# MONITORING OF HEAVY METAL CONTENTS IN CRUSTOSE AND FOLIOSE LICHENS

**SOPHA KEO INPAENG**

**A thesis submitted in partial fulfillment of the requirement for**
**the Master of Science degree in Chemistry**
**at Mahasarakham University**
**October 2012**



# MONITORING OF HEAVY METAL CONTENTS IN CRUSTOSE AND FOLIOSE LICHENS

**SOPHA KEO INPAENG**

**A thesis submitted in partial fulfillment of the requirement for**
**the Master of Science degree in Chemistry**
**at Mahasarakham University**
**October 2012**



Mahasarakham University

The examining committee has unanimously approved this thesis, submitted by Mr. Sopha Keo inpaeng, as partial fulfillment of the requirements for the Master of Science degree in Chemistry, Mahasarakham University.

Examining Committee

| | |
|---|---|
| S. Kruanetr ..... | Chairman |
| (Senee Kruanetr, Ph.D.) | (Faculty Graduate Committee) |
| Piyanete ..... | Committee |
| (Asst. Prof. Piyanete Chantiratikul, Ph.D.) | (Advisor) |
| K. Papong ..... | Committee |
| (Kwanruan Papong, Ph.D.) | (Co-advisor) |
| W. Siriangkhawut ..... | Committee |
| (Watsaka Siriangkhawut, Ph.D.) | (Faculty Graduate Committee) |
| R. Burakham ..... | Committee |
| (Asst. Prof. Rodjana Burakham, Ph.D.) | (External expert) |

Mahasarakham University has granted approval to accept this thesis as partial fulfillment of the requirement for the Master of Science degree in Chemistry.

| | |
|---|---|
| La-orsi Sanoamuang ..... | ..... |
| (Prof. La-orsi Sanoamuang, Ph.D.) | (Assoc. Prof. Nathanon Trachoo, Ph.D.) |
| Dean of the Faculty of Science | Acting Dean of the Faculty of Graduate Studies |
| | October 28, .....2012 |

# ACKNOWLEDGEMENTS

I would like to thank the ASIA-UNINET and Mahasarakham University for my Scholarship and financial support. I would also like to thank the Department of Chemistry, Faculty of Science, Mahasarakham University for partial support of chemicals, instruments and facilities, and the Graduate School, Mahasarakham University for guidance in preparing this thesis.

I am grateful to Asst. Prof. Dr. Rodjana Burakham, Department of Chemistry, Faculty of Science, Khon Kaen University, Dr. Senee Kruanetr and Dr. Watsaka Siriangkhawut, Department of Chemistry, Faculty of Science, Mahasarakham University for their kind comments and support as members of my thesis committee.

I would like to express my sincere gratitude and great appreciation to my advisor, Asst. Prof. Dr. Piyanete Chantiratikul for her excellent supervision, inspiring guidance, encouragement and helpful discussion throughout this thesis. Sincere gratefulness is also extended to my co-advisor, Dr. Kwanruan Papong for her helpful suggestions, comments and supports for completion of my thesis.

I would like to thank Mr. Witphon Thosaikham for his helpful, excellent guidance, encouragement and sincerity in this thesis. Thanks for good support from with Miss Sarunya Maneethong, Miss Rossukon Sittipout and all of my friends for their helpful, encouragement, sincerity and impression friendship.

Finally, I would like to acknowledge my family for their tender loves, definite care, encouragement and constant support throughout my study. The usefulness of this thesis, I dedicate to my parents and all the teachers who have taught me since my childhood.

Sopha Keo inpaeng

**ชื่อเรื่อง** การติดตามปริมาณโลหะหนักในไลเคนกลุ่มครัสโตส และ โฟลิโอส
**ผู้วิจัย** นายโสภา แก้วอีนแปง
**ปริญญา** วิทยาศาสตรมหาบัณฑิต **สาขาวิชา** เคมี
**กรรมการควบคุม** ผู้ช่วยศาสตราจารย์ ดร.ปิยะเนตร จันทร์ถิระติกุล
อาจาร ดร.ขวัญเรือน พาป้อง
**มหาวิทยาลัย** มหาวิทยาลัยมหาสารคาม **ปีที่พิมพ์** 2555

## บทคัดย่อ

ในงานวิจัยนี้ได้ศึกษาปริมาณโลหะหนักในไลเคนเพื่อใช้ประเมินมลภาวะทางอากาศจากแหล่งที่มีสภาวะที่แตกต่างกัน 2 แหล่งคือ เขตเมืองและเขตชนบท โดยไลเคนจากจังหวัดมหาสารคามเป็นตัวแทนของเขตเมือง และไลเคนจากจังหวัดมุกดาหารเป็นตัวแทนของ เขตชนบท โดยใช้ไลเคน 2 กลุ่ม คือ ครัสโคส และโฟลิโอส ซึ่งมี 5 ชนิด ได้แก่ *D. picta, P. tinctorum. P. coccifera, L. argentata* และ *L. benguelensis* ที่เก็บจากภูผากู ด อำเภอหนองสูง จังหวัดมุกดาหาร ตั้งแต่เดือนมิถุนายนถึง พฤศจิกายน พ.ศ. 2554 และ มีสปีชีส์เดียว (*D. picta*) ที่พบและเก็บจาก 6 จุดรอบตัวเมืองจังหวัดมหาสารคาม โดยเก็บ ตัวอย่าง ตั้งแต่เดือนพฤศจิกายน พ .ศ. 2554 ถึงกรกฎาคม พ .ศ. 2555 ศึกษาสภาวะ การทดสอบ ที่เหมาะสมของการย่อยตัวอย่าง ด้วยอ่างน้ำร้อน พบว่าอัตราส่วนของตัวอย่างต่อปริมาตรของกรดไนตริก อุณหภูมิในการย่อย และเวลาในการย่อยคือ 0.1 กรัม : 2 มิลลิลิตร, 100 องศาเซลเซียส และ 40 นาทีตามลำดับ แล้วนำสารละลายจากการย่อยมาตรวจหาปริมาณโลหะหนัก ซึ่งเหล็ก แมงกานีส และสังกะสี วิเคราะห์โดยใช้เทคนิค เฟลมอะตอมมิกแอบซอร์พชันสเป กโทรเมทรี ส่วนทองแดง โครเมียม และนิกเกิล วิเคราะห์โดยใช้เทคนิค อินดักทีฟ คัปเปิล พลาสมา-แมสสเปกโทรมิเตอร์ พบว่าโลหะหนักในไลเคนจากจังหวัดมหาสารคามและมุกดาหารมี ความเข้มข้น ในฤดูแล้งสูงกว่าฤดูฝน โดยไลเคนกลุ่มโฟลิโอส มีการปนเปื้อนโลหะหนักมากกว่าไลเคนกลุ่มครัสโคส เมื่อเปรียบเทียบ ความเข้มข้นของโลหะหนักใน *D. picta* จากทั้งสองจังหวัดพบว่า ปริมาณโลหะหนักใน *D. picta* จากจังหวัดมหาสารคามมีการสะสมมากกว่าจากจังหวัดมุกดาหาร จากการศึกษาการเสื่อมสลายของคลอ โรฟิลล์ในไลเคนจากทั้งสองจังหวัดโดยใช้สารสกัด DMSO ตรวจวัดด้วย เทคนิคอัลตราไวโอเล็ต สเปกโทรโฟโตเมตรี ที่ความยาวคลื่น 415 443 และ 665 นาโนเมตร ซึ่งจาก ผลการทดลองพบว่า ไลเคนที่พบ ในจังหวัดมหาสารคาม มีการเสื่อมสลายของคลอโรฟิลล์มากกว่าไลเคนที่อยู่ในเขตมุกดาหาร จากผลการวิเคราะห์หาปริมาณโลหะหนักและการเสื่อมสลายของคลอโรฟิลล์ที่สะสมอยู่ในไลเคนครั้งนี้ชี้ให้เห็น นว่าบรรยากาศในเขตตัวเมืองมีมลภาวะมากกว่าเขตชนบท

**คำสำคัญ:** โลหะหนัก; ไลเคน; ครัสโคส; โฟลิโอส; คลอโรฟิลล์

**TITLE** Monitoring of Heavy Metal Contents in Crustose and Foliose Lichens
**CADIDATE** Mr. Sopha Keo inpaeng
**DEGREE** Master of Science degree in Chemistry
**ADVISORS** Asst. Prof. Piyanete Chantiratikul, Ph.D.
Khwanruan Papong, Ph.D.
**UNIVERSITY** Mahasarakham University **YEAR** 2012

# ABSTRACT

Monitoring of heavy metals content in lichens was studied to assess the atmospheric pollution from two different sites from urban and rural sites, in this research. Lichen from Mahasarakham and Mukdahan Provinces were studied as representative of urban and rural sites. Two groups of lichen, crustose and foliose such as *D. picta, P. tinctorum. P. coccifera, L. argentata and L. benguelensis* were used as bio-indicators and collected at Phouphakud, Nongsoung District, Mukdahan Province from June to November, 2011. One lichen species (*D. picta*) was found and collected at six sites surrounding Mahasarakham downtown which was collected from November, 2011 to July 2012. The experimental conditions for sample digestion using water bath was optimized. It was found that optimum ratio of sample to volume of nitric acid, digestion temperature and digestion time were 0.1 g : 2 ml, 100 °C and 40 minute, respectively. The heavy metals in digestion solution were determined. Iron, manganese and zinc were analyzed using AAS. While, copper, chromium and nickel were analyzed using ICP-MS. The result revealed that heavy metals content found in lichens from Mahasarakham and Mukdahan Provinces were high concentration in dry season than rainy season. Whereas, the concentration of heavy metals from foliose were higher than crustose. The concentrations of heavy metals in *D. picta* from both Provinces were compared. The results showed that heavy metals content in *D. picta* from Mahasarakham Province was more accumulated than heavy metals content in *D. picta* from Mukdahan Province. Chlorophyll degradation in lichen from both Provinces were studied. The chlorophyll in DMSO extracts were detected using UV-Visible spectrophotometry at 415, 443 and 665 nm. The result showed that the chlorophyll in

lichen from Mahasarakham was found more degraded than the chlorophyll in lichen from Mukdahan Provinces. The analysis of heavy metal and chlorophyll degradation contents in lichens from present study indicated that the atmospheric in urban site was more polluted than rural sites.

**Key Words:** Heavy metal; Lichen; Crustose; Folose; Chlorophyll

# CONTENTS

**PAGE**

# LIST OF TABLES

**PAGE**



# LISTS OF FIGURES

**PAGE**

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic absorption spectrometry |
| cm | Centimeter |
| °C | Degree Celsius |
| g | Gram |
| ICP-MS | Inductively coupled plasma-mass spectrometry |
| l $min^{-1}$ | Liter per minute |
| mA | Milliampare |
| µl | Microliter |
| mg | Milligram |
| mg $l^{-1}$ | Milligram per liter |
| mg $kg^{-1}$ | Milligram per kilogram |
| min | Minute |
| ml | Milliliter |
| mm | Millimeter |
| ppm | Part per million |
| ppb | Part per billion |
| psi | Pounds per square inch |
| S/A | Ratio amount of sample per volume of nitric acid |
| RSD | Relative standard deviation |
| SD | Standard deviation |
| Volt | Voltage |

# CHARTER 1

# INTRODUCTION

## 1.1 Background

Currently, the earth suffers from global warming and climate change, which are caused by anthropogenic factors such as construction, agriculture and transportation. These activities lead to air pollution and environmental change (Mitchell and Gu, 2010). All living organisms, plants and animals on the earth are faced with worse them lives. Some species of organism are very sensitive to air pollution, such as lichen. Around 6% of the earth's land is covered by lichen dominated vegetation (Gadd, 2010).

Lichen are symbiotic organisms consisting of two components: a fungal partner or mycobiont and algal partner or photobiont (Nash, 1996). The algae is a producer and the fungi is a consumer (Tehler, 1996). Lichens can grow in areas where it is moist with warm air or other appropriate conditions. Consequently, lichens are a parasite attack on the plants, rocks, walls and soil. There are traditionally divided to three groups: the foliose lichens are leaf-like, fruticose lichens are bush or shrub like and crustose lichens are tight substrate (Büdel and Scheidegger, 1996).

Nutrients are very important for the metabolism of lichens, existing as positively charged ions (cation) or negatively charged ions (anion). Negatively charged anionic binding sites, such as carboxylic and hydroxycarboxylic acid, are in structural polysaccharides form cell walls, and positively charged cationic binding sites are assumed to be responsible for the cell wall exchange capacity in lichens (Nash, 1996). Lichen can retain and distribute nutrient and trace elements to the environment. Furthermore, lichen can accumulate metals, such as lead, copper, iron and other elements in the environment (Gadd, 2006).

Sensitivity of lichens to pollution is related to their biology and nitrogen oxides, sulphur oxides, pesticides and heavy metals (Bajpai *et al*, 2011), alteration of symbiotic balance between the photobiont and mycrobiont may readily breakdown the association. Therefore, different kinds of lichen are exposed to different toxic nutrients depending on the inhibitor of lichen metabolism processes (Gries, 1996).

There are several metal-polluted sources, including rocks, soil, water and airborne derived from anthropogenic activities, some lichen associated with heavy metals rich substrata are commonly able to tolerate metals, other lichen species are restricted to heavy metals-rich substrata (Bačkor *et al*., 2010). The heavy metals that the lichen are tolerant to depends on the characteristic lichen species and type of heavy metals as well as the concentrations of the metals in their environment (Mendil *et al*., 2009).

Two localities were collected lichens at Mukdahan and Mahasarakham provinces. Samples were classified according the areas and lichen species. Atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) were used to determine heavy metals in lichen from this study. The chlorophyll in individual lichens was tested for environmental evaluation and comparison between rural and urban areas. Sample preparation in this study could be high enriched analytes (% yield) with very high efficiency.

## 1.2 Purposes of Research

This research aimed to study the follows:

1.2.1 To identify lichen species obtained using the proposed method.

1.2.2 To optimize sample digestion conditions such as amount of acids (65% nitric acid), digestion temperatures and digestion times.

1.2.3 To determine heavy metals (copper, chromium, iron, manganese, nickel and zinc) contents in lichens using the AAS and ICP-MS techniques.

1.2.4 To test chlorophyll in lichens using UV-Visible spectrometry.

1.2.5 To compare amounts of heavy metals from individual lichen species, places and months by using one-way ANOVA.

## 1.3 Hypothesis of Research

1.3.1 The proposed digestion method should be effected for heavy metals determination and have high digestion efficiency, good percentage recovery (% R) and low percent relative standard division (% RSD).

1.3.2 Different lichen species, sites and months should absorb different amounts of heavy metals.

1.3.3 Different lichen species, sites and months should have different amount of chlorophyll degradation.

1.3.4 The urban sites should be contaminated with more pollutants than the rural site.

## 1.4 Benefits of Research

1.4.1 High enrichment of metals from sample, high digestion efficiency, low chemical consumption and cost analysis could be obtained from the proposed method.

1.4.2 The amounts of heavy metals in individual lichen species, sites and months could be known.

1.4.3 Accumulation heavy metals in lichens from Mukdahan and Mahasarakham province could be known.

1.4.4 Chlorophyll degradation in individual lichens could be known.

1.4.5 The results of this research could be used to evaluate environmental conditions as well as given an assessment of air pollution and be used as a reference for further study.

## 1.5 Scope of Research

1.5.1 Identification of obtained lichen species.

1.5.2 Lichens from Mukdahan and Mahasarakham provinces were selected and used to determine heavy metals by the AAS and ICP-MS techniques and testing chlorophyll by UV-Visible spectrometry.

1.5.3 Optimization of conditions of digestion method such as amount of nitric acid, temperature and time for heavy metals contamination in lichens were studied.

1.5.4 A suitable digestion method was applied to the determination of heavy metals in lichens.

1.5.5 Heavy metals and chlorophyll contents in individual lichen species from, different sites and different months were compared.

# CHARTER 2

# LITERATURE REVIEW

## 2.1 Histories of lichens

Lichens were recognized in previous centuries by many lichenologists, they endeavored to describe and classify lichen species. At the beginning of the 20$^{th}$ century, Zahlbruckner studied the classification of lichenized fungi and published his results, unfortunately his system was similar to Vainio's. Nevertheless, his study had a very good impacted on the classification of lichenized fungi. *Euascomycetous* fungi were divided at the systematic level into two separations about equal systems, one of the system of lichen fungi had the 13,500 species of the *Euascomycetous*. Therefore, nearly half of the approximate 28,000 *Euascomycetes* species are lichenized (Tehler, 1996).

The lichens based on their sysbiotic life form and composite thalli. The name of lichens was set by convenience, but there were some inaccurately named that need to be reclassified (Galun, 1988). The components of lichens are organisms, consequently they, can not be distinguished into natural systems as they have phylogeny. At the present time, lichen fungi are classified together with other *chitinous* fungi and incorporated into common fungal system (Tehler and Wedin, 2008).

## 2.2 Symbiosis

Symbiosis is composed of two words derived from Greek "*sym*": together and "*biosis*": living, which is described as two or more dissimilar organism living together in the same situation to extend their period of life. It is generally consisted to be between a fungal partner (mycobiont) and photosynthetic partner (photobiont); sometimes, lichens are symbioses involving three or more partners (Nash, 1996).

### 2.2.1 Algae (photobiont)

There are nearly forty genera of algae and cyanobacteria that have been reported as photobionts in lichen; however, three genera *Trebouxia*, *Trentepohlia* and *Nostoc*, are the most frequent photobionts (Figure 2.1). The genera of *Trentepohlia* and *Trebouxia* are eukaryotic and belong to the green algae, the genus *Nostoc* belongs to the oxygenic photosynthetic bacteria (cyanobacteria). Eukaryotic photobionts are referred to as "phycobiont", the cyanobacterial photobionts are sometimes called "cyanobionts", as shown in Table 2.1 (Nash, 2008). The functionally eukaryotic photobionts belong to the green algae (phylum Chlorophyta) which have many similar cytological features and pigmentation. There are two genera of eukaryotic photobiont containing chlorophyll A and C that have been reported as *Heterococcus*, *Xanthophyceae*, *Petroderma* and *Phaephyceae* (Friedl and Büdel, 2008).

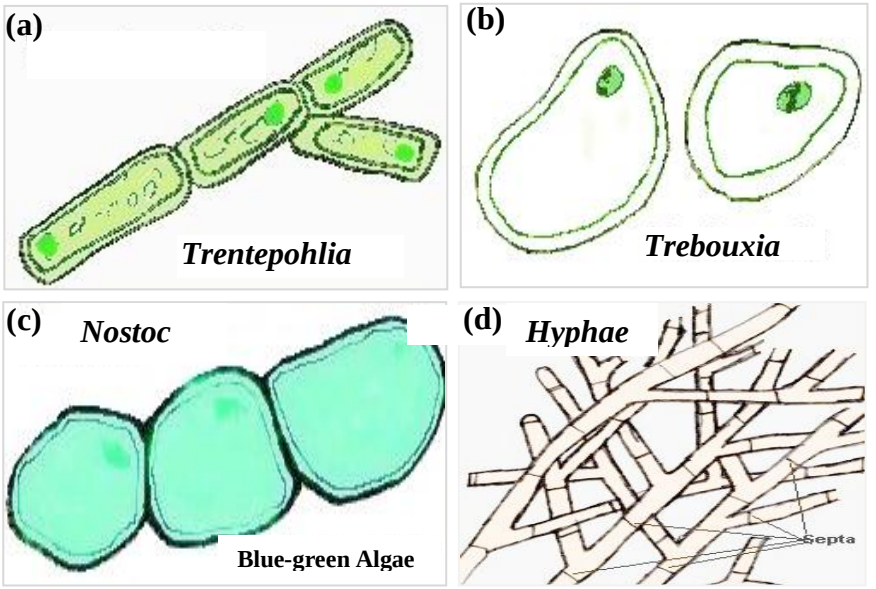


**Figure 2.1** The forms of photobiont; (a) *Trentepohlia*, (b) *Trebouxia*, (c) *Nostoc* and (d) *Hyphae* (Source: Ramel, 2000).

**Table 2.1** Genera of cyanobacteria identified from lichen arranged according to taxonomix characters (Source: Nash, 2008).

| Taxonomical characters | "Botanical" System | "Bacteriological" System |
|---|---|---|
| 1. Unicellular | | |
| a. Binary division only<br>*Gloeocapsa, Chroococcus*<br>*Cyanosarcina, Entophysalis* | Chroococcales[a] | Chroococcales |
| b. Binary division + multiple fission<br>*Nanocytes immotil*<br>*Chroococcidiopsis*<br>*Nanocytes motile*<br>*Myxosarcina, Hyella* | Chroococcales[a] | Pleurocapsales |
| 2. Filamentous with heterocyste | Nostocales | |
| a. Nonbranched<br>*Nostoc* | | |
| b. False branching, no tapering<br>Trichomes *Nostocale, Scytonema* | Nostocales | |
| c. False branching, tapering trichomes<br>*Calothrix, Dichothrix* | Nostocales | |
| d. True branching<br>*Stigonema, Hyphomorpha* | Stigonematales | |

[a] The order chroococcales is subdivided into seven families in the ''botanical'' system.

### 2.2.2 Cyanobacteria

Cyanobacterias are prokaryotic in nature and they do not have chloroplasts, mitochondria or a nucleus. In the cyanobacteria, thylakoids live free in the cytoplasm, often more than restricted to the periphery. The circular DNA is not associated with histones and is concentrated in the areas of the cytoplasm that are free of thylakoids, which is sometimes called the nucleoplasm (Friedl and Büdel, 2008). The filamentous form cannot be recognize within the lichen thallus in the genus *Dichothrix* (Figure 2.2 a). The branch of the filamentous cyanobacterial genus *Stigonema* (Figure 2.2 b) and non branch genus *Nostoc* (Figure 2.2 c) can often be identified within the lichen thallus. Moreover, cyanobacteria do not show all stages of their life cycles when lichenized

(Honegger, 2000). Isolation and cultivation of the cyanobacterial photobiont are necessary steps for positive identification. Cyanobacteria with heterocysts like *Nostoc* (Figure 2.2 c) increase their heterocysts five times when lichenized, compared with the free-living genera *Gloeocapsa* (Figure 2.2 d). An increase of cell size can be a result of very close mycobiont / photobiont contact, as in the deep penetrating haustoria.

This can be seen clearly in the vegetative trichome cells of *Scytonema* within the lichen *Dictyonema* sericeum (Figure 2.2 e). The uni-cellular cyanobacterial photobiont genera *Chroococcidiopsis* and *Myxosarcina*, very rarely show their specific mode of reproduction when lichenized, but frequently show these stages when cultured (Figure 2.2 f) (Friedl and Büdel, 2008).

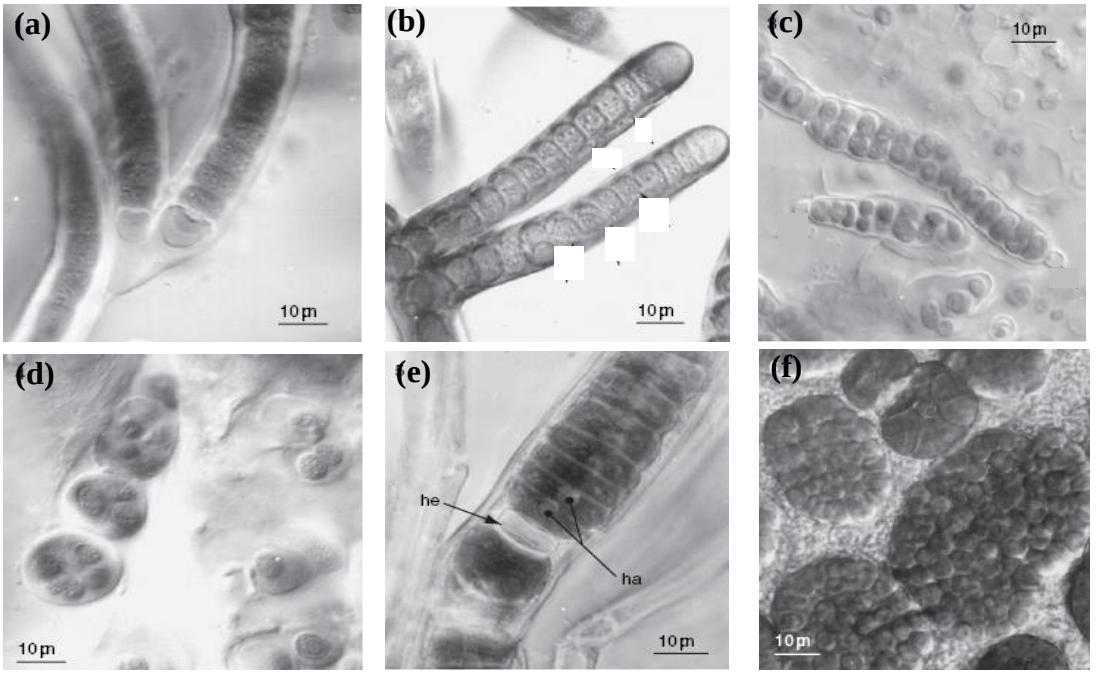


**Figure 2.2** Light microscopy of cyanobacterial photobionts; (a) *Dichothrix*, (b) *Stigonema ocellatum*, (c) *Nostoc*, (d) *Gloeocapsa sanguinea*,(e) *Scytonema* and (f) *Myxosarcinas* (Source: Nash, 2008).

