutinata*	2
	Total frequency	**20**	6	*Dirinaria appleanata*	2
				Total frequency	**30**

Table C.7 Lichen frequency of Ubon Ratchathani (F = frequency, UBNi = inside municipality of Ubon Ratchathani and UBNo = outside municipality of Ubon Ratchathani, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
UBNi2			**UBNo2**		
1	*Pyxine cocoes*	8	1	*Pyxine cocoes*	6
2	*Hyperphyscia adglutinata*	3	2	*Trypethelium tropicum*	5
3	*Hyperphyscia granulata*	1	3	*Hyperphyscia adglutinata*	2
4	*Rinodina cinereovirescens*	2	4	*Dirinaria appleanata*	2
	Total frequency	**14**	5	*Anthracothecium prasinum*	3
				Total frequency	**18**
UBNi3			**UBNo3**		
1	*Pyxine cocoes*	6	1	*Pyxine coccifera*	5
2	*Hyperphyscia adglutinata*	6	2	*Rinodina cinereovirescens*	4
3	*Hyperphyscia granulata*	5	3	*Trypethelium tropicum*	6
	Total frequency	**17**		**Total frequency**	**15**
UBNi4			**UBNo4**		
1	*Pyxine cocoes*	7	1	*Pyxine cocoes*	5
2	*Chrysothrix xanthina*	3	2	*Trypethelium tropicum*	1
	Total frequency	**10**	3	*Arthonia incospicua*	4
				Total frequency	**10**
UBNi5			**UBNo5**		
1	*Pyxine cocoes*	5	1	*Pyxine coccifera*	7
2	*Graphis* sp.1	3	2	*Rinodina cinereovirescens*	6
3	*Dirinaria pica*	2	3	*Lecanora tropica*	4
	Total frequency	**10**		**Total frequency**	**17**
UBNi6			**UBNo6**		
1	*Pyxine cocoes*	5	1	*Pyxine cocoes*	6
2	*Graphis* sp.1	3	2	*Graphis* sp.1	4
3	*Dirinaria pica*	2	3	*Arthonia cinnabarina*	2
4	*Graphis* sp.2	3	4	*Graphis* sp.2	4
5	*Graphis* sp.8	3	5	*Graphis* sp.8	2
6	*Sarcographa* sp.	3	6	*Caloplaca diplacia*	2
7	*Anthracothecium prasinum*	1	7	*Dirinaria confluens*	3
	Total frequency	**20**		**Total frequency**	**23**

Table C.7 Lichen frequency of Ubon Ratchathani (F = frequency, UBNi = inside municipality of Ubon Ratchathani and UBNo = outside municipality of Ubon Ratchathani, 1-10 refer to number of selected tree in each plot) (Cont.).

| Plot | Lichens | | Plot | Lichens | |
	Species	F		Species	F
UBNi7			UBNo7		
1	*Pyxine cocoes*	7	1	*Pyxine coccifera*	5
2	*Graphis* sp.1	2	2	*Rinodina cinereovirescens*	5
3	*Dirinaria pica*	1	3	*Graphis* sp.24	5
	Total frequency	10	4	*Graphis* sp.9	2
			5	*Dirinaria confluens*	3
				Total frequency	20
UBNi8			UBNo8		
1	*Pyxine cocoes*	7	1	*Pyxine cocoes*	9
2	*Arthonia cinnabarina*	4	2	*Graphis* sp.1	4
3	*Dirinaria pica*	1	3	*Chrysothrix xanthina*	3
4	*Pyxine consocians*	3	4	*Arthonia cinnabarina*	1
	Total frequency	15	5	*Graphis* sp.19	2
				Total frequency	19
UBNi9			UBNo9		
1	*Pyxine cocoes*	5	1	*Pyxine cocoe*	4
2	*Arthonia cinnabarina*	2	2	*Rinodina cinereovirescens*	6
3	*Rinodina* cinereovirescens	1	3	*Chrysothrix xanthina*	2
	Total frequency	8	4	*Arthonia cinnabarina*	2
				Graphis sp.19	3
				Total frequency	17
UBM10			UBR10		
1	*Pyxine cocoes*	8	1	*Pyxine coccifera*	10
2	*Chrysothrix xanthina*	4	2	*Graphis* sp.1	2
3	*Arthonia cinnabarina*	2	3	*Hyperphyscia adglutinata*	4
4	*Anthracothecium prasinum*	1	4	*Lecanora tropica*	3
	Total frequency	15	5	*Dirinaria* appleanata	1
			6	*Lecanora leprosa*	4
			7	*Dirinaria pica*	3
				Total frequency	27

Table C.8 Lichen frequency of Yasothon (F = frequency, YSTi = inside municipality of Yasothon and YSTo = outside municipality of Yasothon, 1-10 refer to number of selected tree in each plot).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
YSTi1			**YSTo1**		
1	*Pyxine cocoes*	5	1	*Caloplaca diplacia*	3
2	*Rinodina cinereovirescens*	2	2	*Pyxine cocoes*	3
3	*Chrysothrix xanthina*	3	3	*Lecanora leprosa*	2
4	*Lecanora tropica*	2	4	*Chrysothrix xanthina*	5
5	*Dirinaria appleanata*	2	5	*Graphis* sp.1	4
6	*Arthonia tumidula*	2	6	*Graphis* sp.25	2
	Total frequency	**16**	7	*Arthonia tumidula*	2
			8	*Pyxine subcinerea*	4
			9	*Dichosporidium boschianum*	3
			10	*Trypethelium tropicum*	3
			11	*Lecanora achrosa*	2
				Total frequency	**33**
YSTi2			**YSTo2**		
1	*Pyxine cocoes*	6	1	*Pyxine cocoes*	2
2	*Sarcographa* sp.	3	2	*Lecanora leprosa*	2
3	*Graphis* sp.2	2	3	*Chrysothrix xanthina*	7
4	*Graphis* sp.20	2	4	*Trypethelium eluteriae*	2
5	*Graphis* sp.24	2	5	*Pyxine subcinerea*	2
6	*Chrysothrix candelaris*	2	6	*Dichosporidium boschianum*	4
	Total frequency	**17**	7	*Physcia atrostriata*	2
			8	*Arthonia cinnabarina*	1
			9	*Rinodina intrasa*	1
				Total frequency	**23**
YSTi3			**YSTo3**		
1	*Pyxine cocoes*	7	1	*Pyxine cocoes*	2
2	*Chrysothrix xanthina*	3	2	*Lecanora leprosa*	2
3	*Graphis* sp.2	1	3	*Chrysothrix xanthina*	8
4	*Graphis* sp.20	4	4	*Anthracothecium prasinum*	5
5	*Graphis* sp.1	3	5	*Lecanora achrosa*	2
6	*Graphis* sp.13	1	6	*Chrysothrix candelaris*	2
	Total frequency	**19**	7	*Arthonia cinnabarina*	1
				Total frequency	**22**

Table C.8 Lichen frequency of Yasothon (F = frequency, YSTi = inside municipality of Yasothon and YSTo = outside municipality of Yasothon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
YSTi4			YSTo4		
1	*Pyxine cocoes*	4	1	*Pyxine cocoes*	2
2	*Chrysothrix xanthina*	3	2	*Lecanora leprosa*	2
3	*Graphis* sp.2	1	3	*Graphis* sp.1	3
4	*Graphis* sp.24	2	4	*Graphis* sp.3	5
5	*Graphis* sp.9	2	5	*Graphis* sp.25	3
	Total frequency	12	6	*Arthonia tumidula*	3
			7	*Sarcographa* sp.	1
			8	*Graphis* sp.4	1
			9	*Porina eminentior*	2
				Total frequency	22
YSTi5			YSTo5		
1	*Rinodina cinereovirescens*	4	2	*Lecanora leprosa*	3
2	*Lecanora leprosa*	4	3	*Arthonia tumidula*	3
3	*Graphis* sp.2	3	4	*Trypethelium eluteriae*	3
4	*Graphis* sp.24	2	5	*Anthracothecium prasinum*	4
5	*Graphis* sp.13	1	6	*Dirinaria appleanata*	3
	Total frequency	14	7	*Cryptothecia candida*	1
				Total frequency	17
YSTi6			YSTo6		
1	*Pyxine cocoes*	2	1	*Pyxine cocoes*	1
2	*Rinodina cinereovirescens*	5	2	*Lecanora leprosa*	2
3	*Lecanora leprosa*	4	3	*Chrysothrix xanthina*	4
4	*Lecanora achrosa*	4	4	*Arthonia tumidula*	3
	Total frequency	15	5	*Lecanora helva*	5
			6	*Porina eminentior*	1
				Total frequency	16