### 2.2.3 Green-algae

The green-algae photobiont is known as *coccoid* (Figure 2.3 a-g). There are Sarciniod or filamentous forms (Figure 2.3 i-n), and in terms of green algae, filamentous (Figure 2.3 m) forms are often reduced to short filaments becoming unicells (Figure 2.3 f) within thalli (Lumbsch and Leavitt, 2011). Some algae are unicellular and others multi cellular, sometimes being make up of a million cells. The distribution of

algae in nature is vast with most groups growing in aquatic habitats. Algae can be categorized by where they grow; some algae grow on plants, soil or animal, whereas epiphytic algae grow on fungi (Friedl and Büdel, 2008).

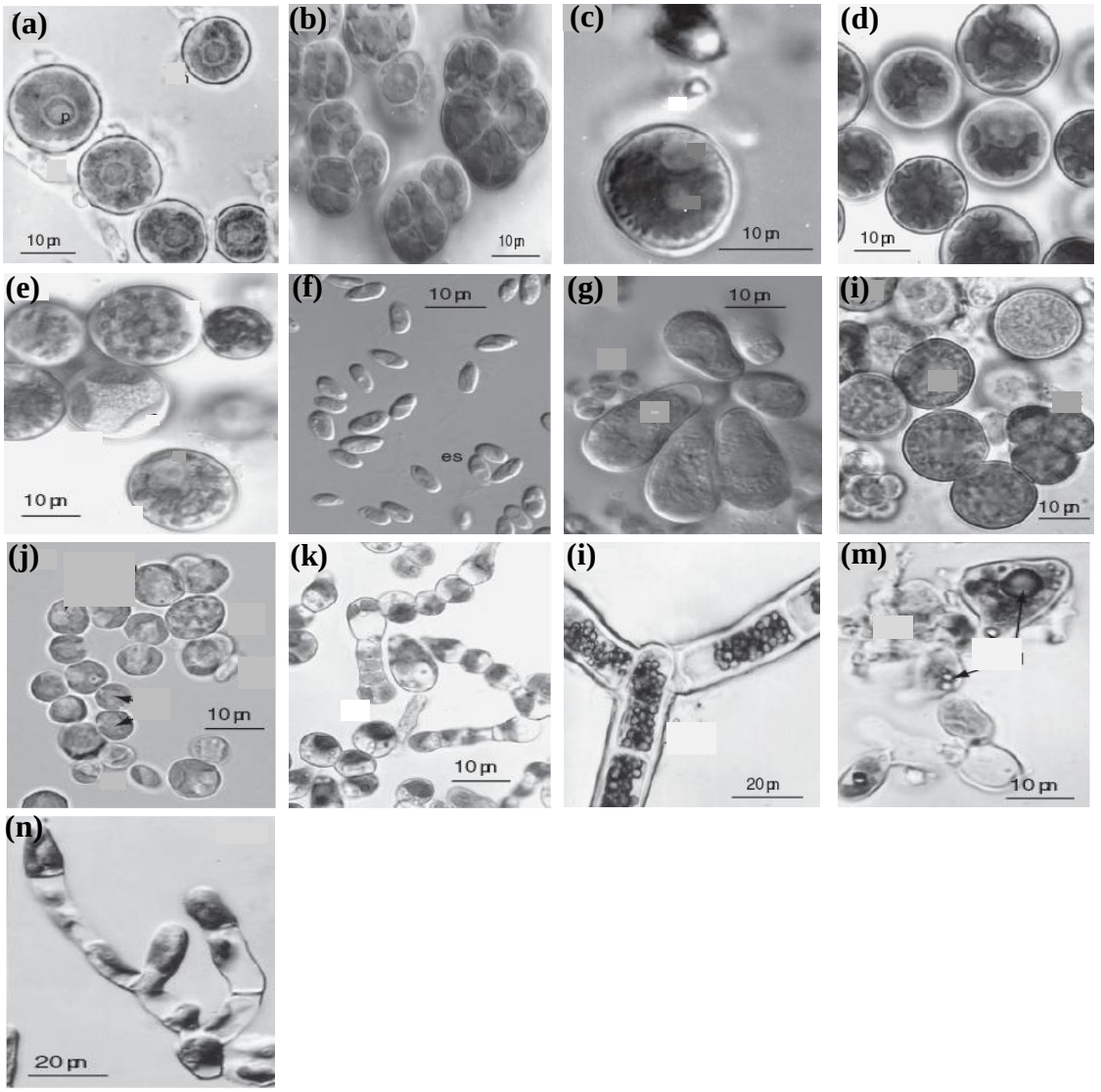


**Figure 2.3** *Trebouxia* and other *coccoid* species, lichenized and in culture; (a) *Trebouxia gigantea*, (b) *Trebouxia sp*, (c) *T. arboricola*, (d) *Gelatinosa*, (e) *Asterochloris* (f) *Coccomyxa subellipsoidea*, (g) M*yrmecia biatorellae*, (i) *Dictyochloropsis*, (j) *Elliptochloris* bilobata, (k) *Leptosira obovata*, (l) *Trentepohlia*, (m) *Trentepohlia sp* and (n) *Dilabifilium arthropyreniae* (Source: Nash, 2008).

## 2.3 Lichen classification

The main characteristics of lichens can be arranged in to three orders, *Ascolichenes, Basidiolichenes* and *Gasterolichenes* (Crespo et al., 2010). *Ascolichenes* are spore-sacs formed by a process of free cell formation, *Basidiolichenes* are formed exogenously by the abstriction of the end of specialized hyphse and *Gasterolichenes* are formed exogenously on basidia with in specialized cavities (Nash, 2008). According to the basic nature of lichens, they are divided into two groups:

**Group 1: Ascolichens**

Lichen in this group are composed with fungal as an ascomycete, they are subdivided into two subgroups:

a). *Gymnocarpae-Ascocarp (Apothecium type)*

b). *Pyrenocarpae-Ascocarp (Perithecium type)*

**Group 2: Basidiolichens**

This group is composed of the fungal partner *Basidiomycete*, division of this group refer to habitat that can be used to divided them into three groups:

a). *Saxicolous*-predominantly grows on stone or rock

b). *Corticolous*-generally grows on the bark of trees

c). *Terricolous*-terrestrial in the nature

The following terms are also sometimes adopted by lichenologists, lichens can be distinguish into the groups that are homoeomerous or heteromerous, according to the nature of algae, lichen can be divided into three groups as crustaceous, foliose and fruticose (Mukerji et al., 1999).

### 2.3.1 Crustose or Crustaceous

Crustose lichens are tightly attached lower substrate surface such as bark and rock as shown in Figure 2.4 (Büdel and Scheidegger, 1996). Water loss is restrict primarily to the upper and exposed surface. The subtype of the crustose as powdery, endolithic, endophloeodic, squamulose, peltate, pulvinate, lobate, effigurate and suffruticose crusts (Printzen *et al*., 2008). Powdery crustoses are found in lichen genera *Lepraria* which was simplest and lack organism in the thallus. *Endolithic* and *endophloeodic* lichen are organism, the upper cortex consist of dense hyphae forming a layer named “lithocortex” *Acrocordia conoidea*, *Petractis clausa*, *Rinodina immersa*,

*Verrucaria baldensis* and *Verrucariamarmorea*; other endolithic lichens like *Verrucaria rubrocincta* form a micrite layer with only a few hyphae. The effigurate is a kind of thallus where the margins are prolong as in many genera *Caloplaca*, *Dimelaena*, *Acarospora* and *Pleopsidium* (Lumbsch and Leavitt, 2011). The squamulose type of crustose lichens often develop overlapping genera *Catapyrenium*, *Peltula*, *Psora* and *Toninia*. The peltate type is developed colonize soils or rock surface. A lobate thallus is developed by some species within the genera *Caloplaca* and *Lecanora*. Most crusrtose lichen are drough-resistance and well adapt dry climate (Büdel and Scheidegger, 2008)

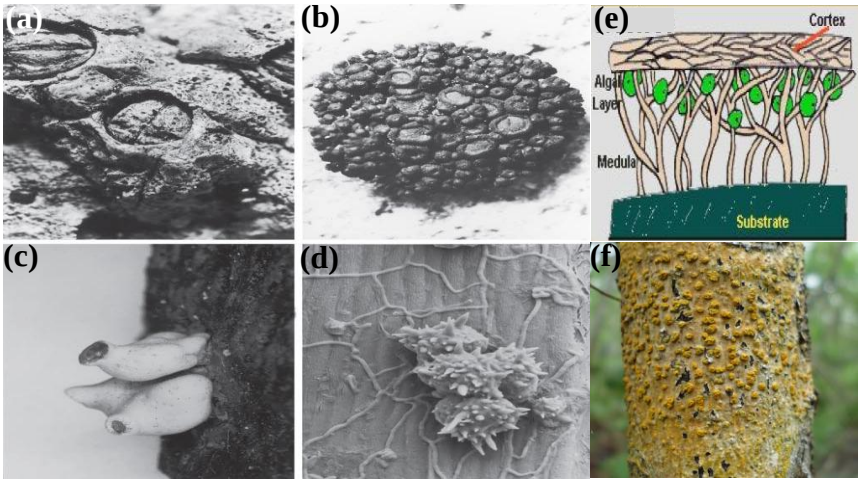


**Figure 2.4** The forms of crustose lichens; (a) *Arthopyrenia halodytes*, (b) *Anemaum mularnium*, (c) *Mobergia calculiformis*, (d) *Vezdaea rheocarpa*, (e) Intracellular and (f) *Laurera benguelensis* (Source: Büdel and Scheidegger, 1996; Ramel, 2000).

### 2.3.2 Foliose or foliaceous

Foliose lichens are leaf-like, flat attached to the substrate surface, either homoiomerous (*gelatinous* lichens) or heteromerous, the foliose thalli develop showing a great deal of size and diversity (Bechtel *et al*., 2002). *Laciniate* lichens are typical foliose lichens that vary considerably size; they maybe either gelatinous-homoiomerous (*Collema, Leptogium, Physmam* in Figure 2.5 d) or in most cases heteromerous (Plata and Lumbsch, 2011). The lobe in the lichen can be arranged (Parmelia species Figure 2.5. a, b and f) or overlapping like tile, in some genera, thallus lobe can become inflated have a hollow medullar center (*Menegazzia* in Figure 2.5. c) (Büdel and Scheidegger, 2008). *Umbilicate* lichens have circular thalli consisting either one single un branched

lobe or multi lobate thalli with limited branching patterns. *Umbilici* have apparently evolved several times such unrelated group as the Dermatocarpaceae, Parmeliaceae, Physciaceae and Umbili-cariaceae. Foliose (*Foliaceous, Fron-dose*) type contain expanding different thalli having an upper outside cover layer, this description includes warty isidiod and squamose thalli (Zotz and Schleicher, 2003). The foliose thallus is variable which is a small lens for detection in *Dermatocarpon* thalli reaching maximum development in *Gyrofhora* and *Umbilicaria*. Foliose thalli are typically of *Dorsiventral*, the upper portion turns toward the sunlight are primarily adapted to perform the function assimilation. In contrast, the lower portion is adapted to absorb food nutrients (Albert, 1897).

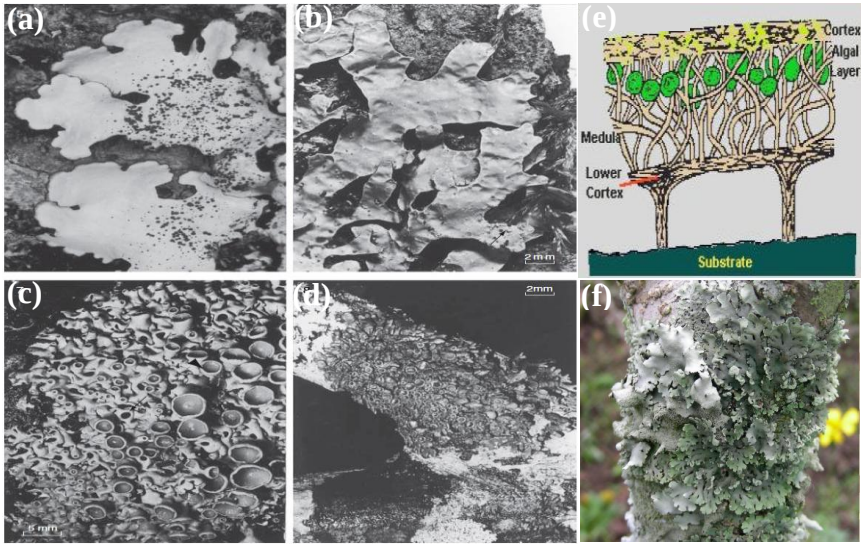


**Figure 2.5** The forms of foliose lichens; (a) *Parmelia pastillifera*, (b) *Parmelia sulcata*, (c) *Menegazzia pertransita*, (d) *Physma byrsaem*, (e) Intracellular and (f) *Parmotrema tinctorum* (Source: Büdel and Scheidegger, 1996; Ramel, 2000).

### 2.3.3 Fruticose or filamentous

The fruticose lichens are hair-like, strap-shaped or shrubby and the lobes, in some, are flat or cyclindrical. Some group have dorsiventrally arranged thalli (*Sphaerophorus melanocarpus, Evernia prunastri*), but the majority possess radial symmetric thalli (Sphaerophorus globosus, Usnea species Ramalina species; Figure 2.6. c, d and f) (Büdel and Scheidegger, 1996). The branching pattern of lobe varied among distinct groups and a single genus, the fruticose lichens are found in a wide climate,

such as desert, humidity of wet rain forest and on various types of substances, highly branched fruticose lichens have a high substrate ratio which benefits in more rapid drying and wetting pattern compared to lichens having lower surface to volume proportion (Büdel and Scheidegger, 2008).

Inside the fruticose thalli of lichens are able to conduct substances by physical mechanism along with hyphae, this tissue is formed hollow cylinder in *Podetia* or *Cladonia* or solid substance as *Usnea*. The cells are distributed in all part of fruticose branches. Also the cell-wall of the fruticose tissue are much less gelatinized than those of the cortical cells (Papong *et al*., 2011). There are several well-marked differences between the foliose and fruticose types. The most important of the fruticoses are best adapted structural function than foliose thalli. There is little doubt that the fruticose thallus has been phylogeneticall derived from higher crutaceous forms (Albert, 1897).

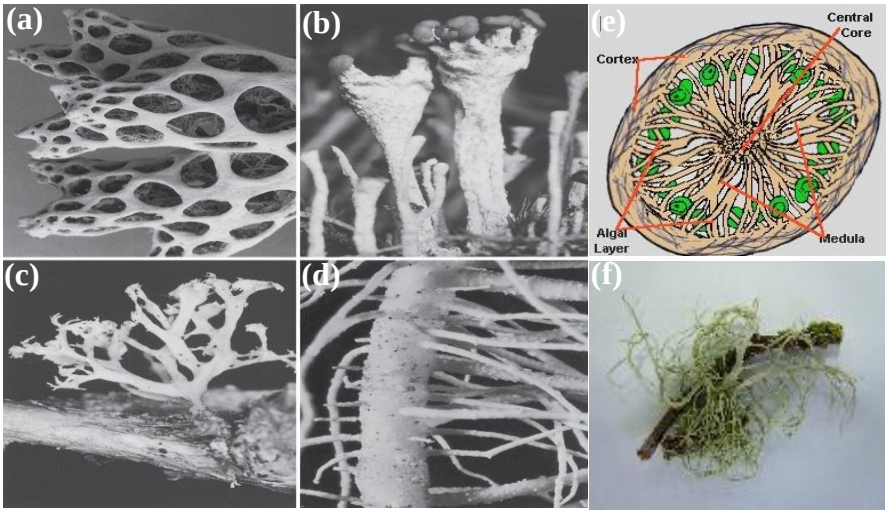


**Figure 2.6** The forms of fruticose lichens; (a) *Cladia retipora*, (b) *Cladoniacoccifera*, (c) *Ramalina pollinaria*, (d) *Usnea filipendula*, (e) Intracellular and (f) *Ramalina pacifica* (Source: Büdel and Scheidegger, 1996; Ramel, 2000).

## 2.4 Elemental accumulation and mineral

Many organism accumulate and process both micronutrient and macronutrients important for life, the physical and chemical function of main nutrient to grow and develop lichens. Lichen do not have procession roots (Řezanka and Guschina, 2000).

Nutrient came to lichen by absorption system similar to vascular plants from the atmospheric source of nutrient or from soil and airborne contamination of chemical ions (Aslan *et al*., 2004). There are several ways to receive nutrient from the source of chemical where are factories, nuclear industry and agriculture, all chemical or chemical ion spread out to the atmosphere, water, soil and food chain. In more recently years, scientists interested in using lichens to study the region deposition metals and other atmosphere contaminates (Nash, 1996).

### 2.4.1 Mineral cycling ecosystem

Mineral recycling can be divided into two phases of intra-system and intersystem cycling (Bennett and Wetmore, 2003). The intra-system cycle concerns nutrients moving within ecosystem and intersystem cycle involves the flux of nutrients between ecosystem and their interconnection with global cycles as shown in Figure 2.7 (Nash, 2008).

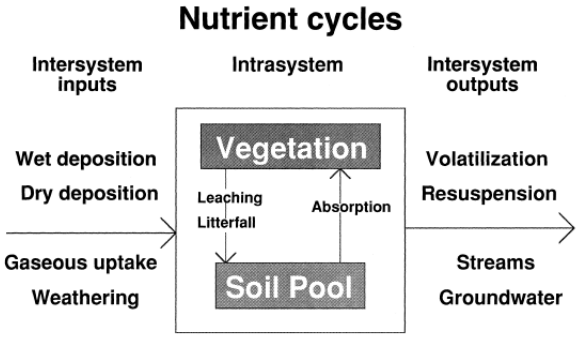


**Figure 2.7** Conceptual diagram of the intra and inter system nutrient cycles of an ecosystem (Source: Nash, 2008).

### 2.4.2 Chemical properties of nutrients and metals

Nutrients are essential for organisms and plants existing primary as ions, either positively charged cations or negatively charged anions, Negatively charged anions are formed by carboxylic and hydro carboxylic acid ions binding with structural polysaccharides in the cell wall (Andrzej *et al*., 2007). Positively charged cations binding site are assumed to be responsible for the cell walls exchange capacity in

lichens. Absorption from outside to the cytoplasm occurs as well as external solution, metabolism of the nutrient with in the cytoplasm is covered basic physiology and biochemistry (Nash, 2008).

**2.4.3 Sources of nutrients**

Atmospheric interaction of lichen ecosystem occurs in precipitation and accumulation, absorption and sedimentation; precipitation is very important to lichen both nutrients and moisture (Simonetti *et al*., 2002). The concentration of contamination and nutrients in precipitation may be substantially higher than in rainfall because more dilution occurs in the formation rain. Sedimentation of large amount aerosol contamination in lichen thalli is demonstrated by comparisons chemical properties. Atmospheric aerosols directly from the air and nutrients particle attack the cortex of lichens (Nash, 1996).

Many lichens occur on tree, rock or soil. Therefore, they are contact with the source of nutrient, it can affect weathering of rock and bark surfaces by both mechanical and chemical. The higher pH and greater availability in calcium of limestone is different compared to acid rocks. The solubility of nutrient is effected by pH and availability of nutrients may cause differences between acidic and limestone rock. That different lichens communities occur on limestone versus acidic rocks is not surprise (Toppia *et al*., 2007). Therefore, on gypsum, surface a few lichens occur at high concentration such as *Acarospora, clauzadena* (Sensen and Richardson, 2001). Most lichens which is occur on rocks and soil are affected by blown dust, This soil can be rapidly incorporated intracellular within lichens and results in relatively high concentration of aluminium, iron, scandium, titanium and other element of lithic origin with the thallus.

Solubility of these particles is potential of nutrients but this process is very low and most elements is very low remain unavailable (Boamponsem *et al*., 2010). It is essential separate between total elemental concentration as ions and solubilized concentrations. The contribution of soil particles to total element loading of lichens can be assessed by comparing ratios of micro or macronutrients to inert elements as scandium or titanium; element such as potassium readily leach from foliage subsequently taken up by epiphyte (Nash, 2008).

### 2.4.4 Accumulation metabolism

Lichen lacked vascular system and root. Consequently, most lichens do not have upper cortex layer of interwoven fungal hyphae. Elemental exchange in the lichen happens across the entire lichen surface (Woess *et al*., 2005). First of all, cation uptake is a rapid physicochemical process occurring extracellular in lichens. Saturated levels are reached in a matter of time as, capillaries retains cations with the cell wall. For example, the ionic uranyl complex ($UO_2L_2^{2-}$) accumulated much more slowly than uranyl cations (Nash, 1996). The study of metallic deposition in lichens was done with several source of metallic smelted far way from industrials area; mostly metals deposition in lichens were used to evaluated atmospheric quality (Carreras and Pignata, 2001). When lichens deposited toxic metals, they grow and maintains uniform morphology with time. Consequently, the vascular plant do not shed. The lack of waxy and associated stomata, which occur on vascular plant leaves are related to many contaminants absorbed over the entire lichen surface (Gonzalo, *et al*., 2012).

#### 2.4.4.1 Ion exchange

The primary of metallic ions are cations, their uptake is rapid, passive and physicochemical process occur extracellular (occurring outside the cell) in lichen. Saturate levels and capacity to retain cations in the lichen occurs in the cell wall are evaluated vary between 6 to 77 µmol $g^{-1}$, these cations can be retained in the external photobiont or mycrobiont's plasma at cation exchange sites (Schmull and Hauck, 2002). Anion are absorbed by lichen but this had only been investigated to a limited degree. Anion absorption is a slower process than cation exchange and produces a lower total accumulation. For example, anionic uranyl complex ($UO_2L_2^{2-}$) accumulated less than uranyl cation, the total uptake of anions were (1.6 µmol $g^{-1}$ and 49 µmol $g^{-1}$) less than cations. In contrast, the uptake of arsenic anion form $H_2AsO_4^-$ was greater than 10 µmol $g^{-1}$ (Nash, 1996).

#### 2.4.4.2 Intracellular uptake

In contrast to ion exchange uptake, intracellular uptake concerned with a flux than external anion exchange process. For example, intracellular absorbed cadmium ion less than 10% of total uptake according to Brown and Beckett investigation in 1985 (Nash, 1996). It takes several hours because intracellular uptake

showed saturation kinetic with increasing external concentration of cadmium (Mendil *et al*., 2009).

**2.4.5 Accumulation of smelted lichen**

Smelting is source of high metal concentration to organism or lichen (Armstrong, 1996). In most case, concentrations are found near the pollutants source, the concentration will decrease or increase depending on the distance from the source (Pacheco *et al*., 2007). Another ways of assessing possible effect of earth crustal (Equation 2.1) element contribution loading within atmosphere (Andrzej *et al*., 2007). One solution is to use procedure for calculating enrichment factors (EF) for any element (X) in the lichen relative crustal rock or soil following this formula:

$$EF = \frac{(X/\text{reference element}) \text{ in lichen}}{(X/\text{reference element}) \text{ in crustal rock}} \qquad (2.1)$$

Scandium and titanium are used as indicator of crust material because they have no biological function and aluminium may use the same function (Nash, 1996). Puckett and Finegan used scandium in their investigation in Canada; they found that the elements aluminium, chromium, cobalt, iron, sodium, titanium and vanadium had means of EF proportion less than 5. In contrast, EF value of other elements were higher for chloride in *Thamnolia subuliformis*. EF value of general elements are 100 for 14 common lichen species including antimony, chloride, lead and sulfur (Grangeon *et al*., 2012).

**2.4.6 Deposition of inorganic ions to lichens**

The source of metals is not limited deposition but also include anion deposition from acid rain and organic association from agriculture, industrial activities. For example, both Takala (1985) in Finland and Bruteig (1993) in Norway reported sulfate deposition gradient from the south to the north of their countries (Nash, 2008). While southern regions of USA, nitrogen pollutants from vehicular traffic in Los Angeles was reported by Boonpragob (1989) which deposited to levels lichens over 170 mmol $g^{-1}$ ten weeks during the summer caused by nitric acid (Riddella *et al*., 2007).