Table C.8 Lichen frequency of Yasothon (F = frequency, YSTi = inside municipality of Yasothon and YSTo = outside municipality of Yasothon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens	Plot	Lichens

	Species	F			Species	F
YSTi7				**YSTo7**		
1	*Pyxine cocoes*	3		1	*Pyxine cocoes*	3
2	*Sarcographa* sp.	3		2	*Lecanora leprosa*	3
3	*Lecanora achrosa*	2		3	*Graphis* sp.1	4
4	*Arthonia tumidula*	2		4	*Graphis* sp.3	5
5	*Dirinaria pica*	1		5	*Sarcographa* sp.	3
	Total frequency	11		6	*Graphis* sp.4	1
				7	*Ocellularia crocea*	4
				8	*Cryptothecia candida*	2
					Total frequency	25
YSTi8				**YSTo8**		
1	*Pyxine cocoes*	3		1	*Pyxine cocoes*	4
2	*Rinodina cinereovirescens*	5		2	*Lecanora leprosa*	2
3	*Dirinaria appleanata*	2		3	*Graphis* sp.1	5
4	*Hyperphyscia adglutinata*	4		4	*Graphis* sp.3	5
	Total frequency	14		5	*Graphis* sp.25	3
				6	*Sarcographa* sp.	1
				7	*Graphis* sp.4	2
					Total frequency	22
YSTi9				**YSTo9**		
1	*Pyxine cocoes*	2		1	*Pyxine cocoes*	5
2	*Rinodina cinereovirescens*	2		2	*Lecanora leprosa*	2
3	*Lecanora tropica*	2		3	*Trypethelium eluteriae*	6
4	*Dirinaria appleanata*	2		4	*Pyxine subcinerea*	2
5	*Physcia atrostriata*	4		5	*Porina eminentior*	1
	Total frequency	12			**Total frequency**	16

Table C.8 Lichen frequency of Yasothon (F= frequency, YSTi = inside municipality of Yasothon and YSTo = outside municipality of Yasothon, 1-10 refer to number of selected tree in each plot) (Cont.).

Plot	Lichens		Plot	Lichens	
	Species	F		Species	F
YSTi10			YSTo10		
1	*Pyxine cocoes*	1	1	*Rinodina intrasa*	1
2	*Sarcographa* sp.	3	2	*Physcia poncinsii*	3
3	*Lecanora tropica*	3	3	*Pyxine cocoes*	5
4	*Cryptothecia candida*	4	4	*Lecanora leprosa*	2
5	*Physcia poncinsii*	2	5	*Chrysothrix xanthina*	4
	Total frequency	13	6	*Graphis* sp.1	3
			7	*Graphis* sp.25	4
			8	*Graphis* sp.4	1
			9	*Trypethelium tropicum*	2
			10	*Hyperphyscia adglutinata*	4
			11	*Physcia atrostriata*	2
			12	*Lecanora tropica*	3
				Total frequency	34