### 2.4.7 Deposition organic to lichens

Most organic pollutants do not appear or occur in nature (Nash, 2008). From the South to the North of Canada, the gradient of several chemicals as chlorinated hydrocarbons, polychloronate biphenyls (PCBs) and chlorocyclohexane (HCHs) isomers was established by Muri in 1993 (Edwards et al., 2003). Similarly case of Carlberg of Norway it was found several gradient of chlorinated hydrocarbons, phthalates and polyaromatic hydrocarbons (PAHs). Polychlorinated biphenyls (PCBs) have been investigated by Garty *et al*., 1982 in Israel (Nedić and Stankovi, 1999). Chlorinate hydrocarbon are widely used as pesticide and it has entered the earth's biologeochemical pathway with accumulation in lichens such as in Antarctica Organic matter decomposition is most important microbial activity in the biosphere; this activity ranges from simple compound such as sugars, organic acid and amino acid to more complex molecule that need extracellular enzyme to break down their structure (Gadd, 2006; 2010).

### 2.4.8 Metals toxicity

Toxic metals mostly occur near mines, factories or agriculture, all of these activities are source of toxicity; they have leaked toxic metals to environment areas. The toxic effect depends on amount of metals contamination and limits of tolerant lichens (Sanchez, 2008).

There are several toxic elements such as zinc, cadmium, mercury (Nash, 2008). Accumulation toxic metals in plants or lichen depends upon several factors such as the availability of elements, characteristics of lichen or plants as species, age, state of health, type of reproducibility and temperature, moisture. These factors direct and indirect effect to plants and lichens (Conti and Cecchetti, 2000).

#### 2.4.8.1 Chromium (Cr)

Chromium is commonly used in industrial applications, such as in tanning processes, electroplating, pigmentation, textile dyeing, or as a catalyst for corrosion inhibitors and wood preservatives (Bingol *et al*., 2009). Chromium is also essential nutrient that potentiates insulin action and thus influences carbohydrate, lipid and protein metabolism (Anderson, 1988). It probably works closely with the hormone insulin to help cells take up glucose and break it down for energy (Mitchell and Gu, 2010). Most adult human tissues contain chromium levels at 0.02-0.04 mg $kg^{-1}$ on dry

basis. The total content in the body of adult man is estimated to be less than 6 mg (Underwood, 1971). Chromium concentrations in vegetable tissues range from 0.01 to 1 mg $kg^{-1}$, with levels in most plants lying between 0.1 and 0.5 mg $kg^{-1}$ (Garty,1987).

Chromium exists in several oxidation states, the most stable and common forms are the trivalent Cr (III) and the hexavalent Cr (VI) species, which display quite different chemical properties. Cr (VI), considered the most toxic form of chromium, is usually associated with oxygen as chromate ($CrO_4^{2-}$) or dichromate ($Cr_2O_7^{2-}$) ions (Uluozlu *et al*, 2008. The effect of Cr (VI) is toxic to plant and lichen. Lichens have a remarkable ability to absorb the heavy metal (Cr) from their external and on the surface of thalli to accumulate in the internal cellular. The toxic chromium is related to oxidative damage, which can be promote both by a direct increase in the cellular concentration and a decrease in the cellular antioxidant capacity which decrease lichen diversity (Unala *et al*., 2010).

**2.4.8.2 Copper (Cu)**

Copper is widely distributed in biological tissues, where it occurs largely in the form of organic complexes, many of which are metalloproteins and function as enzymes (Monnet *et al*., 2006). In humans, acute copper poisoning is rare and usually results from contamination of foodstuffs or beverages by copper containers or from the accidental ingestion of copper salts (Chaignon *et al*., 2002). Dietary sources of copper (> 2 mg $kg^{-1}$) include seafood, organ, legumes and nuts (Gregory, 1990). Recent studies based on the analysis of duplicate diets suggest that the actual intake of adults may be in the range 1-1.5 mg $day^{-1}$ (Garty and Ammann, 1987)

Copper present in industrial wastes is primarily form of bivalent Cu (II) as a hydrolysis product, $CuCO_3$ (aq) or organic complexes (Guschina and Harwood, 2006). Copper (II) ions in water may cause toxic and harmful effects to living organisms and consumers (Ekmekyapar *et al*., 2006).

Several industries, for example, dyeing, paper, petroleum, copper brass-plating and copper-ammonium rayon, release undesired amounts of Cu (II) (Kováčik et al., 2010). Cu (II) concentrations approach 100-120 mg $kg^{-1}$, this value is very high in relation to water quality standards and Cu (II) concentrations in wastewaters should be reduced to a value of 1.0-1.5 mg $kg^{-1}$ (Carreras and Pignata, 2007).

Heavy metal ions are responsible for photobiont and mycrobiont of the lichen at cell wall and hymenium, the tolerance to heavy metal of lichens depend on their species (Bačkor *et al*., 2010). Low molecular weight of metallothioneins of lichen bind heavy metals through cysteine residues and in the algae sulfhydryl of compound reverse the toxicity of Cu (II) by reducing to Cu (I) and forming a Cu (I)-S-complex (Bačkor *et al*., 2003), whereas increasing copper uptake in lichen cell can reduced potassium uptake resulting in reduced lichen species (Wang and Robert 2005).

**2.4.8.3 Iron (Fe)**

There is a total of about 4 g of iron in the body. About 60-70% of this iron is found in haemoglobin and the related myoglobin (Rowsell *et al*., 1989). Vitamin $B_{12}$, ascorbic acid and copper also play an important role in the effective utilization of iron, consequently have an influence on daily requirement (Aslan *et al*., 2004). Good dietary sources of iron are meat, which on average provides 25% of the intake (especially liver and kidney), egg yolk, bread, flour and other cereal products, potatoes and vegetables (Lodenius, 1983). Iron is found in cereals, but due to refining, 50% may be lost. Some natural drinking water contains quite high levels of iron (Garty and Ammann, 1987). If water is stored in iron tanks or passes through cast-iron pipes, concentrations of iron may be reached (Fleck, 1972).

Lichens are asses photobiont and mycrobiont. Therefore, iron bearing-metal mineral are mostly in the medulla and attached between hyphae and algae but not accumulate in the cell and polysacharides. Metal (iron) can be bound to melanin like pigment of plant or lichen, this reaction will be disturbed in physical and chemical aspects of the lichens (Takeshi *et al*., 2003)

**2.4.8.4 Manganese (Mn)**

Manganese has been shown to be an essential element in every animal species (Underwood, 1971). It is found in the liver, skin, bones, kidney, organs and muscles of animals, the human body has about 12 to 20 mg manganese in a 70 kg man (Garty and Ammann, 1987). This quantity is only one-fifth of the estimated total copper and one hundredth of the zinc (Hauck *et al*., 2002)

Whole grains and cereal products are the richest dietary sources of manganese, then fruits and vegetables with somewhat less. Dairy products, meat, fish and poultry are poor sources (Nash, 2008). Tea is a rich source of manganese, but

typical drinking water consumed at the rate of 2 l daily contributes only about 40 to 64 μg or about 2- 3% of the amount furnished by diet. A provisional daily dietary manganese intake of 2 to 5 mg is recommended for adults (Nakayama *et al*., 2001).

In plant and lichen, manganese can reduce the concentration of chlorophyll a and b and soredia in foliose lichen (Hauck *et al*., 2006). The chlorophyll content correlated with the ability of the soredia grow, when the increasing manganese concentration is conducted to reduce the folios (Hauck *et al*., 2003).

**2.4.8.5 Nickel (Ni)**

The nickel containing enzymes found in plants and microorganisms, namely urease, hydrogenase, methylcoenzyme reductase and carbon monoxide dehydrogenase (Kirchgessner *et al*., 1985). Ni (II) intake over permissible levels results in different types of disease such as acute and chronic disorder in man, pulmonary fibrosis and renal edema, and skin dermatitis such as severe damage to lungs and kidney, and gastrointestinal distress, different types of biomass different amounts (Sari *et al*., 2007).

Total dietary nickel intakes of humans vary greatly with the amounts and proportions in food of animal (low-nickel) or plant (high-nickel) consumed. Approximately half the total daily intake of nickel is usually derived from the consumption of bread, cereals and beverages (Garty and Ammann, 1987). A recent report indicate that diets often provide less than 150 μg $kg^{-1}$ of nickel daily. Available information indicates that most monogastric animals have a nickel requirement of less than 200 μg $kg^{-1}$ (Nielsen, 1988). It is reasonable to suggest a basal nickel requirement of less than 100 μg $kg^{-1}$ daily for adults (Carreras and Pignata, 2007).

Nickel is an essential “ultramicronutrient” found to be the active center of urease and cofactor of one superoxide dismutase isoform (Hebe *et al*., 2007). Its “natural” (baseline) content in plants is usually low, ranging from 0.05 to 10 mg $kg^{-1}$ plant dry weight (Medil *et al*., 2009). The toxicity of nickel to lichens has been investigated and studied to observe a negative correlation between the total nickel content in thalli of *Ramalina lacera* transplanted close to a pollution source and chlorophyll integrity (Bačkor *et al*., 2010).

**2.4.8.6 Zinc (Zn)**

Zinc is generally found in the body in the liver, muscle, bones, skin and blood (Pawlik-Skowronska and Bačkor 2011). It has very important function in the reaction of certain enzymes. The whole body of a 70 kg man is estimated to contain 1.4 to 2.3 g of zinc. Good sources of zinc include red meat, liver, kidneys, whole grain cereals, eggs, shellfish, nuts and cheese (Fleck, 1971).

Zinc absorption is better from animal sources than plant sources owing to phytic acid (Nash, 2008). An evaluation of the most reliable balance studies indicates that at least 12 mg of zinc in a mixed US diet is required to maintain the existing zinc status of a healthy young man (Shiel *et al*., 2010). The recommended allowance for men is set at 15 mg $day^{-1}$, the allowance for women, owing to of their lower body weight is set at 12 mg $day^{-1}$ (Györyoá *et al*., 1995). In term of plant and lichen accumulated level of zinc in excess of physical requirement in close correlation with atmospheric concentration levels, the amount of zinc accumulated by lichens depends on morphological and structural features well as eco-physiological properties (Godinho *et al*., 2009).

## 2.5 Environmental role of lichens

The role of lichens is appearance in the biosphere and ecosystem of nature effecting the environment, their roles are potential organisms of the micro and macro-organisms unique to develop atmosphere as well as air and, soil (Gonzfilez and Pignata,1997). The role biological weathering (Guschina *et al*., 2003). The lichen do not appearance where is atmosphere has large amount of pollutants, lichens are natural sensors of our changing environment the sensitivities of particular lichen species and assemblages are a very broad spectrum of environmental conditions (Rogers and Ryel, 2007). Therefore, lichens are used increasingly in evaluating threatened habitats in environmental perturbations, particularly those resulting from a disturbingly large and growing number of chemical pollutants (Krishna *et al*., 2003). Moreover, lichen undoubtedly represent one of the most successful forms of symbiosis in nature (Seaward, 1996).

## 2.6 Lichens as indicator of air pollution

All lichens are recognized as indicator of air pollution as they very sensitive air pollutants (Jovan *et al*., 2009). This procession occurred in 1800s in European cities such London, Munich, Stockholm and Paris when lichens disappeared in urban areas (Paoli *et al*., 2012). At the beginning of 1900s, these cities were affected pollutant from coal soot distribution (Gries, 1996). Colorless gas and sulfur dioxide became recognized as principle phytotoxic agent (Käffer *et al*., 2012). In recent years air pollutants have become much wider and include oxidant, hydrogen fluoride, some metals, acid rain and organics, certainly document list of potential toxic substances have not yet clearly described (Szczepaniak and Biziuk, 2003). The high sensitivity of lichens is related to their biology. Most organisms include perennials and animals, they are target of the accumulative effect of pollutants (Fleck,1971).

Lichens sensitive to pollutants because there is no vascular system for conducting water or nutrients unlike plants (Ayrault *et al*., 2010). Therefore, they have developed efficient mechanisms water and nutrients from atmospheric sources (Loppi *et al*., 2001). Fog and dew are major water source for lichens, often have much high pollutant concentrations than precipitation (Demiraya and Yolcu, 2012).

Unlike many vascular plants. Lichens do not have deciduous parts. Hence, they cannot avoid pollutants by shedding parts. Consequently, aerosols may be absorbed over the entire thallus surface (Saipunkaew *et al*., 2005). Thus, lichens have little biological control over gas exchange and air pollutants can diffuse down to the photobiont layer. Meanwhile, dehydration of lichen occur (Zhanga *et al*., 2002). This has led to high concentration solution that reach a toxic point, finally alteration of the symbiotic equilibrium between the photobiont and mycrobiont may lead to breakdown of the lichen association (Nash, 2008).

### 2.6.1 Cause of lichen death

When healthy lichen change environmental urban atmosphere with pollution and toxic metals or other materials. The green-algae will fade in a few months and lichen dies (Carballal *et al*., 2001). There are many pollutants in the cities such as fume from vehicles and houses, waste, soot, etc, but one major toxic chemical is sulfur dioxide ($SO_2$) which was usually considered as primary causative agent (Hauck *et al*.,

2009). The deleterious effect of sulfur dioxide had been proved in laboratory experiment by Rao and Leblance in Canada, they tried to control amount of sulfur dioxide at various humidity and found the algal components were extremely sensitive to injury by sulfurous acid ($H_2SO_3$) and sulfur dioxide (Lee and Parsons, 1999).

Chlorophyll-A was converted to phaeophy by the loss of magnesium at very high concentration the direct action of sulfurous acid caused irreversible damage to cells (Ulshöfer and Rosne, 2001).

Since lichens have less chlorophyll than other plants, small amounts of sulfur dioxide can reduce the delicate balance between their efficient mechanisms for accumulation and also balances their susceptibility to atmospheric pollutant (Loppi *et al*., 2001).

### 2.6.2 Determination metals in lichen

Approximately 75% of the elements in the periodic table are metals and can be found every where on the earth crust and atmosphere forming aerosol and particle (Gadd, 2006). Many metals are essential for life such as sodium, potassium, copper, zinc, cobalt, calcium, magnesium, manganese and iron (Hawsworth *et al*., 1979). There are some metals caesium, aluminium, cadmium, mercury and lead not essential metabolic function, all of these metals can accumulated (Michell and Gu, 2010).

There are several method to determine heavy metals or inorganic compounds from the target sample contaminating toxic metals such as water, ground water, soil, vegetables and animal tissue (Bajpai *et al*., 2011). Instrumental analysis technique will be requited after sample preparation. Instrumental technique is one of the famous technique because it has short time analysis, good efficiency and low capacity detection (ppm, ppb and ppt) (Carreras and Pignata, 2001).

## 2.7 Chlorophyll

Photosynthesis in plant, moss and lichen is driven by the energy of light (Bjerke *et al*., 2005), which is collected by the photosynthetic pigments (Hajek *et al*., 2012). Chlorophylls are the pigments essential for photosynthesis because the photochemical reactions exclusively special types of chlorophyll for electron transfer (Valladaress *et al*., 1996). The role of chlorophylls in light capture has sustained a

remarkable interest since plants may increase light absorption by increasing their chlorophyll density (Chettri *et al*., 1997). In term of fluorescence, chlorophyll fluorescence is the absorption of blue or red photons by the chlorophyll molecule and emission of far red photons (Kranner *et al*., 2000).

Chlorophyll is located in the photobiont cells of plant or lichen and decreases when heavy metals enter most their cells (Garty *et al*.,1993); because heavy metals are predominantly bound extracellular cation exchange sites on the cell wall and impair plant or lichen metabolism which is related to reducing chlorophyll in lichen (Kupper *et al*., 1996). Thus, lichen are an indicator that used environmental index of air pollution over the last several centuries until the present time (Deltoro *et al*., 1999).

## 2.8 Instrumental analysis

### 2.8.1 Flame atomic absorption spectrometry (FAAS)

The sample solution is pumped to the spray chamber through a flame, and converted to atom vapor (Pollard *et al*., 2007). The flame contains atoms of elements, some elements are thermally excited by the flame but most remain in the ground state to the excite state ($E_0 \rightarrow E_j$), these ground state atoms can absorb radiation of particular wavelength that is produced by a special source made from the element. The wavelengths of radiation given by the source are the same as those absorbed by the atoms in the flame (Lajunen, 1992). The main composition of AAS instrument includes a hollow cathode lamp, beam chopper, burner, grating (mono-chromator), photomultiplier and readout shown in Figure 2.8 (Pollard *et al*., 2007).

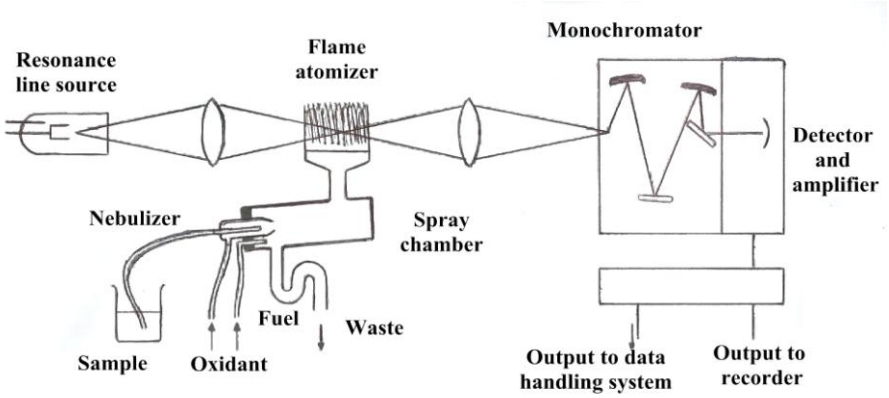


**Figure 2.8** Schematic of atomic absorption spectrometer (Source: Lajunen, 1992)

### 2.8.2 Inductively coupled plasma-mass spectrometry (ICP-MS)

ICP-MS has been used as a very popular method of analysis because it is modern instrument and several advantages over other techniques, rapid, with simultaneous and quasi simultaneous instruments being available, it offers improved sensitivity over many of the other techniques for most analytes and the mass spectrum produced from any sample is simple than that obtained from an emission instrument (Pollard *et al*., 2007).

Inductively coupled plasma-mass spectrometry was developed in the 1970s and used an atmospheric pressure microwave plasma and commercial instrumentation was available in the 1980s. It is a coupling of an ICP with mass spectrometric detection (Ebdon *et al*., 1998); as the principles behind the sample introduction and the processes of plasma formation and dissolution, dissociation, atomization and ionization within the plasma. The sample introduction systems can be used both ICP-MS and ICP-OES. Similarly, plasma procession in these techniques are also identical (Karakas, 2007). However, all ICP-MS instruments detect the analytes according to a mass to charge ratio (m/z). For any signal to be detected, the analytes must become ionized within the plasma. Once ions have been formed, they must pass atmospheric pressure through several chambers of increasingly high vacuum to the mass separation and detection stages. Several different types of mass filters and detectors exist, a more detailed account of how the ions pass from the plasma through the expansion chamber and ion lens system to the mass filter prior to determined trace elements in sample with suitable detector shown in Figure 2.9 (Thomas, 2008).

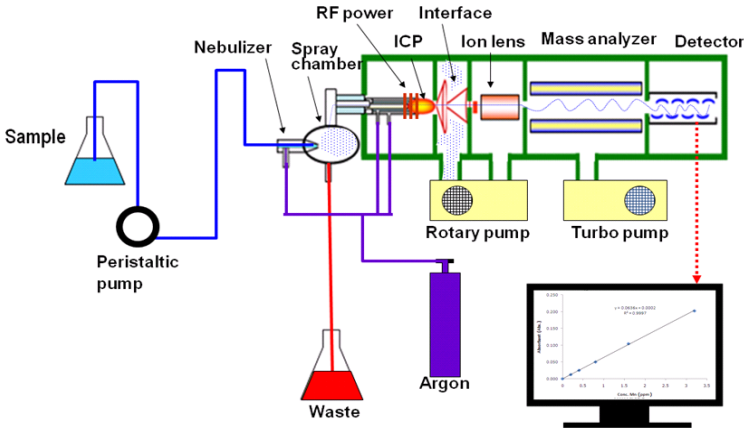


**Figure 2.9** Schematic of ICP-MS (Source: Thosaikham *et al*., 2009).

### 2.8.3 UV-Vis Spectrometry

Spectrometry use since the past time until recent years, the principle of this technique is included light source supplies several wavelength of light through the monochromator. The monochromator will select only one wavelength the same as analyst absorption (Garty *et al*, 2004). The single wavelength will then continued through the sample container (cuvette) containing analytes, the selected wavelength is absorbed by target element and go to the detector converted to spectrums as a read out signal (Kenkel, 2003).

The basic principle of UV-Vis spectrometry includes five parts light source, monochromator, sample, detector and read out as shown in Figure 2.10.

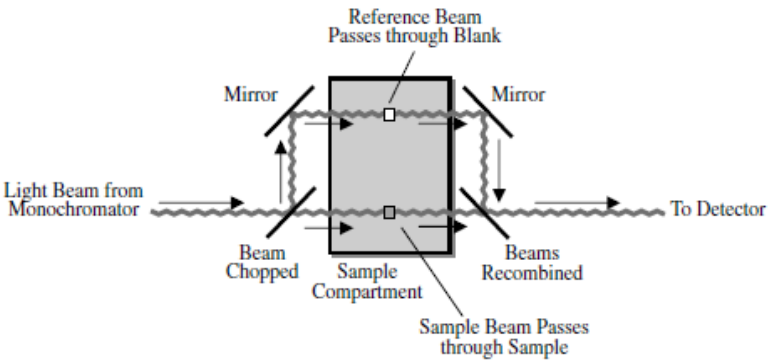


**Figure 2.10** Schematic of UV-Vis spectrophotometer (Source: Kenkel, 2003)

## 2.9 Sample preparation

### 2.9.1 Sample digestion

There are several sample preparation techniques which individual techniques are difference, but all techniques aim to the same goal. The sample digestion is a technique which make analytes to release from samples for reliable analysis (Sooylaki *et al*., 2004). Small amounts of samples are representative of all large sample population, ecosystem and environment that analytes have in their contamination. The method must exact extraction analytes such as acid digestion which coupled instrumental assistant (stream, microwave and heater). Some time anlytes in the sample solution convert to complex compounds by adding some regent (Beyer and Biziuk, 2007).

Digestion method is approximate exaction of the metals analysis because this procedure uses acidic digestion at high concentration under high temperature and instrumental delegate (Nóbrega *et al*., 2006). In general, digestion technique have been classified into two groups; wet digestion and combustion technique as shown in Table 2.2 (Flores *et al*., 2007).

**Table 2.2** Reagent commonly used in the sample dissolution or digestion (Source: Flores *et al*., 2007)

| Reagent | Sample type |
|---|---|
| Water | Soluble |
| Diluted Acid | Dry ashing sample residues, easily oxidized metals and alloys, salts |
| Concentrated acid | Less readily oxidized metals and alloys, steels, metal oxides |
| Concentrated acid with added oxidizing agent | Metals, alloys, soils, particulates from air, refrace-tory minerals, vegetable mater |
| Hydrofluoric | Silicates and other rock |

### 2.9.2 Wet acid digestion procedures

Preparation sample acid treatment releases the element of interest from the target sample and transfer it to a liquid matrix for subsequent analysis is commonly use in many laboratories (Motrenko *et al*., 2000). Therefore, a variety of techniques are required for wet digestion such as digested in a beaker on hotplate or heating block and high pressure microwave heating assistant (Rosenfeld, 2004).

Dissolution is often define as the simple process of dissolving a substance in a suitable liquid at low temperature, with or without chemical reaction (Tinggi *et al*., 1997). The term of decomposition gives more complex process and is usually performed at high temperature and increased pressure with acid reagents and special apparatus (Kenkel, 2003).

#### 2.9.2.1 Opened vessel wet acid digestion

Opened vessel wet acid digestion is one of the oldest technique used to decompose sample of organic and inorganic material in chemical laboratories. This method is low cost analysis and routine use for sample preparation because it can easily control all relevant such as time, temperature, reagent (Mitra, 2003). System of this type are limited by low maximum digestion temperature which can not exceed pressure boiling point of the corresponding acid or acid mixture (Lomonte *et al*., 2008). For instance, the oxidizing power of nitric acid with many matrices is in sufficient at low temperature, one possible to avoid this problem is addition of sulfuric acid which significantly increases the temperature of the digestion solution depending on the matrix and determination method (Rosenfeld, 2004).

High protein and high fat samples are not subject complete digestion at atmospheric pressure. Other disadvantage relate to the risk of contamination through laboratory air, the large amounts of required reagent (very often employing expensive reagents), and the danger of losses of trace elements ((Mitra, 2003). Losses can kept low by using an excess of acid (nitric acid) combined with a reflux condenser and by optimization of the temperature and duration. Therefore, systems operated at atmospheric pressure are preferred from the standpoint of workplace safety. Reagent used in the opened vessel wet acid digestion is shown in Table 2.4 (Mester and Stergeon, 2003).