APPENDIX D

PICTURES OF LICHENS OF NORTHEAST OF

THAILAND IN 2010
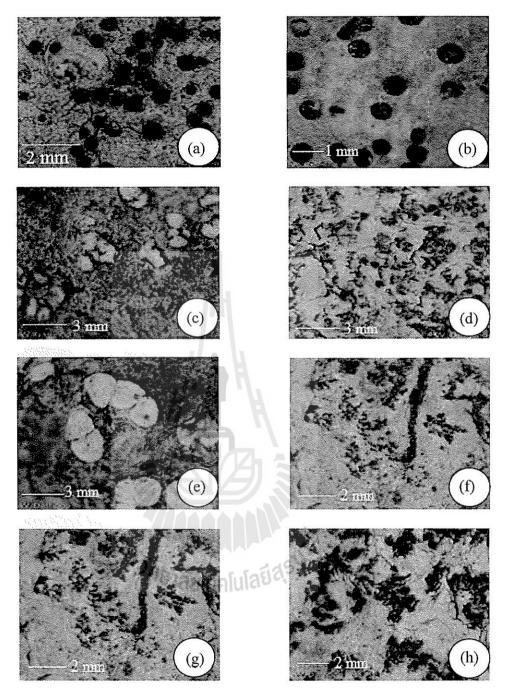
Figure D.1 Pictures of lichens found in the northeast of Thailand in 2010 (1); (a)
Amandinea extunata (NMAo9-3), (b) *Anthracothecium prasinum*
(LEIi1-5), (c) *Arthonia cinabarina* (LEIi1-4), (d) *Arthonia catenatula*
(NMAi2-4), (e) *Arthonia inconspicua* (LEIi1-4), (f) *Arthonia catenatula*
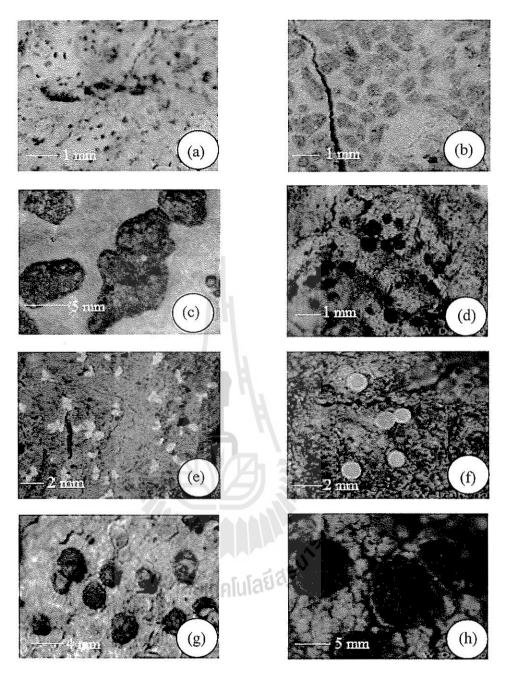(LEIi9-2A), (g) *Arthonia catenatula* (LEIi9-2A), (h) *Arthonia
cinabarina* (LEIo2-1).