### 2.9.2.2 Closed-vessel wet acid digestion

Closed vessel wet acid digestion has been used in last the decade for sample preparation in laboratories (Mester and Stergeon, 2003). The closed system was used as it is isolated from laboratory atmosphere to reduce contamination (Flore *et al*., 2007). The procession of sample digestion is essentially ensured by a common wet acid digestion which is performed under the synergistic effects of elevated temperature and pressure (Sastre *et al*., 2002). Digestion occurs at relatively high temperature due to boiling point elevation. These techniques are generally much more efficient than conventional wet acid digestion in open systems, these technique also avoid the loss of volatile elements and any contribution to blank values (Mester *et al*., 1999).

Closed system digestion is particularly suitable for trace and ultra trace analysis, especially when the supply of sample is limited because the oxidizing power of a digestion reagent shows a marked dependence on temperature, an arbitrary distinction should be made between low pressure digestion and high pressure digestion (Moraes *et al*., 2007).

Low pressure digestions (20 bar) are limited to temperature 1808 °C, whereas with high pressure apparatus (70 bar) the digestion temperature may exceed 3008 °C. Reagent used in closed vessel wet acid digestion are shown in Table 2.3 (Mitra, 2003).

**Table 2.3** Schemes for wet digestion methods (Adapted from Mitra, 2003).

| Digestion technique | Required reagents | Application |
|---|---|---|
| **Open systems** | | |
| Conventional | $HNO_3$, HCl, HF, $H_2SO_4$, $HClO_4$, $H_2O_2$ | Inorganic/organic |
| Microwave | $HNO_3$, HCl, HF, $H_2SO_4$, $HClO_4$, $H_2O_2$ | Inorganic/organic |
| Ultraviolet digestion | $K_2S_2O_8$, $H_2O_2$ | Water, slurries |
| **Closed system** | | |
| Conventional | $HNO_3$, HCl, HF, $H_2O_2$ | Inorganic/organic |
| Conventional | $HNO_3$, HCl, HF, $H_2O_2$ | Inorganic/organic |

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Research design

This study was designed with the aim to determine heavy metals content in lichens and chlorophyll degradation. It was shown in Figure 3.1.

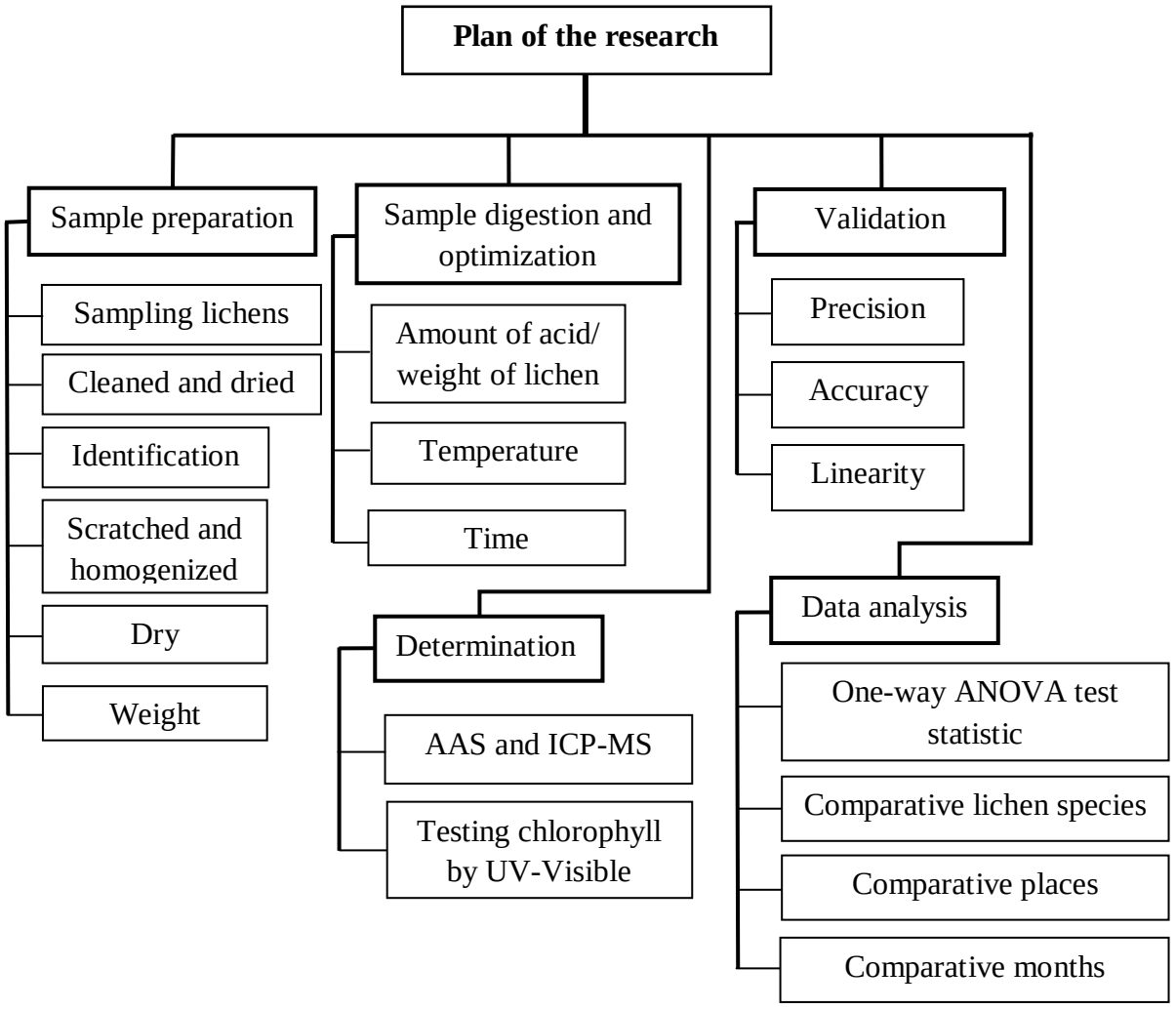


**Figure 3.1** Studied procedure

### 3.2 Sampling sites

Two sampling sites within the Northeast of Thailand were selected (Figure 3.2). The first was located in downtown of Mahasarakham province. Lichen samples from Mahasarakham province were collected from six sites surrounding Mahasarakham downtown such as Public Park, Rajabhat Mahasarakham University, Mueng Campus of Mahasarakham University, Museum of Mahasarakham University, Stadium of Mahasarakham University and Kukeo Temple (Table 3.1). Second site was collected at Phouphakud mountain, Nongsoung district, Mukdahan province which around 60 km southwest from Mukdahan downtown, altitude 238 m, 16º 43' 16,0" N, 104º 43' 12,4" E, with the dipterocarp forest. Five lichens were collected from Mukdahan province and one species was collected from Mahasarakham province (Table 3.2).

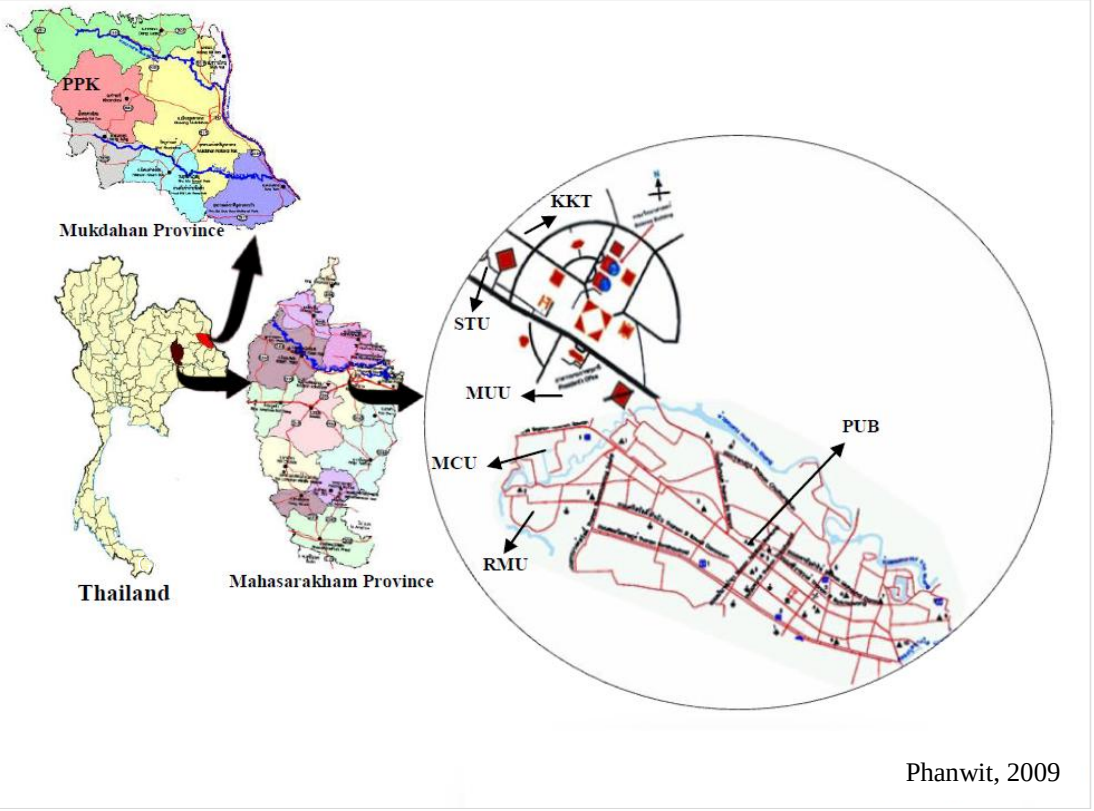


**Figure 3.2** Sampling sites; Public Park (PUB), Rajabhat Mahasarakham University (RMU), Mueng Campus of MSU (MCU), Museum of MSU (MUU), Stadium of MSU (STU), Kukeo Temple (KKT) and Phouphakud (PPK).

**Table 3.1** Collecting localities of lichen.

| Location | Altitude |
|---|---|
| Public Park | 155 m, 16º 11' 14.6" N, 103º 17' 59.0" E |
| Rajabhat Mahasarakham University | 143 m, 16º 11' 58.8" N, 103º 16' 20.2" E |
| Mueng Campus of MSU | 143 m, 16º 12' 17.2" N, 103º 17' 01.0" E |
| Museum of MSU | 165 m, 16º 14' 31,2" N, 103º 14' 57.8" E |
| Stadium of MSU | 166 m, 16º 14' 57.8" N, 103º 14' 43.0" E |
| Kukeo Temple | 158 m, 16º 15' 12,0" N, 103º 15' 03.1" E |

Foliose and crustose groups were focused in this study as shown in Table 3.2. Sample collecting took place from early June to November 2011 for Mukdahan province and November 2011 to July 2012 for Mahasarakham province.

**Table 3.2** List of lichen species collected in this study.

| Species | Groups | Nature of sites | Locations (Provinces) |
|---|---|---|---|
| *D. picta* | Foliose | Rural/Urban | Mukdahan/Mahasarakham |
| *P. tinctorum* | Foliose | Rural | Mukdahan |
| *P. coccifera* | Foliose | Rural | Mukdahan |
| *L. argentata* | Crustose | Rural | Mukdahan |
| *L. benguelengsis* | Crustose | Rural | Mukdahan |

*Dirinaria picta (D. picta), Parmotrema tinctorum (P. trmatinctorum), Pyxine coccifera (P. coccifera), Lecanora argentata (L. argentata)* and *Laurera benguelengsis (L. benguelensis).*

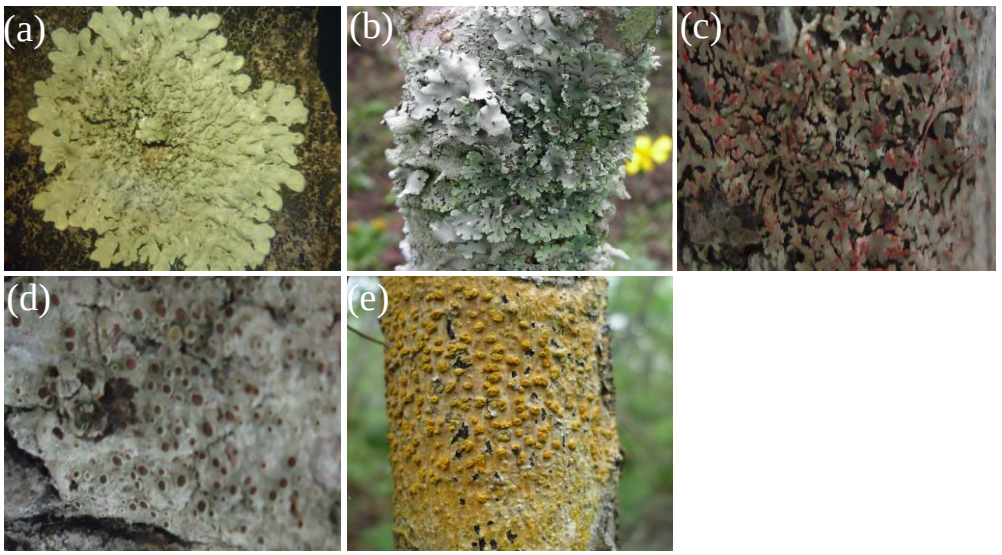


**Figure 3.3** Habitat of lichens; (a) *D. picta,* (b) *P. tinctorum,* (c) *P. coccifera,* (d) *L. argentata* and (e) *L. benguelensis.*

### 3.3 Chemicals and reagents

All chemicals and reagents used in this study were of analytical or AAS grade. Nitric acid, standard solutions of copper, chromium, iron, manganese, nickel and zinc were obtained from Carlo Erba (Italy). The description of all chemicals and reagents shown in Table 3.3.

**Table 3.3** Description of chemical and reagents used in this study.

| Name | Symbol | Molar mass | Density (g $ml^{-1}$) | Grade |
|---|---|---|---|---|
| Chromium standard solution | Cr | 51.99 | - | AAS |
| Copper standard solution | Cu | 63.54 | - | AAS |
| Deionized water | $H_2O$ | 18.02 | - | 18.2 MΩ $cm^{-1}$ |
| Dimethyl sulfoxide (DMSO) | $C_2H_6OS$ | 78.13 | 1.12 | AR |
| Iron standard solution | Fe | 55.84 | - | AAS |
| Manganese standard solution | Mn | 54.93 | - | AAS |
| Nitric acid | $HNO_3$ | 63.01 | 1.40 | AR |
| Nickel standard solution | Ni | 58.69 | - | AAS |
| Zinc standard solution | Zn | 65.38 | - | AAS |

### 3.4 Instrument and apparatus

An Olympus stereo microscope (SZ-PT, Japan) was used for scratching lichen.

An electric balance (TC-254, USA) was used for weighting dry powder lichen.

A water bath (TW 12, Julabo, Germany) was used as heating source for sample digestion. Filter paper No.1 (Circles, 125 mm, Whatman, England) was used to filter the sample solution.

An inductively coupled plasma-mass spectrometer (Perkin-Elmer SCIEX, Norwalk, USA) with a double-pass scott spray chamber fitted with a cross-flow nebulizer was used to determine total trace elements of copper, chromium and nickel in lichen samples. Operating parameters of ICP-MS using standard and DRC mode to determine chromium, copper and nickel, they were shown in Table 3.4 and 3.5, respectively.

A flame atomic absorption spectrometer (AA-680, Shimadzu, Japan) coupled with a 10 cm long slot-burner head, lamps of determinate metal and an air-acetylene flame were used for determination of iron, manganese and zinc. The operating parameters for FAAS were shown in Table 3.6.

A UV-Visible spectrophotometer (HP-8453, Agilent, Germany) with three wavelengths (415, 443 and 665 nm) was used for examining chlorophyll content in lichens.

**Table 3.4** Operating parameters of ICP-MS for standard mode.

| Parameters | Setting |
|---|---|
| Instrument | Elan DRC-e (Perkin-Elmer SCIEX, Norwalk, CT, USA) |
| Standard mode | External standard |
| Nebulizer gas flow | 0.95 l $min^{-1}$ |
| Auxilary gas flow | 1.10 l $min^{-1}$ |
| Plasma gas flow | 17 l $min^{-1}$ |
| Lens voltage | 5 volt |
| ICP RF power | 1200 watt |
| Pulse state voltage | 1200 volt |
| Interface | Ni cone |
| Mass analyzer | Quadrupole |

**Table 3.5** Operating parameters of ICP-MS for dynamic reaction cell (DRC) mode.

| Parameters | Setting |
|---|---|
| Instrument | Elan DRC-e (Perkin-Elmer SCIEX, Norwalk, CT, USA) |
| Standard mode | External standard |
| Nebulizer gas flow | 0.95 l $min^{-1}$ |
| Auxilary gas flow | 1.10 l $min^{-1}$ |
| Plasma gas flow | 17 l $min^{-1}$ |
| Lens voltage | 5 volt |
| ICP RF power | 1200 watt |
| Pulse state voltage | 800 volt |
| Interface | Ni cones |
| Mass analyzer | Quadrupole |
| Analyte masses | $^{63}Cu$, $^{52}Cr$, $^{60}Ni$ |
| RPa | 0.10 |
| RPq | 0.70 |
| Cell gas A | 0.60 |

**Table 3.6** Operating parameters of flame atomic absorption spectrometer (FAAS).

| Parameters | Fe | Mn | Zn |
|---|---|---|---|
| Wavelength (nm) | 248.3 | 279.5 | 219.3 |
| HC lamp current (mA) | 5 | 8 | 5 |
| Slit width (nm) | 0.2 | 0.5 | 0.5 |
| Fuel gas flow rate (l $min^{-1}$) | 2.0 | 1.9 | 2.0 |
| 1% absorption concentration (mg $l^{-1}$) | 0.1 | 2 | 0.02 |
| Type of flame | Air/$C_2H_2$ | Air/$C_2H_2$ | Air/$C_2H_2$ |

## 3.5 Experimental

The material contaminations were removed from lichens using Olympus stereo microscope. Lichens were then cleaned and dried to a constant weight at 100 °C. The dried lichen samples were homogenized to powders and then accurately weight (0.1 g) in triplicates. After that, lichen were digested with nitric acid on stream water (water bath modification). The solutions were filtered through Whatman filter paper No 1. Filtrated solutions were diluted to desired volume at 20 ml with deionized water. Iron, zinc and manganese contents of the solution were analyzed by FAAS, while nickel, copper and chromium contents of the solution were analyzed by ICP-MS.

### 3.5.1 Identification of lichen species

There are several techniques to identify lichen species by morphological, anatomical and chemical. In this study, morphological and anatomical techniques were used to identify lichen species from both provinces.

### 3.5.2 Optimization of digestion conditions

There are several parameters affected the sample digestion efficiency such as amounts of nitric acid, digestion temperatures and digestion times. Therefore, the effect of these digestion conditions were studied as follows:

#### 3.5.2.1 Effect of amounts of nitric acid

Nitric acid was used for decomposition of large molecules into free ions or aqueous solution. The optimization of amounts of nitric acid was studied by varying the ratio of sample weight to amount of 65% nitric acid. Digestion procedure of

individual ratios were optimized at 0.1:0.5, 0.1:1, 0.1:1.5, 0.1:2, 0.1:2.5 and 0.1:3 by gram of dried weight (DW) of lichen sample (g) to amount of acid (ml).

**3.5.2.2 Effect of digestion temperature**

The temperature is an important parameter for sample digestion with acid. The appropriate ratio of sample weight to amount of 65% nitric acid in section 3.5.2.1 was used for optimization of digestion temperature which was optimized at 50, 60, 70, 80, 90 and 100 ºC.

**3.5.2.3 Effect of digestion time**

The digestion time is also important for chemical reaction. Therefore, the appropriate ratio of amount of acid to weight in section 3.5.2.1 and appropriate temperature in section 3.5.2.2 were applied to the optimization of digestion time which was optimized at 10, 20, 30, 40, 50 and 60 minutes.

**3.5.3.4 Validation of digestion procedure**

**1) Precision**

The precision of the proposed digestion procedure was presented in term of repeatability and reproducibility. The relative standard deviation (RSD) was the target value for quantitation see equation 3.1. The repeatability was calculated from three replications for each samples. The reproducibility was calculated from the experiment in three days (inter-day precision, n = 3 x 5) and repeatability was performed from ten replicates within a day (intra-day precision, n = 10) for both *P. tinctorum* and *L. benguelensis*.

$$RSD = \frac{SD}{Average} \times 100 \qquad (3.1)$$

**2) Accuracy**

A recovery test was used to evaluate the accuracy of the proposed digestion procedure which was calculated using equation 3.2. In this study, mixed standards solution of copper, chromium, iron, manganese, nickel and zinc was used to assess the accuracy; the concentration of individual stock standards showed in the Table 3.7. The 100 µl of mixed standards solution were spiked into the sample digestion.

**Table 3.7** Concentration of heavy metals in standard solution for spiking into samples digestion.

| Stock-Standard solution | Spiking concentration (μg $g^{-1}$) |
|---|---|
| Copper | 10 |
| Chromium | 4 |
| Iron | 200 |
| Manganese | 400 |
| Nickel | 4 |
| Zinc | 200 |

$$\% \text{ Recovery} = \frac{X_f - X_i}{X_s} \text{ x100} \qquad (3.2)$$

Where: $X_f$ = Amount of heavy metals found in the sample digestion with spiking mixed standard solution (μg)

$X_i$ = Initial amount of heavy metals found in real sample (μg)

$X_s$ = Amount of spiked standard solution added in real sample (μg)

**3) Linearity range**

The linearity was used to estimate the relationship between absorbance (from AAS) or intensity (from ICP-MS) of copper, chromium, iron, manganese, nickel and zinc on y axis and concentration of heavy metals on x axis. They were plotted by the concentration of standard solutions of copper, chromium, iron, manganese, nickel and zinc. The concentrations of copper, chromium and nickel were varied in range of 0.001 to 0.1 μg $g^{-1}$, while concentration of iron, manganese and zinc were varied in range of 0.05 to 8.0 μg $g^{-1}$. The solutions were prepared by diluting from stock standard solutions.

### 3.6 Chlorophyll examination

Fifty milligram of dry weight lichen powder was extracted with 15 ml of dimethyl sulfoxide (DMSO) and left to stand for one hour at room temperature. The solution was filtered through Whatman filter paper No 1. The filtrated solutions were diluted to volume at 10 ml with deionized water. Chlorophyll in the solution was analyzed by UV-Visible spectrophotometer at wavelength of 415, 443 and 665 nm, respectively.

### 3.7 Data analysis

All experimental data was expressed as means. The data from sample measurements were analyzed by one-way ANOVA test, the significant difference among the means from triplicate analysis was set at $p < 0.05$ using Duncan's new multiple range test. This data was determined by general linear model multivariate range test using the Statistical Program for Social Science (SPSS, Chicago, IL, USA) Version 15.0 for windows.

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 Identification of lichen species

The thallus of *D. picta* is foliose form, appressed to agglutinated up to the lobe tips 2-8 cm in diameter. Lobes are radiating, confluent, flat or convex but concave near apex, 0.5-1 mm wide. Upper surface is gray, bluish gray. Soredia perform farinose and pseudocyphellae are present, but not distinct. Medulla is white to orange. Lower surface is black in center and apothecia are very rarely present, laminal on thallus is 0.7-1.3 mm wide. Spot tests: upper cortex is K+ yellow, P+ yellow; medulla upper and lower part are K-, P- (Figure 4.1 a).

Thallus of *P. tinctorum* is foliose form with green to gray, 3.2-23 cm radius and 76-190 µm thick; agglutinated lobe tips 0.02-1.5 cm; isidia is dense in the centre thallus and 0.03-0.45 mm long; *Trebouxia* is 6-40 µm; medulla is white and 30-190 µm thick, the thallus surface is white brown to black, the rhizine is black and single 0.05-1 mm, picmedia and ascomata are not found. Cortex is K+ yellow. Medulla is K-, C+ red, KC+ red (Figure 4.1 b).

Thallus of *P. coccifera* is folose form and a gray to green, growth form of thallus 2.5-8 cm in diameter and an independently from 200-206 µm thick, lope is loosely on the small flat to play the long side the pseudo surface rupture. *Trebouxia* components are continuous from 40-42 µm thick, medulla layer is yellow. Lower surface is 32-36 µm with white to brown. Rhizine is white to black and ascomata. Cortex is K+ yellow. Medulla is K-, C-, KC- (Figure 4.1 c).

The thallus of *L. argentata* is crustose form with white, cortex layer is colorless and 6.3-14.7 µm thick, algal layer is a family of *trebouxia* with green. The medulla is white brown 180-300 µm thick, apothecia performs round disc and brown to dark brown which is 0.4-1.0 µm thick, exciple, epihymenium are red, hypothecium is yellow and 52.5-115.0 µm high, hymenium is colorless 52-70 µm high, one ascus contains 8 ascospores (Figure 4.1 d).

Thallus of *L. benguelensis* is crustose with green to yellow and smooth to rough. The core text is 50-70 µm. Green algae from *Trenthepholia* is 30-40 µm thick, layer arrangement in the medulla of white fibers in the interface between 35-50 µm thick. Ascomata is in form of perthercia 0.3-0.7 mm high, from 0.2-0.4 mm with black circle and embedded in the tissue stroma, orange to yellow with 0.5-1.0 mm high. 0.4-0.7 mm above the surface by lifting thallus. One ascus contains 8 ascospores (Figure 4.1 e).

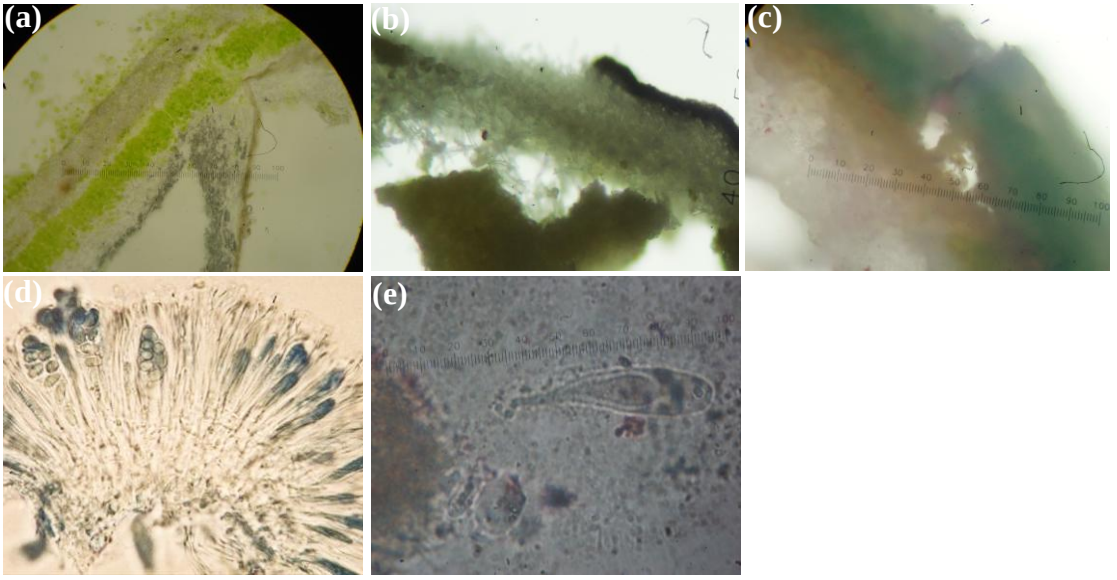


**Figure 4.1** Images of lichens crossed section; (a) *D. picta*, (b) *P. tinctorum*, (c) *P. coccifera*, (d) *L. argentata* and (e) *L. benguelensis*.

### 4.2 Optimization of experimental conditions

The optimization of amount of nitric acid, digestion temperature and digestion time are essential for suitable conditions to achieve high enrichment of analytes from real samples. Appropriate conditions from the optimization of digestion were applied to digest all samples in this study. Two lichen species, *L. benguelensis* and *P. tinctorum* were selected to optimize the conditions. The results of optimization conditions were expressed as recovery test (Table 4.1 to 4.6) for both lichens which recoveries were performed by comparing the spiked volume of stock standard solution at 100 µl from mixed stock standard solution (Table 3.7).

### 4.2.1 Effect of ratio of amount sample to volume of nitric acid

The volume of nitric acid was optimized in the range of 0.5 to 3.0 ml to 0.1 g of dry weight sample. It was presented in term of the ratio of sample to volume of nitric acid (S/A). The results of effect of S/A ratio for *L. benguelensi* and *P. tinctorum* on percentage recovery of heavy metals concentration were shown in Table 4.1 and 4.2, respectively. It can be seen that recovery of the S/A ratio for *L. benguelensi* and *P. tinctorum* were not difference with increasing of S/A ratio excepted some heavy metals from *L. benguelensis* such as copper at 0.1:0.5, 0.1:3.0 and chromium at 0.1:3.0 shown in the Table 4.1. Copper and chromium could be discussed that the S/A ratio of 0.1:0.5 and 0.1:3.0 were not optimum for these metals which were uncompleted digestion with these volume of nitric acid.

The results could be discussed that both lichen samples are in the same order lichens because their cell walls contain polysaccharide and other organic compounds as the major matrices (Kabata-Pendias, 2001). Thus, both of these samples preparation were completely digested with nitric acid in the same S/A ratio. The optimum selection of S/A for both sample digestions had performed by observing the appearance of the digestion. Consequently, the optimum S/A ratio for *L. benguelensis* and *P. tinctorum* were completely digested at S/A 0.1:2.0. The selection of S/A ratio of samples were agreed with 100% of percentage recovery.

**Table 4.1** Percentage recovery (%R) of ratio sample to volume of nitric acid for *L. benguelensis*.

| Ratio of S/A ($g\ ml^{-1}$) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 0.1:0.5 | 69.61 ± 1.18 | 108.05 ± 0.08 | 89.50 ± 0.16 | 102.10 ± 0.90 | 101.42 ± 0.11 | 98.50 ± 0.28 |
| 0.1:1.0 | 90.54 ± 0.84 | 103.87 ± 0.03 | 99.90 ± 0.06 | 108.60 ± 1.56 | . 8657 ± 0.14 | 87.09 ± 0.26 |
| 0.1:1.5 | 94.39 ± 0.08 | 99.64 ± 0.06 | 101.96 ± 0.88 | 100.19 ± 1.28 | 86.02 ± 0.02 | 79.97 ± 1.12 |
| 0.1:2.0 | 109.25 ± 1.03 | 100.53 ± 0.05 | 97.92 ± 0.43 | 98.62 ± 1.8 | 91.76 ± 0.00 | 95.72 ± 0.84 |
| 0.1:2.5 | 115.46 ± 0.03 | 117.59 ± 0.07 | 101.77 ± 1.2 | 101.01 ± 1.98 | 96.81 ± 0.12 | 97.01 ± 0.04 |
| 0.1:3.0 | 136.48 ± 2.77 | 132.2161 ± 0.03 | 96.65 ± 1.28 | 98.94 ± 0.34 | 84.47 ± 0.01 | 85.65 ± 0.10 |

Ratio of S/A; amount of sample (S) to volume of nitric acid (A)

**Table 4.2** Percentage recovery (%R) of ratio sample to volume of nitric acid for *P. tinctorum*.

| Ratio of S/A ($g\ ml^{-1}$) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 0.1:0.5 | 106.22 ± 0.14 | 75.78 ± 0.02 | 123.22 ± 0.29 | 90.01 ± 2.42 | 126.55 ± 0.01 | 107.96 ± 0.37 |
| 0.1:1.0 | 116.92 ± 0.80 | 112.36 ± 0.00 | 78.32 ± 3.42 | 86.06 ± 1.11 | 112.63 ± 0.10 | 86.35 ± 0.07 |
| 0.1:1.5 | 85.11 ± 0.63 | 62.57 ± 0.16 | 86.72 ± 4.25 | 81.07 ± 0.66 | 91.47 ± 0.14 | 83.90 ± 0.06 |
| 0.1:2.0 | 109.26 ± 2.21 | 93.68 ± 0.06 | 105.74 ± 1.91 | 89.03 ± 2.24 | 95.68 ± 0.06 | 104.42 ± 0.24 |
| 0.1:2.5 | 94.57 ± 1.22 | 112.45 ± 0.14 | 111.27 ± 1.74 | 93.78 ± 2.65 | 99.42 ± 0.12 | 105.26 ± 0.36 |
| 0.1:3.0 | 93.90 ± 0.34 | 105.09 ± 0.20 | 112.27 ± 5.16 | 86.55 ± 2.55 | 103.91 ± 0.04 | 97.54 ± 0.12 |

Ratio of S/A; amount of sample (S) to volume of nitric acid (A)

### 4.2.2 Effect of digestion temperature

Temperature is the one of important parameter to improve the reaction procedure because it requires enough energy for complete sample digestion. In this study, the digestion temperature for digesting both *L. benguelensis* and *P. tinctorum* were optimized by varying in the range of 50 to 100 °C. The selected optimum temperature of these sample digestion were performed in term of percentage recovery.

The results found that both lichens were not difference with completely digested at maximum digestion temperature showed in Table 4.3 and 4.4. The results could be discussed that matrices in both samples with nitric acid were completely digested by using high temperature because the appearance of solution were homogeneous at this temperature (Vriesmanna *et al*., 2011), the complete digestion temperature could be freed in the solution when the matrices were completely destroyed. This study was considered to select the optimum digestion temperature at 100 °C for both samples.

**Table 4.3** Percentage recovery (%R) of digestion temperatures for *L. benguelensis*.

| Temperature (°C) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 50 | 118.99 ± 2.21 | 101.47 ± 0.00 | 110.52 ± 0.76 | 86.86 ± 0.67 | 111.61 ± 0.14 | 105.19 ± 0.85 |
| 60 | 84.85 ± 2.47 | 90.50 ± 0.05 | 113.03 ± 0.97 | 97.59 ± 0.10 | 80.37 ± 0.07 | 107.18 ± 0.71 |
| 70 | 72.26 ± 2.74 | 107.23 ± 0.09 | 92.78 ± 4.12 | 91.23 ± 0.02 | 81.31 ± 0.02 | 106.16 ± 0.61 |
| 80 | 78.16 ± 0.82 | 105.39 ± 0.01 | 88.57 ± 0.09 | 87.90 ± 3.87 | 89.15 ± 0.24 | 107.90 ± 0.62 |
| 90 | 122.02 ± 1.17 | 97.56 ± 0.05 | 83.52 ± 2.86 | 86.06 ± 1.45 | 98.14 ± 0.16 | 103.16 ± 0.00 |
| 100 | 100.61 ± 1.88 | 88.61 ± 0.09 | 80.87 ± 4.98 | 84.46 ± 2.05 | 87.68 ± 0.11 | 99.32 ± 0.02 |

**Table 4.4** Percentage recovery (%R) of digestion temperatures for *P. tinctorum*.

| Temperature (°C) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 50 | 113.76 ± 0.26 | 76.62 ± 0.18 | 85.24 ± 1.17 | 92.91 ± 1.65 | 110.44 ± 0.02 | 100.51 ± 0.64 |
| 60 | 103.23 ± 3.37 | 83.93 ± 0.18 | 104.11 ± 3.23 | 91.95 ± 1.74 | 97.05 ± 0.12 | 94.29 ± 0.68 |
| 70 | 78.98 ± 1.78 | 109.38 ± 0.18 | 87.54 ± 0.65 | 91.03 ± 0.31 | 87.39 ± 0.03 | 98.49 ± 0.60 |
| 80 | 100.04 ± 3.48 | 93.88 ± 0.06 | 97.00 ± 1.69 | 94.55 ± 0.12 | 79.36 ± 0.09 | 89.75 ± 2.26 |
| 90 | 72.22 ± 3.15 | 76.17 ± 0.15 | 97.38 ± 4.09 | 94.97 ± 0.00 | 70.05 ± 0.11 | 91.59 ± 2.86 |
| 100 | 71.68 ± 0.89 | 94.38 ± 0.00 | 92.05 ± 2.16 | 93.92 ± 0.06 | 110.80 ± 0.06 | 86.48 ± 0.70 |

### 4.2.3 Effect of digestion time

The digestion time is an important factor in sample digestion. This parameter was used for completion of digestion reaction and homogeneity of sample solution (Vriesmanna et al., 2011). In order to investigation the optimum digestion time. The digestion time was varied in the range of 10 to 60 minute for *L. benguelensis* and *P. tinctorum*.

The results of *L. benguelensis* and *P. tinctorum* (Table 4.5 and 4.6) showed that percentage recoveries of both lichens were not difference with increasing of the digestion time. The results observed that both lichens were completely digested among the 40 to 60 minutes. The results could be discussed that digestion time of samples solution were homogeneous with enough time because pigment of both samples could be completely digested when using enough digestion time. The complete digestion could be performed in the solution when the matrices were completely destroyed with nitric acid. This study considered and selected the optimum digestion time at 40 minute for both samples.

**Table 4.5** Percentage recovery (%R) of digestion of times for *L. benguelen**s**is*.

| Time (min) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 10 | 84.12 ± 0.90 | 114.78 ± 0.17 | 113.64 ± 4.58 | 86.81 ± 1.78 | 113.80 ± 0.25 | 85.80 ± 0.01 |
| 20 | 74.18 ± 0.83 | 100.93 ± 0.13 | 95.84 ± 3.69 | 79.41 ± 1.66 | 103.90 ± 0.15 | 82.99 ± 0.11 |
| 30 | 88.41 ± 1.96 | 100.34 ± 0.04 | 86.52 ± 3.06 | 83.41 ± 1.71 | 120.29 ± 0.04 | 78.80 ± 0.49 |
| 40 | 91.69 ± 0.26 | 88.53 ± 0.13 | 97.97 ± 1.75 | 81.18 ± 3.10 | 84.51 ± 0.19 | 80.96 ± 0.85 |
| 50 | 92.23 ± 2.16 | 91.02 ± 0.26 | 96.93 ± 2.06 | 83.80 ± 1.63 | 102.03 ± 0.02 | 115.07 ± 0.61 |
| 60 | 119.97 ± 2.19 | 108.22 ± 0.11 | 107.59 ± 0.15 | 83.04 ± 1.62 | 114.79 ± 0.06 | 93.19 ± 0.42 |

**Table 4.6** Percentage recovery (%R) of digestion of times for *P. tinctorum*.

| Time (min) | Percentage recovery (%R ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| 10 | 89.24 ± 2.56 | 82.21 ± 0.02 | 95.37 ± 4.51 | 96.98 ± 0.24 | 99.65 ± 0.01 | 93.15 ± 0.12 |
| 20 | 104.42 ± 1.13 | 76.92 ± 0.39 | 89.93 ± 0.07 | 86.11 ± 2.24 | 99.30 ± 0.07 | 102.22 ± 0.18 |
| 30 | 95.63 ± 1.24 | 77.27 ± 0.10 | 80.69 ± 0.87 | 87.68 ± 3.64 | 64.17 ± 0.07 | 96.63 ± 1.04 |
| 40 | 115.56 ± 1.04 | 90.79 ± 0.03 | 76.82 ± 0.6 | 82.52 ± 1.89 | 110.80 ± 0.06 | 90.61 ± 0.59 |
| 50 | 98.61 ± 0.20 | 79.99 ± 0.16 | 80.60 ± 0.60 | 91.02 ± 3.48 | 106.44 ± 0.00 | 97.05 ± 0.71 |
| 60 | 111.94 ± 0.35 | 76.94 ± 0.39 | 101.80 ± 0.49 | 91.09 ± 1.72 | 89.95 ± 0.05 | 94.83 ± 0.24 |

## 4.3 Precision

The repeatability and reproducibility of optimization conditions were investigated in terms of relative standard deviation (RSD). The results showed that the RSD of repeatability and reproducibility of almost heavy metals in two lichens were revealed a bit lower and higher 10%. Excepted repeatability of nickel in *P. tictorum* was expressed 18.10. These results indicated that the optimization procedure could be good procedure and agreed with this precision (Table 4.7).

**Table 4.7** Repeatability and reproducibility of heavy metals in lichens.

| Lichen species | Heavy metals | Concentration of heavy metals (μg $g^{-1}$ ± SD) | | | |
|---|---|---|---|---|---|
| | | Repeatability | RSD | Reproducibility | RSD |
| *L.benguelensis* | Cu | 54.75 ± 1.80 | 3.29 | 52.13 ± 3.08 | 5.90 |
| | Cr | 3.78 ± 0.39 | 10.36 | 3.44 ± 0.28 | 8.15 |
| | Fe | 94.41 ± 5.77 | 6.11 | 94.95 ± 7.09 | 7.74 |
| | Mn | 74.25 ± 4.12 | 5.55 | 72.93 ± 2.57 | 3.51 |
| | Ni | 4.77 ± 0.48 | 10.01 | 4.17 ± 0.25 | 5.90 |
| | Zn | 37.74 ± 2.23 | 5.91 | 37.25 ± 1.45 | 3.87 |
| *P. tinctorum* | Cu | 55.26 ± 2.69 | 4.88 | 55.90 ± 2.38 | 4.28 |
| | Cr | 4.68 ± 0.31 | 6.55 | 4.54 ± 0.28 | 6.25 |
| | Fe | 80.28 ± 5.01 | 5.01 | 80.31 ± 4.51 | 5.62 |
| | Mn | 68.35 ± 7.31 | 10.70 | 70.28 ± 3.96 | 5.73 |
| | Ni | 3.78 ± 0.68 | 18.10 | 3.96 ± 0.14 | 10.54 |
| | Zn | 48.14 ± 4.77 | 9.90 | 46.69 ± 1.89 | 4.05 |

Repeatability (intra-day precision; $n = 10$)
Reproducibility (inter-day precision; $n = 5 \times 3$)

## 4.4 Accuracy

The recovery test was used to evaluate the accuracy of the proposed method. Therefore, concentration of mixed standard solution was spiked into real sample of optimization of conditions showed in the Table 3.7. Sufficiency optimization of S/A

ratio, digestion temperature and digestion time were shown percentage recoveries in Table 4.1 to 4.6, respectively. The results of percentage recoveries of heavy metals were greed with 100%. These results were indicated that the proposed method provided good accuracy.

## 4.5 Application to real samples

The optimum conditions of S/A ratio, digestion temperature and digestion time from the optimization procedures were applied to digest real samples which were collected from two provinces as mention in chapter 3. The results of heavy metals concentration from individual lichen species, sites and months from Mudahan and Mahasarakham provinces were discussed as follows.

### 4.5.1 Heavy metals contents in lichens in Mukdahan Province

Mukdahan province is located in a rural site and surrounded by agricultural land, water canal and small river, which provides humidity to the air, consequently supporting growth of many lichens. The concentration of heavy metals in lichens from this site were interesting. The results showed that different concentrations of heavy metals in each lichens were found. The comparison and evaluation of the concentration of heavy metals are discussed months and species.

#### 4.5.1.1 Distribution of heavy metals in months

The greatest accumulation of heavy metals in each lichen species was found in June followed by August, October and November (Table 4.8 and Figure 4.2). However, manganese in *D. picta* was low in June (Figure 4.2 d). Among the six heavy metals, iron was found highest accumulation followed by Mn > Zn > Cu > Ni > Cr.

The concentrations of copper in each lichen were found in the range of 15.71 ± 0.09 to 56.98 ± 0.26 µg $g^{-1}$ DW (dry weight) (Figure 4.2 a), this metal revealed low accumulation when compared to other metals in this study. The concentrations of chromium were found in the range of 2.57 ± 0.09 to 5.31 ± 0.04 µg $g^{-1}$ DW (Figure 4.2 b) and results shown that this metal was found to have the lowest accumulation of the heavy metals. In contrast, the concentrations of iron were found highest accumulation, ranging from 79.15 ± 2.98 to 281.68 ± 1.37 µg $g^{-1}$ DW, because iron is the most abundant in the earth crust and commonly used in several materials. Therefore, the dust

from this metal could be distributed to the environment more than other heavy metals (Godinho et al., 2009).

The concentrations of manganese were found in the range of 66.63 ± 1.74 to 73.93 ± 2.61 µg $g^{-1}$ DW (Figure 4.2 d). While, concentrations of nickel were revealed 4.08 ± 0.06 to 9.01 ± 0.05 µg $g^{-1}$ DW (Figure 4.2 e). In another observation, the concentrations of zinc were found in the range of 20.30 ± 0.75 to 43.15 ± 0.40 µg $g^{-1}$ DW (Figure 4.2 f).

Individual lichens, the results showed that concentration of each heavy metals in various months were significantly different at $p < 0.05$. It was observed that the concentration of these heavy metals in June was maximum accumulated and decreased from August to November because June was contaminated high heavy metals pollutant from previous months in dry season. Whereas, August, October and November were low contaminated heavy metals caused by pollutant were diluted by water from the rainy season. These results indicated that high humidity in rainy season and different climatic conditions from each months were found different heavy metals concentration from lichens.

**4.5.1.2 Distribution of heavy metals in lichen species**

The results of heavy metals contents in individual lichens are shown in Table 4.8. The concentration of heavy metals in each lichen species were considered and evaluated. Copper was found high concentration in *P. tintorum* followed by *P. coccifera, L. benguelensis, D. picta* and *L. argentata* (Figure 4.2 a).

The maximum concentrations of chromium was found *L. benguelensis* followed by *P. coccifera, P. tinctorum, D. picta* and *L. argentata* (Figure 4.2 b).

Iron was found higher than other metals. *P. coccifera* was found highest concentration of this metal followed by *D. picta, L. argentata* and *L. benguelensis, P. tinctorum* (Figure 4.2 c).

The high concentrations of manganese were found in *P. tinctorum* followed by *D. picta, P. coccifera, L. argentata* and *L. benguelensis* (Figure 4.2 d).

The concentrations of nickel were found high in *P. coccifera* followed by *D. picta, P. tinctorum, L. argentata* and *L. benguelensis* (Figure 4.2 e); the highest concentrations of zinc were found in *D. picta* followed *P. tinctorum, P. coccifera, L. benguelensis* and *L. argentata* (Figure 4.2 f).

The observed levels of heavy metals in lichen species were found significantly different at $p < 0.05$. The result could be interoperated to show that heavy metals can be taken up by lichens actively and passively. In the active process the amount of heavy metals penetrating the cell depend on propinquity of respective ions with specific carriers in plasmatic membranes. In passive process the main role is being played by physical-chemical properties of the heavy metals for ion absorption (complete structure ion exchange groups) (Kularatne and Freitas, 2012). Moreover, Bosiacka in 2001 reported that the functional group of actively participant accumulating process are phosphate, carboxyl, phenolic, ammino and sulphydryl groups of cell wall protein. A role in the passive uptake cations has been attributed to the presence of galacturonic acid (Puckett, 1976). Thus, different lichen species could be absorbed different concentration of heavy metals.

Foliose lichens were found high concentration of heavy metals than the crustose lichen. The results corresponded to Alirzayeva *et al*., 2006 reported that the different morphology of foliose and crustose lichens resulted in different contamination of heavy metals from their ambient atmosphere. This is due to foliose lichen having a wide thallus and covering a large area of the substrate (Nayaka, 2003); while, the crustose have small thallus and apothecia which cover a limited area. Therefore, foliose group can absorb high heavy metals concentration than crutose (Adamo and Violante 2000).

From the result (Table 4.8), four heavy metals (Cu, Cr, Mn and Zn) were found high concentration in *P. tinctorum*. While, other species were low concentration. This study suggested that *P. tinctorum* would be used for environmental evaluation because this species is more sensitive to air pollution than other lichen species. Furthermore, this species is good homogeneous digestion with nitric acid which it is also found abundant in any where in forest from Thailand.