Figure D.2 Pictures of lichens found in the northeast of Thailand in 2010 (2);
(a) *Arthonia incospicua* (LEIo6-3), (b) *Arthonia parantillarum* (NKIo8-4A), (c) *Arthonia cyrtodes* (YSTo6-2), (d) *Amandinea extunata* (NMAo9-3), (e) *Arthonia incospicua* (LEIo6-3), (f) *Basidia pallidocarnea* (UBMi5-5), (g) *Buellia erubescens* (CPMi 2-2), (h) *Caloplaca diplacioides* (NMAo1-3).
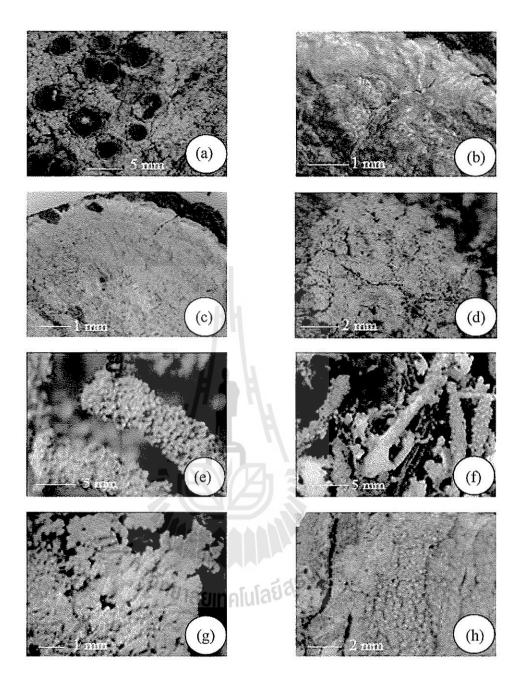
Figure D.3 Pictures of lichens found in the northeast of Thailand in 2010 (3); (a) *Caloplaca diplacia* (NMAo1-1), (b) *Cryptothecia punctosorediata* (NKIo10-3), (c) *Cryptothecia candida* (LEIo6-4), (d) *Chrysothrix xanthina* (LEIi6-1), (e) *Cladonia coccifera* (LEIo6-2), (f) *Cladonia* sp. (SKNo5-2), (g) *Crocynia pyxinoides* (CPMi4-2), (h) *Dichosporidium boschianum* (KKNi1-3).
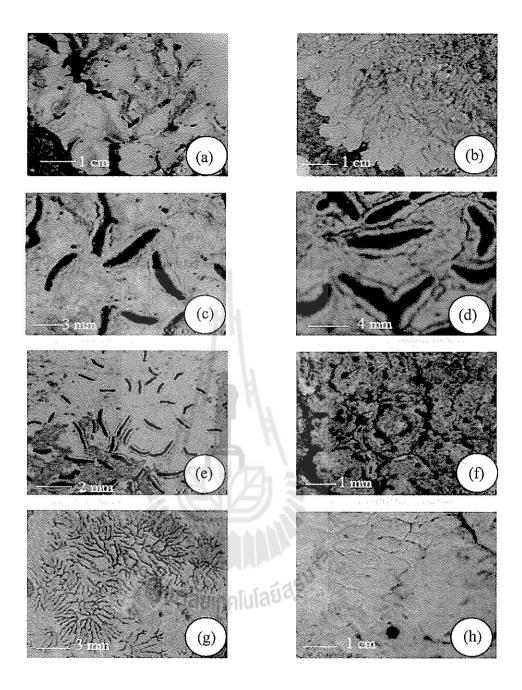
Figure D.4 Pictures of lichens found in the northeast of Thailand in 2010 (4); (a) *Dirinaria pica* (NMAI1-3D), (b) *Dirinaria applanata* (NMAI2-2A), (c) *Graphis* sp.1 (NMAi2), (d) *Graphis* sp.4 (KKNi2-2), (e) *Graphis* sp..3 (NMAo9-1), (f) *Graphis* sp.4 (NMAo6-2), (g) *Graphis* sp.5 (LEIi1-3), (h) *Graphis* sp.6 (LEIi1-7).
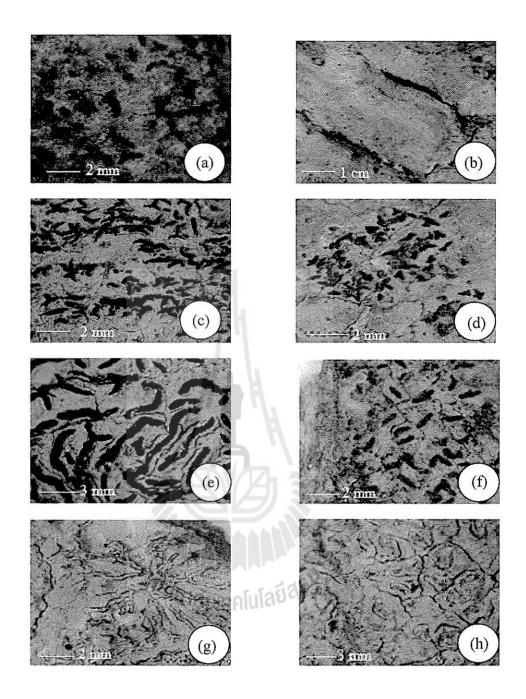