**Table 4.8** Concentration of heavy metals content in lichens in Mukdahan Province in 2011.

| Lichen species | Months | Concentration of heavy metals (µg $g^{-1}$ ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | Cr | Fe | Mn | Ni | Zn |
| *D. picta* | June | 15.71 ± 0.09$^{a}$ | 2.94 ± 0.04$^{a}$ | 262.53 ± 1.46$^{a}$ | 73.93 ± 2.61$^{a}$ | 5.70 ± 0.30$^{a}$ | 27.70 ± 0.64$^{a}$ |
| | August | 14.71 ± 0.53$^{b}$ | 2.31 ± 0.04$^{b}$ | 255.06 ± 0.20$^{a}$ | 69.98 ± 2.63$^{a}$ | 5.42 ± 0.04$^{ab}$ | 26.46 ± 0.10$^{a}$ |
| | October | 13.75 ± 0.60$^{c}$ | 2.03 ± 0.20$^{b}$ | 126.57 ± 9.79$^{b}$ | 55.31 ± 2.97$^{b}$ | 5.12 ± 0.12$^{bc}$ | 21.69 ± 0.48$^{b}$ |
| | November | 10.66 ± 0.35$^{d}$ | 1.98 ± 0.01$^{b}$ | 137.63 ± 8.40$^{b}$ | 64.06 ± 2.46$^{c}$ | 5.03 ± 0.20$^{c}$ | 20.87 ± 0.63$^{b}$ |
| *L. argentata* | June | 17.08 ± 0.60$^{a}$ | 2.57 ± 0.09$^{a}$ | 124.61 ± 0.67$^{a}$ | 71.93 ± 0.81$^{a}$ | 4.38 ± 0.17 | 20.30 ± 0.75$^{a}$ |
| | August | 12.97 ± 0.45$^{b}$ | 1.95 ± 0.04$^{b}$ | 117.81 ± 2.36$^{ab}$ | 67.83 ± 0.45$^{b}$ | 4.27 ± 0.16 | 12.58 ± 0.52$^{b}$ |
| | October | 11.45 ± 0.52$^{c}$ | 1.51 ± 0.07$^{c}$ | 117.58 ± 2.41$^{ab}$ | 62.48 ± 2.17$^{c}$ | 4.08 ± 0.19 | 11.54 ± 0.61$^{b}$ |
| | November | 11.42 ± 0.53$^{c}$ | 1.35 ± 0.04$^{d}$ | 109.95 ± 5.04$^{b}$ | 60.30 ± 3.60$^{c}$ | 4.06 ± 0.22 | 9.01 ± 0.39$^{c}$ |
| *L. benguelengsis* | June | 56.98 ± 0.26$^{a}$ | 3.11 ± 0.13$^{a}$ | 104.64 ± 0.85$^{b}$ | 73.11 ± 0.57$^{a}$ | 4.46 ± 0.19$^{a}$ | 30.27 ± 0.19$^{a}$ |
| | August | 18.31 ± 0.18$^{b}$ | 2.53 ± 0.11$^{b}$ | 99.20 ± 2.18$^{b}$ | 68.51 ± 2.88$^{ab}$ | 4.09 ± 0.04$^{b}$ | 17.92 ± 0.47$^{b}$ |
| | October | 17.60 ± 0.19$^{c}$ | 2.23 ± 0.04$^{c}$ | 98.63 ± 5.66$^{b}$ | 67.98 ± 3.28$^{ab}$ | 4.02 ± 0.12$^{b}$ | 14.95 ± 0.21$^{c}$ |
| | November | 13.24 ± 0.52$^{d}$ | 1.95 ± 0.06$^{d}$ | 94.66 ± 3.64$^{a}$ | 65.23 ± 4.36$^{b}$ | 3.99 ± 0.06$^{b}$ | 13.82 ± 0.32$^{d}$ |
| *P. tinctorum* | June | 54.97 ± 1.04$^{a}$ | 5.31 ± 0.04$^{a}$ | 82.23 ± 2.44 | 66.63 ± 1.74$^{d}$ | 4.08 ± 0.06$^{a}$ | 43.15 ± 0.40$^{a}$ |
| | August | 48.16 ± 1.82$^{b}$ | 2.54 ± 0.13$^{b}$ | 81.30 ± 3.66 | 94.53 ± 1.41$^{a}$ | 4.50 ± 0.22$^{b}$ | 26.86 ± 0.30$^{b}$ |
| | October | 47.65 ± 2.62$^{b}$ | 2.45 ± 0.14$^{b}$ | 81.44 ± 4.51 | 91.43 ± 3.41$^{b}$ | 4.42 ± 0.17$^{ab}$ | 21.25 ± 0.93$^{c}$ |
| | November | 47.03 ± 1.51$^{b}$ | 2.24 ± 0.05$^{c}$ | 79.15 ± 2.98 | 83.66 ± 2.21$^{c}$ | 4.35 ± 0.23$^{ab}$ | 8.85 ± 0.43$^{d}$ |
| *P. coccifera* | June | 17.52 ± 1.03$^{a}$ | 2.65 ± 0.02$^{a}$ | 281.68 ± 1.37$^{a}$ | 71.70 ± 4.23$^{a}$ | 9.0 1± 0.50$^{a}$ | 27.36 ± 0.59$^{a}$ |
| | August | 17.44 ± 0.56$^{a}$ | 2.78 ± 0.12$^{a}$ | 195.30 ± 0.01$^{b}$ | 70.92 ± 2.92$^{a}$ | 8.72 ± 0.37$^{a}$ | 25.83 ± 0.62$^{b}$ |
| | October | 16.72 ± 0.21$^{ab}$ | 2.26 ± 0.10$^{b}$ | 184.53 ± 11.27$^{b}$ | 65.48 ± 3.93$^{ab}$ | 8.34 ± 0.38$^{ab}$ | 22.20 ± 0.37$^{c}$ |
| | November | 16.04 ± 0.36$^{b}$ | 2.24 ± 0.09$^{b}$ | 182.56 ±11.81$^{b}$ | 63.21 ± 2.07$^{b}$ | 7.81 ± 0.29$^{b}$ | 20.60 ± 0.12$^{d}$ |

a, b, c and d Mean significant differences in the comparison of heavy metals from lichens in column at $P < 0.05$ $(n=3)$

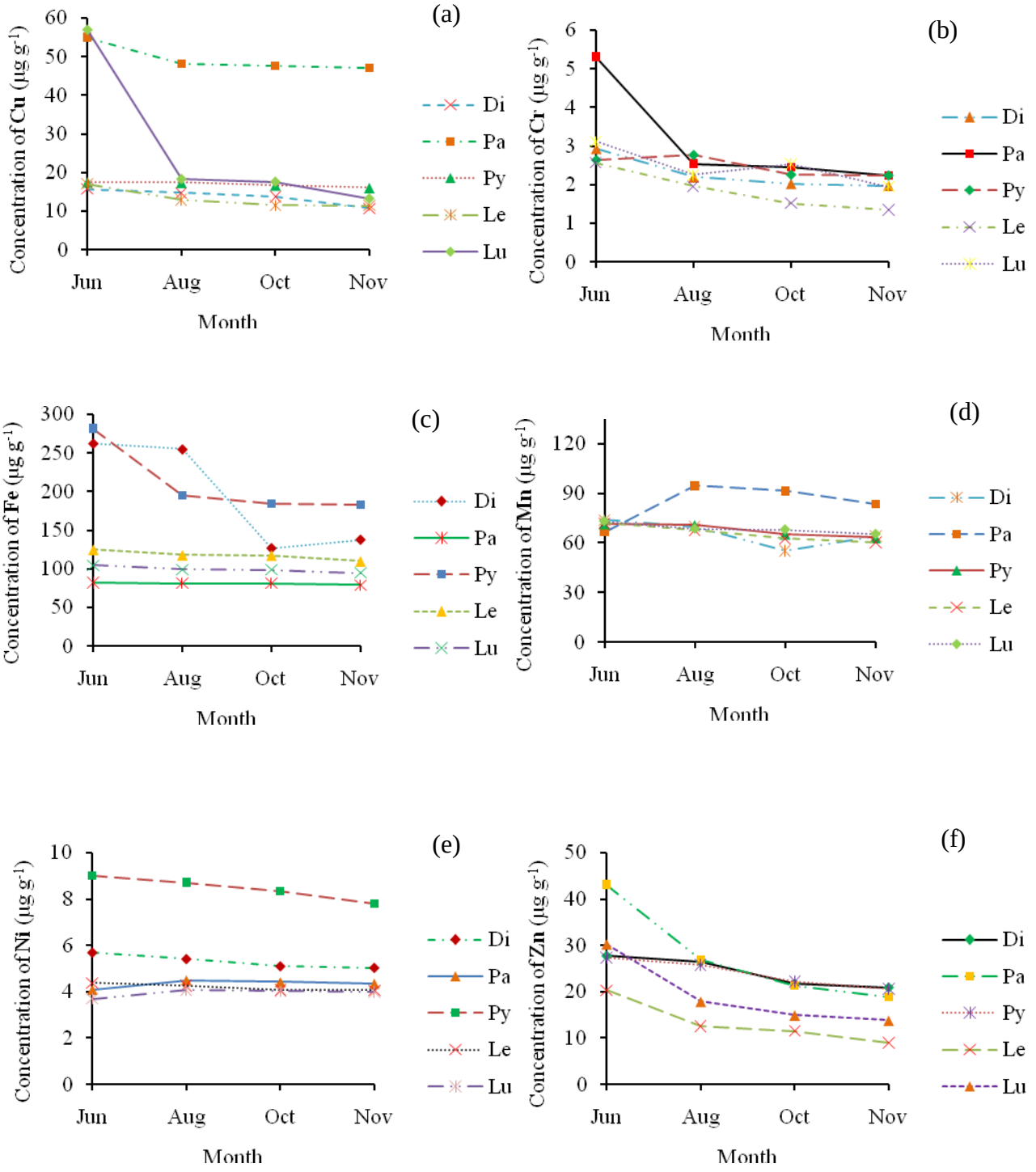


**Figure 4.2** Concentration of each heavy metals by months from Mukdahan province; *D. picta* (Di), *P. tinctorum* (Pa), *P. coccifera* (Py), *L. argentata* (Le), *L. bengurensis* (Lu), (a) Cu, (b) Cr, (c) Fe, (d) Mn, (e) Ni and (f) Zn.

### 4.5.2. Heavy metals contents in lichen in Mahasarakham Province

Mahasarakham is a site that has a high population. Hence, there are many vehicles, transportation and human activities throughout the years. The atmosphere of this downtown might be affected by air pollution such as distribution of heavy metals and toxic gases to the environment. Therefore, the present study was observed the contents of heavy metals in lichen from six sites surrounding Mahasarakham downtown and the result shown in Table 4.9 and Figure 4.3. The concentration of these heavy metals from individual sites and months were considered as follows:

#### 4.5.2.1 Distribution of heavy metals in months

The climatic conditions in individual months were differed. Therefore, the results of heavy metals in this study were observed from November 2011 to July 2012. Among six heavy metals, iron had the highest accumulated followed by Mn > Zn > Cu > Ni > Cr (Table 4.9).

The increasing of accumulation of copper from November to May was observed and this metal was decreased in July excepted Public Park. The concentrations of this metal were found in the range of 10.75 ± 0.10 to 25.72 ± 1.05 μg $g^{-1}$ DW (Figure 4.3 a), dispersion of metals depend on gravity of particular metals a long with speed direction of wind to the lichen thalli (Bajpai *et al*., 2010).

The concentrations of chromium was found to increase from November to May and decreased concentration of this metal was found in July because July is in rainy season which this month could be have more water to dilute this metal concentration than months in the dry season. Therefore, July was low concentration. This metal was also found high concentration in Rajabhat Mahasarakham University in July because this area had been built many construction in this month. The concentration of this metal was found in the range of 1.93 ± 0.04 to 5.87 ± 0.51 μg $g^{-1}$ DW (Figure 4.3 b).

The increasing concentration of iron was found from November to May and decreasing in July. The concentrations of this metal was found in the range of 101.24 ± 0.48 to 307.02 ± 9.51 μg $g^{-1}$ DW. Accumulation of this metal individual sites was differed in each month (Figure 4.3 c).

The increasing concentrations of manganese was found from November to May and decreasing in July. The concentrations of this metal were found in the range of 73.29 ± 0.70 to 170.30 ± 2.70 to μg $g^{-1}$ DW (Figure 4.3 d).

The concentration of nickel was found to increase from November to May and decreasing in July. Moreover, this metal was also found high concentration in Stadium of MSU in July because this area is closed to main road and contained student motorcycle. The concentrations of this metal were found in the range of 3.67 ± 0.34 8.76 ± 0.40 μg $g^{-1}$ DW (Figure 4.3 e).

The accumulations of zinc was found to increase from November to May and decrease in July. The concentrations of this metal were found in the range of 17.66 ± 0.29 to 127.83 ± 2.63 μg $g^{-1}$ DW (Figure 4.3 f). Human activities and wind direction could be probable reason for spread of the heavy metals from their source to lichens in each months. Therefore, zinc could be high accumulated in this month (Bajpai *et al*., 2010).

The result showed that the concentration of heavy metals individual months were found significantly different at $p < 0.05$ demonstrated in the Table 4.9. The increasing concentration of heavy metals in May could be explained as May is in the end of summer. The climate of this season is dry with high temperatures which distributes more pollutant from vehicle exhaust and burning fuel to the atmosphere (Andrzej *et al*., 2007). Therefore, this month could have higher contamination with heavy metals than other months. The distribution of heavy metals were deposited to the atmosphere and come to lichen from November 2011 to May 2012 (Mendil *et al*., 2009).

**4.5.2.2 Distribution of heavy metals in different sites**

The data for heavy metals concentrations is shown by individual metals (Table 4.9). The concentrations of copper were found in the order of Rajabhat Mahasakham University > Public Park > Museum of MSU > Stadium of MSU > Mueng Campus of MSU > Kukeo Temple (Figure 4.3 a). The distribution of copper is released from waste of electrical conductor, wire and bronze because Rajabhat Mahasakham University had constructed building and closed high way. Therefore, waste and dust of this metal could be contaminating these environment and impacting the lichen (Poblet *et al*., 1997).

The concentrations of chromium were found in the order of Public Park > Rajabhat Mahasarakham University > Kukeo Temple > Museum of MSU > Mueng Campus of MSU > Stadium of MSU (Figure 4.3 b). However, the concentration of chromium was also low in Rajabhat Mahasarakham University in November. It was expressed in the sample collected from roadside and urban having heavy metal from vehicular activities because Public Park is located in center of downtown. Therefore, this corresponded to Bajpai *et al*., 2011, chromium is emitted to the atmosphere due to coal and oil combustion especially by diesel-fed vehicles, incineration and frequent use in stainless steel production.

The concentrations of iron were found in the order of Public Park > Museum of MSU > Mueng Campus of MSU > Rajabhat Mahsarakham University > Kukeo Temple > Stadium of MSU (Figure 4.3 c). Iron was accumulated in huge amount in all months in the Public Park. It was abundant in sample collected from site having mixed pollution source, the origin of iron is from vehicles, waste, coal and oil burning activities. According to Bajpai *et al*., 2011, iron contents in lichens is affected by ion originating from fuel and soil dust.

The concentrations of manganese were found in the order of Museum of MSU > Mueng Campus of MSU > Stadium of MSU > Rajabhat Mahasarakham University > Public Park > Kukeo Temple (Figure 4.3 d). The Museum of MSU is located close to a main road which have several student vehicles and traffic throughout the years. Manganese is a part of aluminium alloys, batteries and ceramic (Hauck *et al*., 2003). Therefore, the dispersion of these waste materials is correlated to wind direction, mostly remains east to west (Bajpia *et al*., 2010). Thus, the Museum and Mueng Campus of MSU were located to the west of the resident and main road which manganese could be transplanted following the wind direction.

The concentrations of nickel were found in the order of Stadium of MSU > Rajabhat Mahasarakham University > Mueng Campus of MSU > Museum of MSU > Public Park > Kukeo Temple. The Stadium is located in the University and near resident. Therefore, it corresponded with Bosiacka *et al*., 2001, the dust from nickel waste materials such as vehicle exhaust was distributed to atmosphere ambient lichen (Figure 4.3 e).

The concentrations of zinc were found in the order of Public Park > Rajabhat Mahasarakham University > Mueng Campus of MSU > Museum of MSU > Stadium of MSU > Kukeo Temple (Figure 4.3 f). The accumulation of this metal was recorded from the samples collected in the centre of downtown have several vehicular activities. The concentration of this metal were associated with automobile, car tire and incomplete combustion of fossil fuel. Thus, dust from this metal could contaminate the environment and transplant to lichens (Bajpai *et al*., 2011).

The results of heavy metals accumulation in lichen in Mahasarakham province from individual sites were found significantly different at $p < 0.05$. The results could be discussed that the higher concentration of heavy metals were found in Public Park followed by Rajabhat Mahasarakham University, Mueng Campus of MSU, Museum of MSU, Stadium of MSU and Kukeo Temple, respectively because Public Park is located in center of Mahasarakham downtown which have several human activities such as vehicle exhaust, combustion fuel and using metal materials. Therefore, this site could be high accumulated heavy metals. For other sites, they are located far away from down but some sites are located close to main road such as Rajabhat Mahasarakham University and Mueng Campus of MSU which were low accumulated heavy metal than Public Park because these sites are contain many trees. Museum of MSU, Stadium of MSU and Kukeo Temple are located in the new MSU Campus, there are several student vehicle but University was closed from March to May every years. Therefore, these sites were low concentration of heavy metals than Public Park, Rajabhat Mahasarakham University and Mueng Campus of MSU.

The results indicated that different sites could be different atmosphere and different distribution of heavy metals. Moreover, wind direction and temperature could be concerned with spreading dust of heavy metals to lichen (Bajpai and Upreti 2012).

**Table 4.9** Concentration of heavy metals in lichen in Mahasarakham Province in 2011 to 2012.

| | Months | Concentration of heavy metals (μg $g^{-1}$ ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | Cr | Fe | Mn | Ni | Zn |
| Public Park | November | 17.01 ± 0.94$^{ab}$ | 4.26 ± 0.15$^{a}$ | 280.55 ± 8.51$^{b}$ | 100.39 ± 4.00$^{a}$ | 4.09 ± 0.26$^{a}$ | 59.03 ± 2.32$^{a}$ |
| | January | 17.91 ± 0.36$^{ab}$ | 4.71 ± 0.21$^{b}$ | 291.40 ± 15.13$^{bc}$ | 120.86 ± 1.69$^{ab}$ | 4.41 ± 0.08$^{a}$ | 111.53 ± 2.52$^{c}$ |
| | March | 17.64 ± 0.10$^{a}$ | 5.18 ± 0.08$^{c}$ | 295.79 ± 4.70$^{bc}$ | 108.58 ± 4.04$^{b}$ | 5.16 ± 0.32$^{b}$ | 127.83 ± 2.63$^{d}$ |
| | May | 19.83 ± 0.07$^{b}$ | 5.87 ± 0.33$^{d}$ | 307.02 ± 9.51$^{bc}$ | 126.36 ± 3.80$^{d}$ | 5.21 ± 0.22$^{b}$ | 124.92 ± 1.87$^{d}$ |
| | July | 23.13 ± 0.10$^{c}$ | 3.93 ± 0.77$^{a}$ | 262.25 ± 2.82$^{a}$ | 117.84 ± 1.47$^{c}$ | 5.55 ± 0.28$^{b}$ | 91.65 ± 1.14$^{b}$ |
| Rajabhat Mahasarakham University | November | 15.86 ± 0.73$^{a}$ | 1.93 ± 0.04$^{a}$ | 214.67 ± 8.26$^{a}$ | 120.61 ± 6.51$^{b}$ | 5.71 ± 0.25$^{bc}$ | 52.71 ± 2.53$^{a}$ |
| | January | 19.20 ± 0.42$^{b}$ | 3.50 ± 0.04$^{b}$ | 216.89 ± 4.91$^{a}$ | 122.03 ± 3.87$^{b}$ | 5.60 ± 0.17$^{b}$ | 61.01 ± 1.02$^{b}$ |
| | March | 19.23 ± 0.60$^{b}$ | 4.59 ± 0.19$^{c}$ | 218.96 ± 6.77$^{a}$ | 120.97 ± 3.89$^{b}$ | 6.07 ± 0.26$^{c}$ | 67.90 ± 3.61$^{c}$ |
| | May | 25.72 ± 1.05$^{c}$ | 5.27 ± 0.13$^{d}$ | 249.28 ± 7.81$^{b}$ | 124.34 ± 5.07$^{b}$ | 6.63 ± 0.25$^{d}$ | 72.77 ± 3.70$^{c}$ |
| | July | 18.97 ± 0.21$^{b}$ | 5.87 ± 0.51$^{e}$ | 239.51 ± 0.91$^{b}$ | 95.35 ± 2.70$^{a}$ | 4.06 ± 0.05$^{a}$ | 60.15 ± 2.49$^{b}$ |
| Mueng Campus of MSU | November | 14.30 ± 0.43$^{b}$ | 4.59 ± 0.19$^{b}$ | 227.38 ± 4.48$^{a}$ | 128.55 ± 1.64$^{b}$ | 5.48 ± 0.13$^{a}$ | 30.99 ± 1.23$^{b}$ |
| | January | 14.87 ± 0.38$^{b}$ | 4.53 ± 0.17$^{b}$ | 253.65 ± 9.67$^{b}$ | 156.77 ± 3.59$^{d}$ | 6.13 ± 0.21$^{b}$ | 35.72 ± 0.75$^{c}$ |
| | March | 14.00 ± 0.41$^{b}$ | 4.40 ± 0.11$^{b}$ | 263.46 ± 16.11$^{b}$ | 137.69 ± 1.18$^{c}$ | 6.13 ± 0.24$^{b}$ | 51.21 ± 1.77$^{d}$ |
| | May | 16.24 ± 0.38$^{c}$ | 4.50 ± 0.11$^{b}$ | 277.20 ± 10.32$^{b}$ | 154.95 ± 7.07$^{d}$ | 5.81 ± 0.04$^{ab}$ | 53.17 ± 2.10$^{d}$ |
| | July | 11.66 ± 0.64$^{a}$ | 2.53 ± 0.42$^{a}$ | 233.55 ± 4.09$^{a}$ | 113.28 ± 1.51$^{a}$ | 5.47 ± 0.32$^{a}$ | 45.04 ± 2.16$^{a}$ |

$^{a, b, c \text{ and } d}$ Mean significant differences in the comparison of heavy metals from lichen in column at $P < 0.05$ $(n=3)$

**Table 4.9** Concentration of heavy metals in lichen in Mahasarakham Province in 2011 to 2012 (continue).

| Places | Months | Concentration of heavy metals (μg $g^{-1}$ ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | Cr | Fe | Mn | Ni | Zn |
| | November | 14.06 ± 0.26[a] | 4.87 ± 0.15[b] | 158.57 ± 9.62[a] | 98.19 ± 1.12[a] | 5.81 ± 0.21[b] | 35.86 ± 0.41[a] |
| Museum | January | 18.77 ± 0.61[c] | 4.88 ± 4.88[b] | 202.34 ± 0.17[b] | 137.85 ± 1.09[c] | 6.05 ± 0.23[b] | 37.61 ± 0.16[a] |
| of MSU | March | 18.97 ± 0.67[c] | 5.06 ± 0.05[b] | 179.06 ± 5.12[c] | 167.65 ± 1.89[d] | 5.91 ± 0.11[b] | 36.16 ± 1.36[a] |
| | May | 19.13 ± 0.69[c] | 5.14 ± 0.05[b] | 270.97 ± 5.34[e] | 170.30 ± 2.70[d] | 6.51 ± 0.05[c] | 56.28 ± 1.78[c] |
| | July | 15.32 ± 0.58[b] | 1.91 ± 0.40[a] | 228.15 ± 8.06[d] | 118.34 ± 4.10[b] | 5.20 ± 0.11[a] | 40.97 ± 0.74[b] |
| | November | 15.30 ± 0.10[a] | 3.79 ± 0.01[a] | 101.24 ± 0.48[a] | 88.41 ± 1.30[a] | 4.71 ± 0.02[a] | 30.94 ± 0.29[a] |
| Stadium | January | 17.79 ± 0.53[b] | 4.14 ± 0.18[b] | 214.12 ± 7.38[bc] | 118.36 ± 2.60[b] | 5.00 ± 0.20[ab] | 34.90 ± 1.45[b] |
| of MSU | March | 18.41 ± 0.87[b] | 4.30 ± 0.22[b] | 223.11 ± 4.35[c] | 138.33 ± 5.02[c] | 5.42 ± 0.19[b] | 49.19 ± 1.41[c] |
| | May | 18.97 ± 0.84[b] | 4.51 ± 0.13[b] | 207.47 ± 7.75[b] | 112.80 ± 1.84[b] | 6.58 ± 0.05[c] | 56.02 ± 0.54[d] |
| | July | 11.72 ± 0.21[a] | 3.26 ± 0.04[a] | 208.59 ± 8.19[b] | 91.21 ± 1.01[a] | 8.76 ± 0.40[d] | 50.35 ± 1.92[c] |
| | November | 14.43 ± 0.11[b] | 4.83 ± 0.18[b] | 150.96 ± 7.45[a] | 82.13 ± 1.63[b] | 3.67 ± 0.34[a] | 17.66 ± 0.29[a] |
| Kukeo | January | 14.65 ± 0.65[b] | 5.06 ± 0.10[b] | 222.54 ± 7.21[b] | 86.52 ± 1.94[c] | 4.05 ± 0.06[b] | 34.72 ± 0.91[b] |
| Temple | March | 14.69 ± 0.26[b] | 5.14 ± 0.10[b] | 259.71 ± 7.05[d] | 86.62 ± 1.01[c] | 4.08 ± 0.22[b] | 46.23 ± 3.12[c] |
| | May | 14.87 ± 0.80[b] | 5.82 ± 0.23[c] | 251.27 ± 0.20[d] | 92.66 ± 4.42[d] | 4.08 ± 0.16[b] | 49.22 ± 0.72[c] |
| | July | 10.75 ± 0.10[a] | 2.67 ± 0.18[a] | 192.97 ± 7.60[b] | 73.29 ± 0.70[a] | 4.74 ± 0.25[c] | 44.76 ± 2.63[c] |

[a, b, c and d] Mean significant differences in the comparison of heavy metals from lichens in column at $P < 0.05$ $(n=3)$

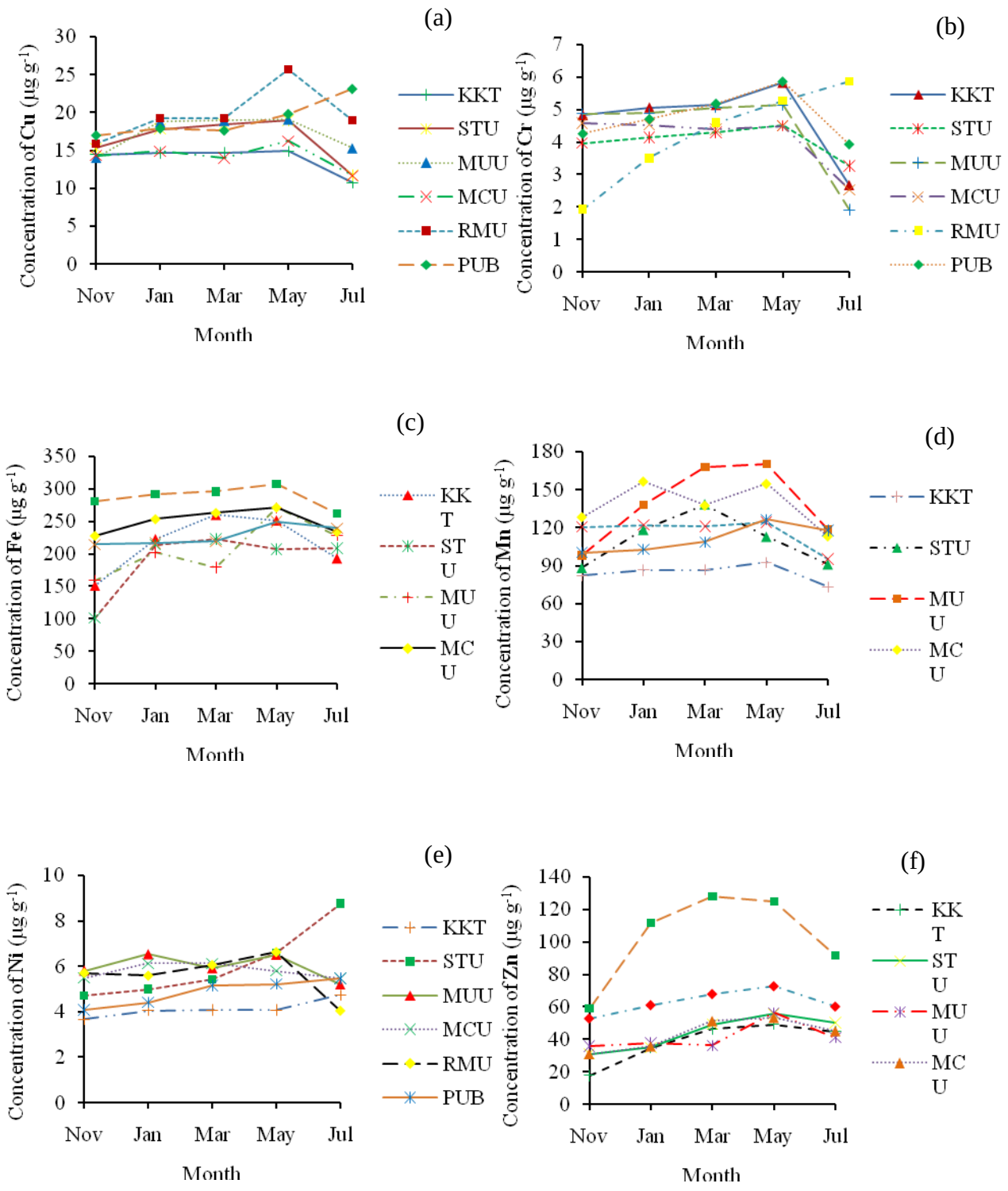


**Figure 4.3** Concentration of each heavy metals in months from Mahasarakham province; Kukeo Temple (KKT), Stadium of MSU (STU), Museum of MSU (MUU), Mueng Campus of MSU (MCU), Rajabhat Mahasarakham University (RMU), Public Park (PUB), (a) Cu, (b) Cr, (c) Fe, (d) Mn, (e) Ni and (f) Zn.