Figure D.5 Pictures of lichens found in the northeast of Thailand in 2010 (5); (a) *Graphis* sp.7 (LEIo7-9), (b) *Graphis* sp.8 (NKIi2-3), (c) *Graphis* sp.9 (NKIi2-3), (d) *Graphis* sp.10 (NKIi2-3), (e) *Graphis* sp.11(LEIo7-8), (f) *Graphis* sp.12 (LEIo7-8), (g) *Graphis* sp.13 (LEIo7-8), (h) *Graphis* sp.14 (LEIo1-10).
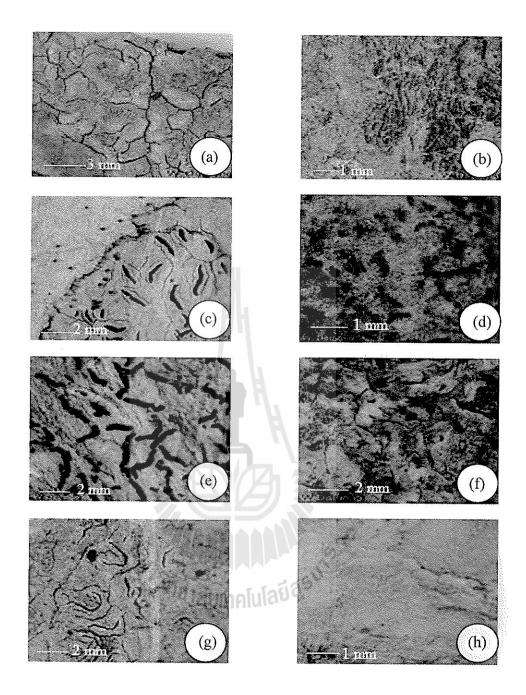
Figure D.6 Pictures of lichens found in the northeast of Thailand in 2010 (6); (a) *Graphis* sp.15 (LEIi5-1), (b) *Graphis* sp.16 (LEIo7-8), (c) *Graphis* sp.17 (NKIi2-5D), (d) *Graphis* sp.18 (NKIo6-3C), (e) *Graphis* sp.19 (NKIo10-3), (f) *Graphis* sp. 20 (UBMi5-2), (g) *Graphis* sp.21 (YSTo3-2), (h) *Graphis* sp.22 (SKNi4-4).
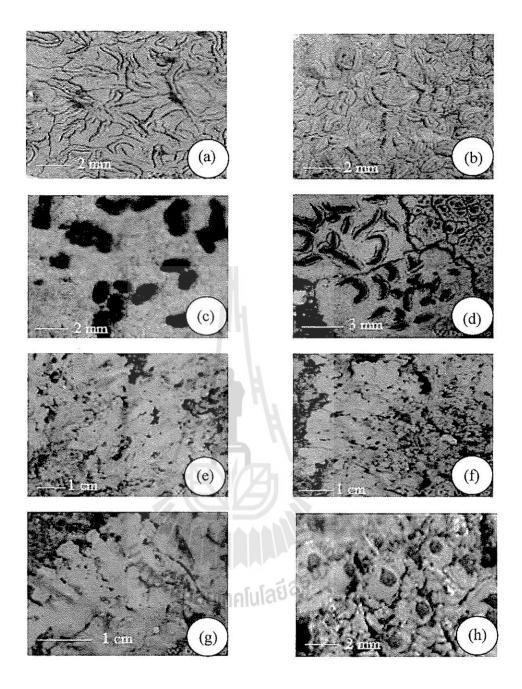
Figure D.7 Pictures of lichens found in the northeast of Thailand in 2010 (7); (a) *Graphis* sp.23 (YSTo9-1), (b) *Graphis* sp. 24 (SKNo52-2), (c) *Graphis* sp.25, (KNo8-2), (d) *Graphis* sp.26 (SKNi6-3), (e) *Hyperphyscia adglutinata* (NKIi1-3A), (f) *Hyperphyscia adsendes* (NRM1-3C), (g) *Hyperphyscia flavida* (SKNo8-1B), (h) *Laurera megasperma* (NKIo4-2A).
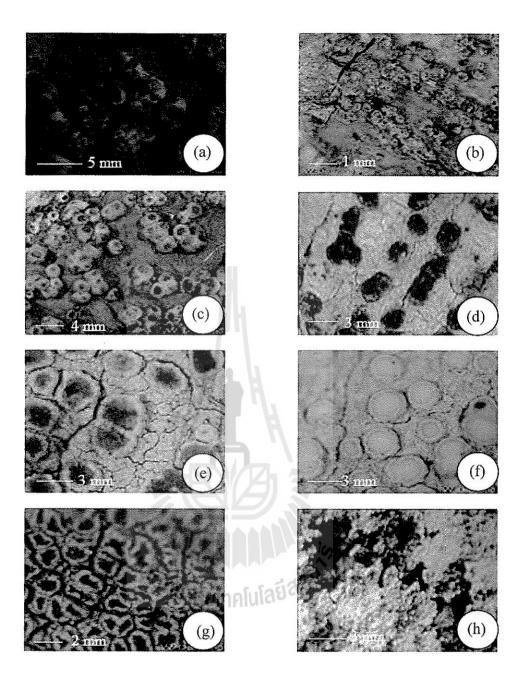
Figure D.8 Pictures of lichens found in the northeast of Thailand in 2010 (8); (a)