### 4.5.3 The concentration of heavy metals in *D. picta* in Mukdahan and Mahasarakham Provinces

The concentration of heavy metals in *D. picta* in both provinces were compared as this species was collected in the same month (November 2011) from both provinces. The concentration of heavy metals in other lichen species were not compared in this study because they were collected in different months. There is only one species (*D. picta*) was found and collected from both provinces. High concentrations of heavy metals were found iron followed by manganese, zinc, copper, nickel and chromium (Table 4.10 and Figure 4.4).

The results of heavy metals content in this lichen differed in both provinces. High heavy metals accumulation was found in the sites of Mahasarakham province in the order of Public Park, Rajabhat Mahasarakham University, Mueng Campus of MSU, Museum of MSU, Stadium of MSU and Kukeo Temple. Lower accumulation of these heavy metals was found from Phouphakud mountain in Mukdahan province. The results of heavy metals concentration from individual sites were found significantly different at $p < 0.05$. The results could be discussed as the lichen collected from Mahasarakham province are exposed to more pollutant throughout the year. The individual sites from this province were located close to the main roads and constructions which have many human activities, vehicle exhaust and combustion fuel. Therefore, these sites were found to have high concentration of each heavy metals. *D. picta* from Phouphakud mountain was found lower concentration of heavy metals this site was located in rural area containing rich forest, conservation area and was far away from highway. There are no vehicle exhaust and human activities. Thus, this site could be clean environment than Mahasarakham province. The result indicated that rural site was low accumulated that city or downtown. It was responded to Bajpai and Upreti reported in 2012.

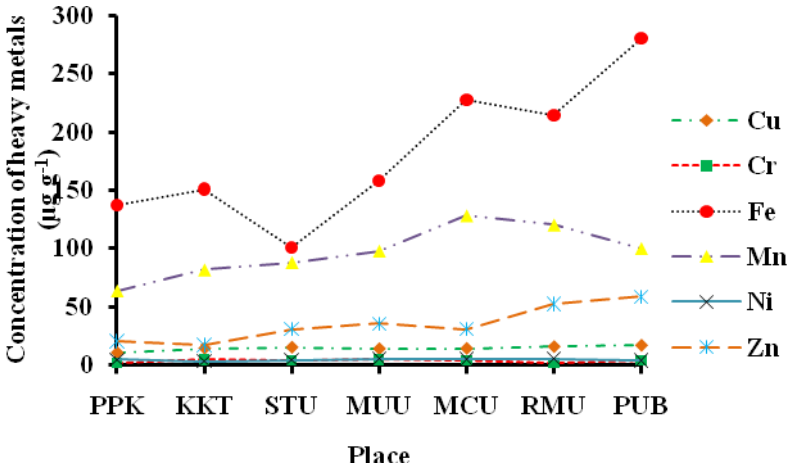


**Figure 4.4** Concentration of heavy metals in sites from Mahasarakham and Mukdahan provinces; Phouphakud mountain (PPK), Kukeo Temple (KKT), Stadium of MSU (STU), Museum of MSU (MUU), Mueng Campus of MSU (MCU), Rajabhat Mahasarakham University (RMU) and Public Park (PUB).

**Table 4.10** Concentration of heavy metals content in *D. picta* from both Mahasarakham and Mukdahan Provinces.

| Stations | Concentration of heavy metals (µg $g^{-1}$ ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Cr | Fe | Mn | Ni | Zn |
| Public Park | 17.01 ± 0.94$^{d}$ | 4.26 ± 0.15$^{c}$ | 280.55 ± 8.51$^{d}$ | 100.39 ± 4.00$^{d}$ | 4.09 ± 0.26$^{b}$ | 59.03 ± 2.32$^{e}$ |
| Rajabhat Mahasarakham University | 15.86 ± 0.73$^{c}$ | 1.93 ± 0.04$^{a}$ | 214.67 ± 8.29$^{c}$ | 120.61 ± 6.51$^{e}$ | 5.71 ± 0.25$^{d}$ | 52.71 ± 2.53$^{e}$ |
| Mueng Campus of MSU | 14.30 ± 0.43$^{b}$ | 4.59 ± 0.19$^{d}$ | 227.38 ± 4.48$^{c}$ | 128.55 ± 1.64$^{e}$ | 5.48 ± 0.13$^{d}$ | 30.99 ± 1.23$^{c}$ |
| Museum of MSU | 14.06 ± 0.26$^{b}$ | 4.87 ± 0.15$^{e}$ | 158.57 ± 9.62$^{b}$ | 98.19 ± 1.12$^{d}$ | 5.81 ± 0.21$^{d}$ | 35.86 ± 0.41$^{d}$ |
| Stadium of MSU | 15.30 ± 0.10b$^{c}$ | 3.97 ± 0.01$^{b}$ | 101.24 ± 0.48$^{a}$ | 88.41 ± 1.30$^{c}$ | 4.71 ± 0.02$^{c}$ | 30.94 ± 0.29$^{c}$ |
| Kukeo Temple | 14.43 ± 0.11$^{b}$ | 4.83 ± 0.18$^{e}$ | 150.96 ± 7.45$^{b}$ | 82.13 ± 1.63$^{b}$ | 3.67 ± 0.43$^{a}$ | 17.66 ± 0.29$^{a}$ |
| Phouphakud | 10.66 ± 0.35$^{a}$ | 1.98 ± 0.01$^{a}$ | 137.63 ± 8.40$^{b}$ | 64.06 ± 2.46$^{a}$ | 5.03 ± 0.20$^{d}$ | 20.87 ± 0.63$^{b}$ |

$^{a, b, c, d \text{ and } e}$ Mean significant differences in the comparison of heavy metals from lichen in column at $P < 0.05$ $(n=3)$

### 4.5.4 Examination of chlorophyll

Lichen was affected by nitrogen oxide, sulfur dioxide and heavy metals in the atmosphere. These toxicity was probably released from vehicle exhaust, waste and burning fuel. Lichens absorbed or dissolved $NO_3$ and $NH_4$ throughout metabolize procedure as well as nutrients. However, excess nitrogen can be toxic. In addition, lichens in urban area fail to produce soredia and apothecia causes death lichens (Deltoro *et al*., 1999). Lichen is widely used as indicator of air quality because they use water and nutrients from ambient atmosphere for growth. Toxic gases and heavy metals pollutants cause chlorophyll to degrade, resulting in a declined in photosynthesis.

Chlorophyll in lichen was extracted with Dimethyl sulfoxide (DMSO) and measured optical density by UV-Visble. Chlorophyll in DMSO solution shows maximum absorption at 665 and 443 nm. Upon chlorophyll degradation into phaeophytin, the absorption at 665 nm is reduced while the absorption at 443 nm is shifted to 415 nm. The results in this study were expressed in term of pheophytin using calculation from different absorbance values between 415 and 443 nm (415-443 nm) showed in the Table 4.11 and 4.12. The degradation of chlorophyll in each lichens from both provinces (Mukdahan and Mahasarakham) were discussed as follows.

In Mukdahan province, the results were compared between individual lichens and months. In monthly comparison (Table 4.11), the level degradation was found different in each months. *D. picta* and *P. tinctorum* were found high chlorophyll degradation at 0.25 ± 0.31 and 0.30 ± 0.04 in November (Figure 4.5 d). While, *P. coccifera* was found at 2.81 ± 0.18 in October (Figure 4.5 c). *L. benguelensis* and *L. argentata* were found at 0.52 ± 0.07 and 0.82 ± 0.48 in June (Figure 4.5 a). The result of chlorophyll degradation in individual months were found significantly different at $p < 0.05$. The results could be observed that high having chlorophyll degradation to pheophytin were found in June and November. Moreover, October was found high chlorophyll degradation only *P. coccifera*. The minimum value of chlorophyll degradation or integrity of chlorophyll was revealed in August. This result could be discussed that August is in rainy season having hard rain and more pollutant diluted than each months.

The observation of individual lichen species found that the high level of chlorophyll degradation was found in *P. coccifera* followed by *L. argentata, L. benguelensis, P. tinctorum* and *D. picta*.

In October 2011, P. coccifera was found high value of chlorophyll degradation at 2.81± 0.18 while *D. picta* was detected lower value at 0.12 ± 0.12, both species were foliose. The results in four months showed that the *P. coccifera* can be used for evaluation of air pollution than *D. picta* and *P.tinctorum* (Table 4.11 and Figure 4.5). Crustose lichens (*L. argentata and L. benguelensis*) were detected high value in June. This study was collected samples in wet season (June to October) and dry season (November). *L. benguelensis* showed the results are different between wet and dry season. While L. argentata was not much difference. In suggestion, crustose lichen for air pollution is *L. benguelensis*.

The result of chlorophyll degradation in individual lichens in Mukdahan province were found significantly different at $p < 0.05$. The results shown that the ability of lichens exposed to heavy metals and toxic gases were different deposition. Moreover, the morphology and physical mechanism in the intracellular of lichens had different affected on their susceptibility pollutant in the ambient atmosphere (Shakya et al., 2008). The results indicated that the foliose lichen is more sensitive to air pollution than the crustose lichen.

In Mahasarakham province, chlorophyll in *D. picta* from individual sites and months in this province were discussed. For monthly comparison, maximum chlorophyll degradation was found in May at 0.61 ± 0.00 followed by July at 0.30 ± 0.11, March at 0.23 ± 0.00, January at 0.21 ± 0.02 and November at 0.13 ± 0.12 (Table 4.12 and Figure 4.6). The result of chlorophyll degradation in individual months were found significantly different at $p < 0.05$. The result could be discussed that May is in the end of dry season, it could be accumulated pollutant from each months in the summer. The environmental conditions during this month changed from dry season to rainy season. Therefore, wind and dust of pollutant could be more distributed to lichens than each months (Garty *et al*., 1993).

For comparison of sites, the high chlorophyll degradations were found in Public Park followed by Rajabhat Mahasarakham University, Stadium of MSU, Museum of MSU, Mueng Campus of MSU and Kukeo Temple. In March and May was

high value of chlorophyll degradation, while Public Park showed highest 0.61± 0.00 in May and 0.57± 0.04 in Stadium of MSU in the same month. All stations were found lower value in November 2011 (Table 4.12 and Figure 4.6. a, b, c and d). The results shown that chlorophyll degradation was found in urban area which was indicated this area high distributed several pollutant such as dust heavy metals and toxic gases than the each sites. The result of chlorophyll degradation in individual sites were found significantly different at $p < 0.05$. The result could be discussed that Public Park is located in center city of Mahasarakham province, there are many human activities throughout the years such vehicle exhaust, combustion fuel, construction and waste while the other site were less these activities than this site.

The results of chlorophyll degradation in this study from Mudahan and Mahasarakham provinces were compared and investigated using one lichen such as *D. picta* which was collected on November 2011 from both provinces. This species was selected to evaluated chlorophyll degradation from both provinces because it was collected same months. The results found that the high chlorophyll degradation was found at 0.61 ± 0.00 in Public Park in Mahasarakham province while Mudahan province was found lower at 0.25 ± 0.31 (Table 4.11 and 4.12 in November 2011). These results indicated that the urban area is high distribution of pollutant than the rural area. The case of this result was mention in the previous section, these results were responded to Manrique et al 1989 and Garty *et al*.,1993 reports. They results found that urban or industries site was expressed high chlorophyll degradation than rural site.

From this study, chlorophyll degradation indicated that foliose lichen such as *P. coccifera* was found high chlorophyll degradation or high sensitive to pollution than crustose lichen which is involved itself morphology and intracellular to pollutant in its ambient atmosphere. This study suggested that *P. coccifera* could be used for testing chlorophyll degradation or environmental evaluation.

**Table 4.11** Absorbance of chlorophyll contents in five lichens from Mukdahan Province in 2011.

| Months | Value of chlorophyll degradation | | | | |
|---|---|---|---|---|---|
| | *D. picta* | *P. tictorum* | *P. coccifera* | *L. argentata* | *L. benguelensis* |
| June | $0.22 \pm 0.00^{b}$ | $0.19 \pm 0.31^{c}$ | $0.26 \pm 0.06^{b}$ | $0.52 \pm 0.07^{b}$ | $0.82 \pm 0.48^{c}$ |
| August | $0.24 \pm 0.10^{c}$ | $0.15 \pm 0.10^{a}$ | $0.36 \pm 0.01^{c}$ | $0.36 \pm 0.02^{a}$ | $0.08 \pm 0.14^{a}$ |
| October | $0.12 \pm 0.12^{a}$ | $0.17 \pm 0.02^{b}$ | $2.81 \pm 0.18^{d}$ | $0.36 \pm 0.48^{a}$ | $0.18 \pm 0.14^{b}$ |
| November | $0.25 \pm 0.31^{c}$ | $0.30 \pm 0.04^{d}$ | $0.17 \pm 0.36^{a}$ | $0.36 \pm 0.26^{a}$ | $0.54 \pm 0.14^{c}$ |

[a, b, c and d] Mean significant differences in the comparison of chlorophyll degradation in column at $P < 0.05$ $(n=3)$

**Table 4.12** Absorbance of chlorophyll contents of *Dirinaria picta* from Mahasarakham Province in 2011 to 2012.

| Months | Value of chlorophyll degradation | | | | | |
|---|---|---|---|---|---|---|
| | PUB | RMU | MCU | MUU | STU | KKT |
| November (2011) | $0.13 \pm 0.12^{a}$ | $0.18 \pm 0.03^{a}$ | $0.04 \pm 0.04^{a}$ | $0.09 \pm 0.07^{a}$ | $0.17 \pm 0.11^{a}$ | $0.04 \pm 0.10^{a}$ |
| January (2012) | $0.21 \pm 0.02^{b}$ | $0.15 \pm 0.11^{a}$ | $0.06 \pm 0.04^{a}$ | $0.12 \pm 0.01^{b}$ | $0.12 \pm 0.05^{a}$ | $0.10 \pm 0.00^{b}$ |
| March (2012) | $0.23 \pm 0.00^{b}$ | $0.25 \pm 0.00^{c}$ | $0.21 \pm 0.05^{c}$ | $0.26 \pm 0.12^{c}$ | $0.42 \pm 0.01^{b}$ | $0.22 \pm 0.11^{c}$ |
| May (2012) | $0.61 \pm 0.00^{d}$ | $0.22 \pm 0.01^{c}$ | $0.26 \pm 0.01^{d}$ | $0.22 \pm 0.00^{c}$ | $0.57 \pm 0.04^{c}$ | $0.23 \pm 0.12^{c}$ |
| July (2012) | $0.30 \pm 0.11^{c}$ | $0.20 \pm 0.01^{b}$ | $0.10 \pm 0.02^{b}$ | $0.10 \pm 0.25^{b}$ | $0.24 \pm 0.01^{d}$ | $0.10 \pm 0.03^{b}$ |

[a, b, c and d] Mean significant differences in the comparison of chlorophyll degradation in column at $P < 0.05$ $(n=3)$

Public Park (PUB), Rajabhat Mahasarakham University (RMU), Mueng Campus of MSU (MCU), Museum of MSU (MUU), Stadium of MSU (STU) and Kukeo Temple (KKT).

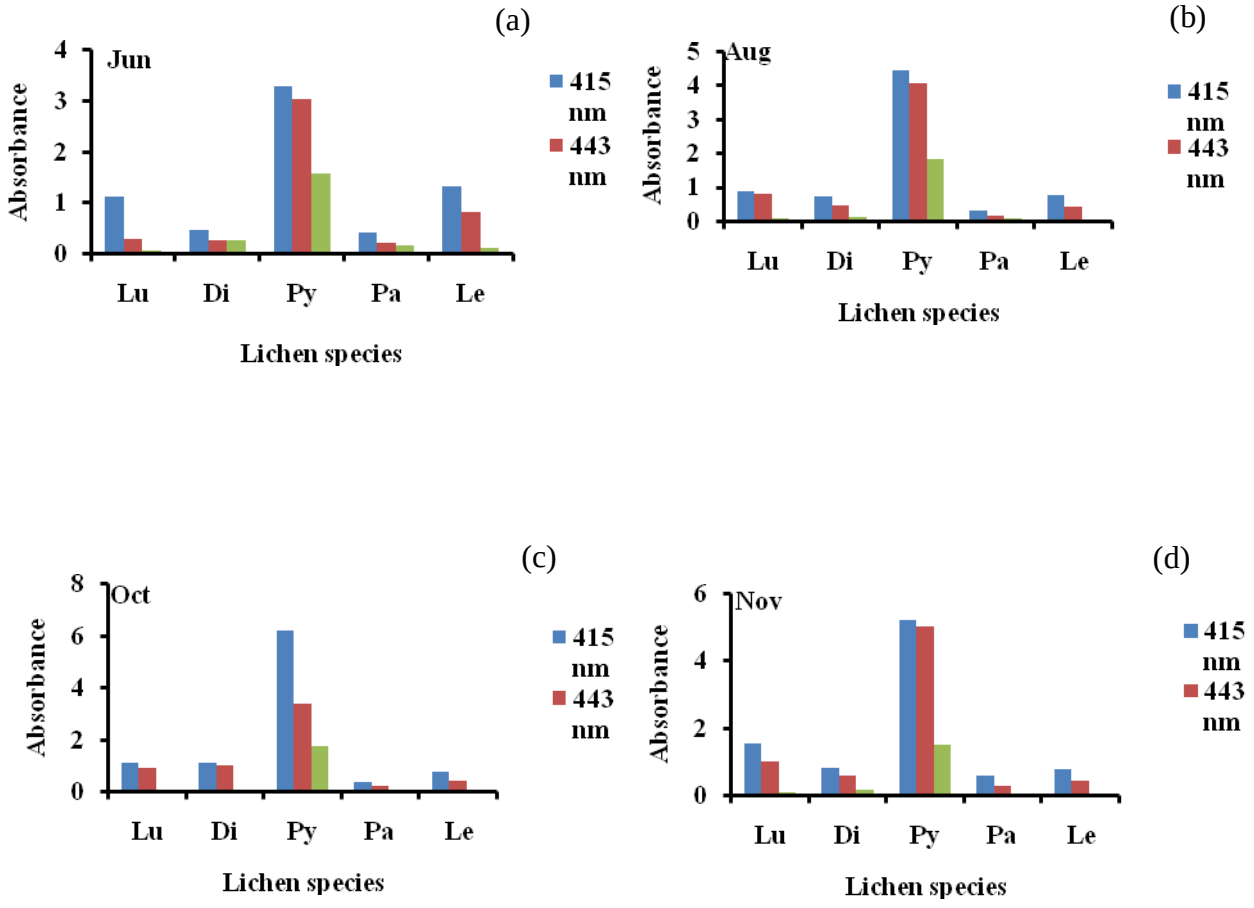


**Figure 4.5** Comparison of chlorophyll absorbance in lichens from Mukdahan Province; (a) June, (b) August, (c) October and (d) November. *D. picta* (Di), *P. tinctorum* (Pa), *P. coccifera* (Py), *L. argentata* (Le), *L. bengurensis* (Lu)

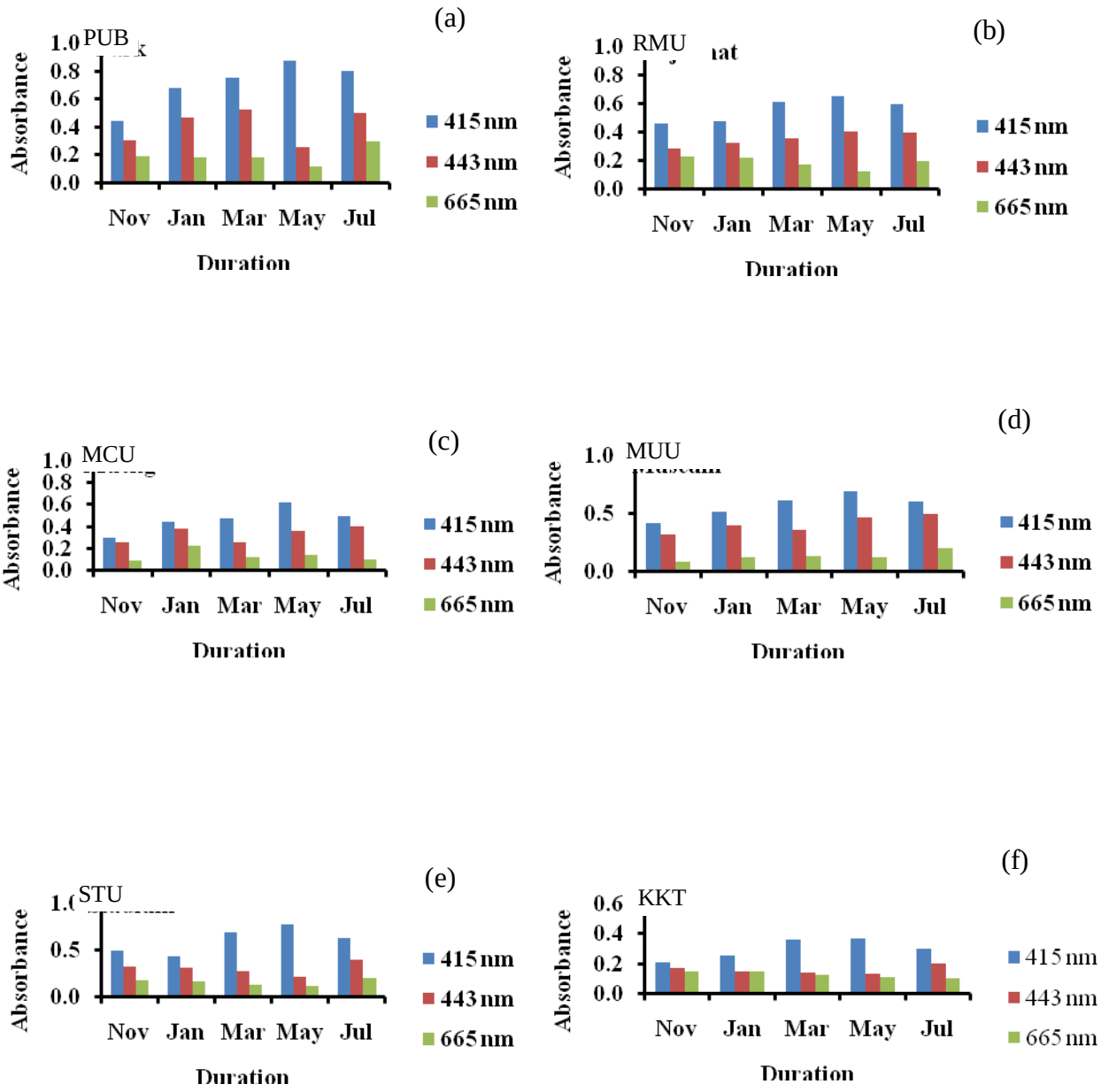


**Figure 4.6** Comparison of chlorophyll absorbance in lichen from Mahasarakham Province; (a) Public Park, (b) Rajabhat Mahasarakham University, (c) Mueng Campus of MSU, (d) Museum of MSU, (e) Stadium of MSU and (f) Kukeo Temple.