Laurera benguelensis (NMAo4-2A), (b) *Trypthelium tropicum* (LEIo1-

6), (c) *Trypthelium eluteriae* (NMAo10-1), (d) *Lecanographa* sp.

(NMAi1), (e) *Lecanora helva* (NKIi8-3), (f) *Lecanora leprosa* (NMAi1-

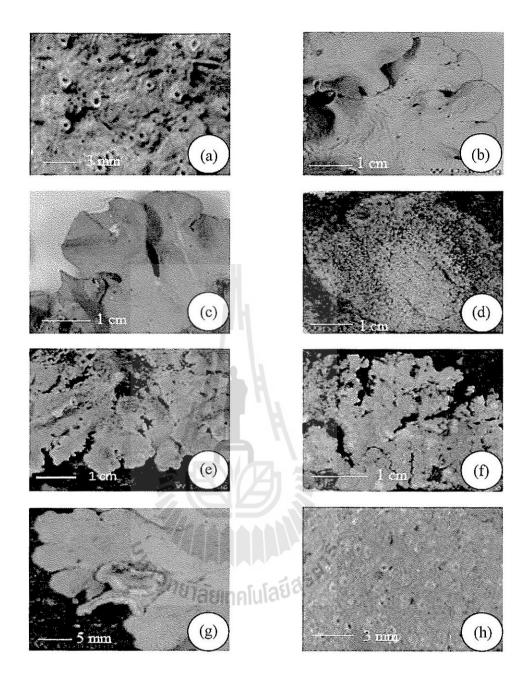2A), (g) *Lecanora tropica* (SKNo2-3), (h) *Lepraria* sp. (CPMi6-2).

Figure D.9 Pictures of lichens found in the northeast of Thailand in 2010 (9); (a)

Myriotrema sp. (LEIi6-4A), (b) *Parmotrema praesodiosum* (SKNo6-1),

(c) *Parmotrema tinctorum* (LEIo6-6), (d) *Phyllopsa sorediata*

(NMAi3-3), (e) *Physcia atrotriata* (NMAi5-3), (f) *Physcia dimidiate*

(KKNi5-2), (g) *Physia poncinsii* (LEIo7-2), (h) *Porina eminentior*
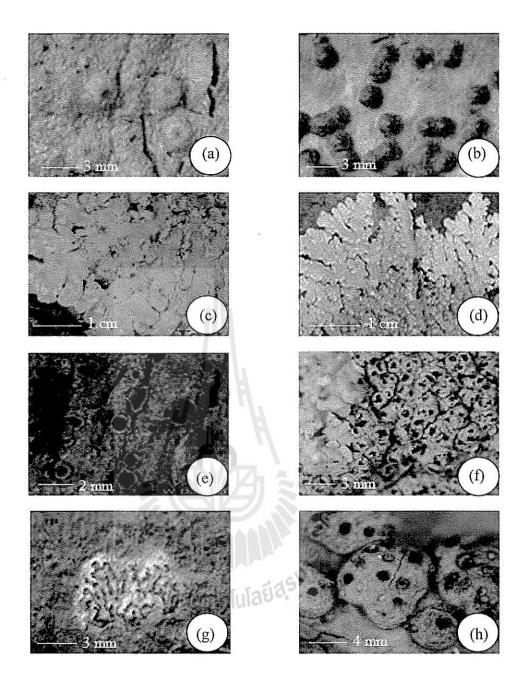
(NMAi7-2A).

Figure D.10 Pictures of lichens found in the northeast of Thailand in 2010 (10); (a)

Porina internigrans (LEIi10-2A), (b) *Pyrenura confinis* (LEIo3), (c)

Pyxine cocoes (SKNi6-2), (d) *Pyxine subcinerea* (NMAi2-5), (e)

Rinodina intrasa (NMAo2-2), (f) *Rinodina cinereovirescens* (LEIi6-1),

(g) *Sarcographa* sp. (YSTi10-1), (h) *Trypethelium eluteriae* (NKIo10-

3).

CURRICULUM VITAE

NAME   MISS WAEWDAO DATHONG

DATE OF BIRTH   14 April 1984

PLACE OF BIRTH   Buriram province, Thailand

EDUCATIONAL BACKGROUND

2006: B.Sc. Applied Biology (First class honor), Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand

GRANTS AND FELLOWSHIPS

2008-2011: CHE-PhD-THA-PROG-49 from the Commission on Higher Education, Thailand