# CHAPTER 5

# CONCLUSIONS

## 5.1 Optimization of digestion and method validation

The S/A ratio was studied in the range of 0.1:0.5 to 0.1:3 g $ml^{-1}$. It was found that the optimum S/A ratio for both lichens was 0.1:2.0 g $ml^{-1}$. The digestion temperature was optimized in the range of 50 to 100 °C for both lichens and the result showed that the concentration of heavy metals increased with increasing temperature. Therefore, the optimum digestion temperature was found at 100 °C. The optimization of digestion time was studied in the range of 10 to 60 min. It was found the optimum for both lichens at 40 min. The optimum conditions for proposed apparatus were validated. It was found that the repeatability and reproducibility of the proposed apparatus given good precision.

## 5.2 Sample determination

The monitoring of heavy metals content in foliose and crustose lichens was studied and completed with testing chlorophyll degradation in the Northeast of Thailand (Mukdahan and Mahasarakham provinces).

In Mukdahan province, five lichen species were found and collected from Phouphakud mountain covering dipterocarp forest in conservation area. This province was studied during the rainy season from June to November 2011. The levels of heavy metals concentration in individual months and lichen species were investigated. In months, high concentration of iron, zinc, manganese, copper, nickel and chromium were found in June followed by August, October and November. As, June is beginning of the rainy season, the accumulation of heavy metals in June could be higher than months in the rainy season. In lichen species, maximum concentration of iron, zinc and manganese were found in *P. tinctorum*. While, maximum concentration of copper, chromium and nickel were found in *D. picta*. The result found that foliose lichen more contaminated heavy metals than crustose lichen.

In Mahasarakham province, one lichen species was found and studied as *D. picta*. The determination of heavy metals accumulated in lichen from this province was investigated in dry season from November 2011 to July 2012. Moreover, individual sites surrounding Mahasarakham downtown were studied such as Public Park, Rajabhat Mahasarakham University, Mueng Campus of MSU, Museum of MSU, Stadium of MSU and Kukeo Temple. The monthly study revealed increasing concentration from November 2011 to May 2012 and a decreased concentration in July, with some exception for some heavy metals and sites. The individual sites in Mahasarakham province had different concentrations. The high accumulation of heavy metals in this study was found in the order of Public Park > Rajabhat Mahasakham University > Mueng Campus of MSU > Museum of MSU > Stadium of MSU > Kukeo Temple. Highest concentration of iron, zinc, copper and chromium were found in Public Park. While, maximum concentration of manganese was found at the Museum. Moreover, maximum concentration of nickel was found at the Stadium.

The comparison of heavy metals concentration from both provinces found that the Mahasarakham province was high concentration of each heavy metals than Mudahan province.

Testing chlorophyll found that chlorophyll degradation in both Mukdahan and Mahasakham provinces were found difference. The chlorophyll degradation in lichens from Mukdahan was discussed for months and species. In months, *P. tinctorum* and *D. picta* were found high chlorophyll degradation. While, *P. coccifera* was found in October. *L. benguelensis* and *L. argentata* were found in June. In species, high chlorophyll degradation was found in *P. coccifera* followed by *L. argentata*, *L. benguelensis*, *P. tinctorum* and *D. picta*, respectively.

For monthly comparison from Mahasarakham province, maximum chlorophyll degradation was found in May followed by July, March, January and November. For sites comparison, the high chlorophyll degradations were found in Public Park followed by Rajabhat Mahasarakham University, Stadium of MSU, Museum of MSU, Mueng Campus of MSU and Kukeo Temple

These results indicated that high chlorophyll degradation was found in urban area (Mahasarakham province); while, rural (Mukdahan province) had less chlorophyll

degradation. Moreover, high degradation of chlorophyll was found in months that were part of the dry season.

### 5.3 Application

The data about heavy metals content in lichens from this study can apply for monitoring of heavy metals in the atmosphere as well as pollutant to evaluate the environmental quality. *P. tinctorum* was found more sensitive to air pollution than each lichen species in this study. Therefore this study suggests that *P. tinctorum* could be used for study of monitoring of heavy metal content and chlorophyll degradation in lichen. This research was first study involving heavy metals in lichen in Northeast of Thailand. This data can be used to investigate and environmental index between dry and rainy season. Therefore, the essential data from this study is information that can be led to environmental conservation activities and environmental protection. The data from this study will be used for further study.

# REFERENCES


Albert W (1897) *General lichenology*. USA: New Era Printing Company Lancaster.

Andrzej K, Małgorzata R, Maria W and Witold W (2007) Heavy metal sorption in the lichen cationactive layer. *Journal of Bioelectrochemistry*, 71, 60-65.

Armstrong RA (1996) Are metal ions accumulated by *Saxicolous* lichens growing in a rural environment. *Journal of Environmental and Experimental Botany*, 38, 73-79.

Aslan A, Budak G and Karabulut A (2004) The amounts Fe, Ba, Sr, K, Ca and Ti in some lichens growing in Erzurum province (Turkey). *Journal of Quantitative Spectroscopy & Radiative Ttransfer*, 88, 423-431.

Anderson RA (1988) *Trace minerals in food*. USA: Marcel Dekker.

Ayrault S, Senhou A, Moskura M and Gaudry A (2010) Atmospheric trace element concentrations in total suspended particles near Paris, France. *Journal of Atmospheric Environment*, 44, 3700-3707.

Alirzayeva EG, Shirvani TS, Yazici MA, Alverdiyeva SM, Shukurov ES, Ozturk L, Ali-zade VM and Cakmak I (2006) Heavy metal accumulation in *Artemisia* and foliaceous lichen species from the Azerbaijan flora. *Journal of Forest Snow Landsc.Res*, 80, 1-10.

Adamo P and Violante P (2000) Weathering of rocks and neogenesis of minerals associated with lichen activity. *Journal of Applied Clay Science*, 16, 229-256.

Büdel B and Scheidegger C (1996) *Thallus morphology and anatomy*. UK: Cambridge University Press.

Bačkor M, Peksa O, Škaloud P and Bačkorová M (2010) Photoboint diversity in lichen from metal-rich substrata based on ITS rDNA sequences. *Journal of Ecotoxicology and Environmental Safety*, 73, 603-612.

Bačkor M, Kováčik J, Piovár J, Pisani T and Loppi S (2010) Physiological aspects of cadmium and nickel toxicity in the lichens *Peltigera rufescens* and *Cladina arbuscula Subsp. Mitis. Journal of Water Air Soil Pollut*e, 207, 253-262.

Bacčkor M, Fahselt D and Davidson RD (2003) Effects of copper on wild and tolerant strains of the lichen photobiont *Trebouxia erici* (chlorophyta) and possible tolerance mechanisms. *Journal of Environmental Contamination and Toxicology*, 45, 159-167.

Bajpai R, Mishra GK, Mohabe S, Upreti DK and Nayaka S (2011) Determination of atmospheric heavy metals using two lichen species in Katni and Rewa cities, India. *Journal of Environmental Biology*, 32, 195-199.

Bajpaia R, Upretia DK, Nayakaa S and Kumari B (2010) Biodiversity, bioaccumulation and physiological changes in lichens growing in the vicinity of coal-based thermal power plant of Raebareli district, north India. *Journal of Hazardous Materials*, 174, 429-436.

Bajpai R and Upreti DK (2012) Accumulation and toxic effect of arsenic and other heavy metals in a contaminated area of west Bengal, India, in the lichen *Pyxine cocoes* (Sw.) Nyl. *Journal of Ecotoxicology and Environmental Safety*, 83, 63-70.

Büdel B and Scheidegger C (2008) *Thallus morphology and anatomy.* USA: Cambridge University Press.

Boamponsem LK, Adam JI, Dampare SB, Nyarko BJB and Essumang DK (2010) Assessment of atmospheric heavy metal deposition in the Tarkwa gold mining area of Ghana using epiphytic lichens. *Journal of Nuclear Instrument and Methods in Physics Rearch B*, 268, 1492-1501.

Beyer A and Biziuk M (2007) Applications of sample preparation techniques in the analysis of pesticides and PCBs in food. *Journal of Food Chemistry*, 108, 669-680.

Bennett JP and Wetmore CM (2003) Elemental chemistry of four lichen species from the Apostle Islands, Wisconsin, 1987, 1995 and 2001. *Journal of Science of the Total Environment*, 305, 77-86.

Bingol A, Aslan A and Cakici A (2009) Bio-absorption of chromate anions from aqueous solution by a cationic surfactant-modified lichen. *Journal of Hazardous Materials*, 161, 747-752.

Bechtel R, Rivard B and Azofeifa AS (2002) Spectral properties of foliose and crustose lichens based on laboratory experiments. *Journal of Remote Sensing of Environment*, 82, 389-396.

Bjerke JW, Jones DJ and Callaghan TV (2005) of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic-alpine lichens. *Journal of Environmental and Experimental Botany*, 53, 139-149.

Bosiacka B and Pienkowski P (2001) Content and localization of heavy metals in thalli of hemerophilous lichen. Journal of Environmental Studies, 4, 213-216.

Carballal R, Bermúdez GP, Biezma MJS and Prieto B (2001) Lichen colonization of coastal churches in Galicia: Biodeterioration implications. *Journal of International Biodeterioration and Biodegradation*,47, 157-163.

Conti ME and Cecchetti G (2000) Biologycalmonitoring: Lichens as bioindicators of air pollution assessment. *Journal of Environmental Pollution*, 114, 471-492.

Carreras HA and Pignata ML (2001) Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. *Journal of Environment Pollution*,117, 77-87.

Chaignon V, Neira SI, Herrmann P, Jaillard B and Hinsinger P (2002) Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Journal of Environmental Pollution*, 123, 229-238.

Chettri MK, Cook CM, Vardaka E, Sawidis T and Lanaras T (1997) The effect of Cu, Zn and Pb on the chlorophyll content of the lichens Cladonia conoluta and Cladonia rangiformis. *Journal of Environmental and Experimental Botany*, 39, 1-10.

Crespo A, Kauff F and Divakar PK (2010) Phylogenetic generic classification of parmelioid lichens (*Parmeliaceae, Ascomycota*) based on molecular, morpho logical and chemical evidence. *Journal of Generic classification of parmelioid liches*, 23, 1735-1753.

Carreras HA and Pignata ML (2007) Effects of the heavy metals $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$, and $Zn^{2+}$ on some physiological parameters of the lichen usnea amblyoclada. *Journal of Ecotoxicology and Environmental Safety*, 67, 59-66.

Demiraya AD and Yolcu (2012) Determination of heavy metals etals using the lichen *Xanthoria parietina* in Kocaeli province, Turkey. *Journal of Ecological Indicators*, 18, 632-643.

Deltoro VI, Gimeno C, Calatayud A and Barreno E (1999) Effects of $SO_2$ fumigations on photosynthetic $CO_2$ gas exchange, chlorophyll a fluorescence emission and antioxidant enzymes in the lichens *Evernia prunastri* and *Ramalina farinacea*. *Journal of Physiologia Plantarum*, 105, 648-654.

Ebdon L, Evans EH, Fisher AS and Hill SJ (1998) *An introduction to analytical atomic spectrometry*. UK: John Wiley & Sons.

Edwards HGM, Newton EM, Wynn-Williams DD and Lewis-Smith RI (2003) Non-destructive analysis of pigments and other organic compounds in lichens using fourier-transform raman spectroscopy: a study of Antarctic epilithic lichens. *Journal of Spectrochimica Acta*, 59, 2301-2309.

Ekmekyapar F, Aslan A, Bayhan YK and Cakici A (2006) Biosorption of copper(II) by nonliving lichen biomass of *Cladonia rangiformis*. *Journal of Hazardous Materials*, 137, 293-298.

Flores MM, Barin JS, Mesko MF and Knapp G (2007) Sample preparation techniques based on combustion reactions in closed vessels-A brief overview and recent application. *Journal of Spectrochimica Acta*, 62, 1051-1064.

Friedl T and Büdel B (1996) *Photobionts.* UK: Cambridge University Press.

Friedl T and Büdel B (2008) *Photobionts.* USA: Cambridge University Press.

Fleck H (1971) *Macrominerals and microminerals, introduction to nutrition*. USA, Macmillan.

Fleck H (1972) *Introduction to Nutrition*. USA: Macmillan.

Gadd MG (2006) Geomycology: Biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Journal of Mycological Research*,111, 3-49.

Galun M (1988) *Hand book of lichenology*. USA: Boca-Raton.

Gadd GM (2006) *Fungi biogeochemical cycles*. UK: Cambridge University Press.

Gadd GM (2010) Metals, minerals and microbes: Geomicrobiology and bioremediation. *Journal of Microbiology*, 156, 609-643.

Guschina IA, Dobson G and Harwood JL (2003) Lipid metabolism in cultured lichen photobionts with different phosphorus status. *Journal of Phytochemistry*, 64, 209-217.

Gonzfilez CM and Pignata ML (1997) Chemical response of the lichen *Punctela subrudecta* (NYL) krog transplanted close to a power station in an urban-industrial. *Journal of Environmental Pollution*, 97, 195-203.

Györyová K, Balek V and Kováŕova J (1995) Thermal properties of zinc butyrate complex compounds. *Journal of Thermochimica Acta*, 269, 425-432.

Gregory J (1990) *The diet and health of Adults in the United Kingdom*. UK: HMSO.

Gries C (1996) *Lichens as indicator air pollution.* UK: Cambridge University Press.

Garty J, Levin T, Lehr H, Tomer S and Hochman A (2004) Interactive effects of UV-B radiation and chemical contamination on physiological parameters in the lichen *Ramalina lacera. Journal of Atmospheric Chemistry*, 49, 267-289.

Gonzalo MA, Bermudez, Jasan R and Plà R (2012) Heavy metals and trace elements in atmospheric fall-out. *Journal of Hazardous Materials*, 2-39.

Godinho RM, Verburg TG, Freitas MC and Wolterbeek HTH (2009) Accumulation of trace elements in the peripheral and central parts of two species of epiphytic lichens transplanted to a polluted site in Portugal. *Journal of Environmental Pollution*,157, 102-109.

Grangeon S, Guédron S, Asta S, 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). *Journal of Ecological Indicators*, 13,178-183.

Garty J and Ammann K (1987) The amounts of Ni, Cr, Zn, Pb, Cu, Fe and Mn in some lichens growing in Switzerland. *Journal of Environmental pollution*, 27, 127-138.

Garty J, Karary Y, and Harel J (1993) The impact of air pollution on the integrity of cell membranes and chlorophyll in the lichen *Ramalina duriaei* (De Not.) Bagl. transplanted to industrial sites in Israel. *Journal of Environmental Contamination and Toxicology*, 24, 455-460.

Hawksworth DL and Rose F (1979) *Lichen as air pollution monitors*. UK: Eward Arnold.

Hauck M, Jürgens SR, Willenbruch K, Huneck S and Leuschner C (2009) Dissociation and metal-binding characteristics of yellow lichen substances suggest a relationship with site preferences of lichens. *Journal of Annals of Botany*, 103, 13-22.

Hauck M, Paul A, Gross S and Raubuch M (2003) Manganese toxicity in epiphytic lichens: chlorophyll degradation and interaction with iron and phosphorus. *Journal of Environmental and Experimental Botany on SciVerse*, 49, 181-191.

Hauck M, Paul A and Spribille T (2006) Uptake and toxicity of manganese in epiphytic cyanolichens. *Journal of Environmental and Experimental Botany*, 56, 216-224.

Hauck M, Hesse V and Runge M (2002) Correlations between the Mn/Ca ratio in stemflow and epiphytic lichen abundance *Flora*, 197, 361-369.

Hebe A, Carreras and Pignata ML (2007) Effects of the heavy metals $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$, and $Zn^{2+}$ on some physiological parameters of the lichen Usnea amblyoclada. *Journal of Ecotoxicology and Environmental Safety*, 67, 59-66.

Honegger R (2000) Great discoveries in bryology and lichenology. *Journal of Bryologist*, 103, 307-313.

Hajek J, Vaczi P, Bartak M and Jahnova L (2012) Interspecific differences in cryoresistance of lichen symbiotic algae of genus Trebouxia assessed by cell viability and chlorophyll fluorescence. *Journal of Cryobiology*, 1-8.

Jovan S, Walf SW and Tkacz B (2009) Lichen indicator and climate change. *Journal of Forst Health Monitoring*, 1.

Krishna MVB, Karunasagar D and Arunachalam J (2003) Study of mercury pollution near a thermometer factory using lichens and mosses. *Journal of Environmental Pollution*, 124, 357-360.

Kenkel J (2003) *Analytical chemistry for technicians third edition*. USA: Acid-Free Paper.

Kabata-Pendias A (2001) *Trace element in soils and plants*. USA: Boca Raton London.

Kirchgessner M, Reichlmayr-Lais A and Maier R (1985) *Trace elements in Man and Animal-TEMA*, Farnham Royal, U.K: CAB International.

Karakas SY (2007) Uncertainty assessment including sampling for determination of metals in lichen samples using ICP-OES. *Journal of Chem. Metrl*,1, 10-24.

Kranner I, Beckett PR and Varma AK (2000) *Protocaols in lichenology*. Germany, Springer-Verlag.

Kováčik J, Klejdus B, Hedbavny J and Baćkor M (2010) Effect of copper and salicylic acid on phenolic metabolites and free amino acids in Scenedesmus quadricauda (Chlorophyceae). *Journal of Plant Science*, 178, 307-311.

Käffer MI, Lemos AT, Apel MA, Rocha JV, Martins SMA and Vargas VMF (2012) Use of bioindicators to evaluate air quality and genotoxic compounds in an urban environment in Southern Brazil. *Jouenal of Environmental Pollution*, 163, 24-31.

Kupper H, Kiipper F and Spiller M (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *Journal of Experimental Botany*, 47, 259-266.

Kularatne KIA and Freitas RC (2012) Epiphytic lichens as biomonitors of airborne heavy metal pollutio. *Journal of Auckland, New Zealand Environmental and Experimental Botany*, 1.

Loppi S, Ivanovb D and Boccardia R (2001) Biodiversity of epiphytic lichens and air pollution in the town of Siena (Central Italy). *Journal of Environmental Pollution*, 116, 123-128.

Lajunen LHJ (1992) *Spectrochemical analysis by atomic absorption and emission*. UK: Royal Society of Chemistry.

Lee MR and Parsons I (1999) Biomechanical and biochemical weathering of lichen-encrusted granite: textural controls on organic mineral interactions and deposition of silica-rich layers. *Journal of Chemical Geology*, 161, 385-397.

Lodenius M (1983) Cd, Fe and Zn content of the epiphytic lichen *Hypogymnia physodes* in a finnish suburb. *Journal* of *The Total Environment on Sci Verse Science Direct,* 81-85.

Lomonte C, Gregory D, Baker AGM and Koleva SD (2008) Comparative study of hotplate wet digestion methods for the determination of mercury in biosolids. *Journal of Chemosphere*, 72, 1420-1424.

Lumbsch HT and Leavitt SD (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Journal of Fungal Diversity*, 50, 59-72.

Mukerji KG, Chamola BP, Upreti DK and Upadhyay RK (1999) *Biology of lichens*. Canada: Wiley-Blackwell.

Mitchell R and Gu JD (2010) *Environmental microbiology*. Canada, Wiley-Blackwell.

Mester Z and Sturgeon R (2003) *Sample preparation for trace element analysis*. Canada: Elsevier.

Manrique E, Rodondo FL, Serina E and Izco J (1989) Estimation of chlorophyll Degradation into phaeophytin in *Anaptychia ciliaris* as a method to detect air pollution. Journal of Lazaroa, 11, 141-148.

Mitra S (2003) *Sample preparation techniques in analytical chemistry*. Canada: John Wiley & Sons.

Mendil D, Celik F, Tuzena M and Soylak M (2009) Assessment of trace metal levels in some moss and lichen samples collected from near the motorway in Turkey. *Journal of Hazardous Materials*, 1344-1350.

Monnet F, Bordas F, Deluchat V and Baudu M (2006) Toxicity of copper excess on the lichen Dermatocarpon luridum: Antioxidant enzyme activities. *Journal of Chemosphere*, 65, 1806-1813.

Motrenko HP, Danko B, Dybczynski R, Ammerlaan AK and Bode P (2000) Effect of acid digestion method on cobalt determination in plant materials. *Journal of Analytica Chimica Acta*, 408, 89-95.

Mester Z, Angelone M, Brunori C, Cremisini C, Muntau R and Morabito R (1999) Digestion methods for analysis of fly ash samples by atomic absorption spectrometry. *Journal of Analytica Chimica Acta*, 395, 157-163.

Moraes DP, Mesko MF, Mello PA, Paniz JNG, Dressler VL, Knapp G and Flores EMM (2007) Application of microwave induced combustion in closed vessels for carbon black-containing elastomers decomposition. *Journal of Spectrochimica Acta,* 62, 1065-1071.

Nakayama T, Honjou N, Minaga T and Yashiki H (2001) Effects of manganese and sulfur contents and slab reheating temperatures on the magnetic properties of non-oriented semi-processed electrical steel sheet. *Journal of Magnetism and Magnetic Materials*, 234, 55-61.

Nielsen FH (1988) *Trace Minerals in Foods,* USA: Marcel Dekker.

Nóbrega JA, Santos MC, Sousa RA, Cadore S, Barnes RM and Tatro M (2006) Sample preparation in alkaline media. *Journal of Spectrochemica Acta Part B61,* 61, 465-495.

Nayaka S, Upreti DK, Gadgil M and Pandey V (2003) Distribution pattern and heavy metal accumulation in lichens of Bangalore city with special reference to Lalbagh garden. *Journal of Curent Science,* 84, 5-10.

Nash TH (1996) *Nutrients, elemental accumulation and mineral cycling*. UK: Cambridge University Press.

Nash TH (2008) *Nutrients, elemental accumulation and mineral cycling*. UK: Cambridge University Press.

Nedić O, Stanković A and Stankovi S (1999) Organic cesium carriers in lichen. *Journal of The Science of the Total Environment***,** 1-6**.**

Pawlik-Skowronskaa B and Bačkor M (2011) Zn/Pb-tolerant lichens with higher content of secondary metabolites produce less phytochelatins than specimens living in unpolluted habitats. *Journal of Environmental and Experimental Botany*, 72, 64-70.

Pacheco AMG, Freitas MC, Baptista MS, Vasconcelos MTSD and Cabral JP (2007) Elemental levels in tree-bark and epiphytic-lichen transplants at a mixed environment in mainland Portugal, and comparisons with an in situ lichen. *Journal of Environmental Pollution,* 151, 326-333.

Pollard M, Batt C, Stern B and Young SMM (2007) *Analytical chemistry in archaeology*. USA: Cambridge University Press.

Paoli L, Corsini A, Bigagli V, Vannini J, Bruscoli C and Loppi S (2012) Long-term biological monitoring of environmental quality around a solid waste landfill assessed with lichens. *Journal of Environmental Pollution*, 161, 70-75.

Papong K, Kantvilas G and Lumbch HT (2011) Morphological and molecular evidence places *Maronina* into synonymy with *Protoparmelia* (Ascomycota: *Lecanorales*). *Journal of The Lichenologist*, 43, 561-567.

Plata ER and Lumbsch HT (2011) Parallel evolution and phenotypic divergence in lichenized fungi: A case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). *Journal of Molecular Phylogenetics and Evolution*, 61, 45-63.

Printzen C, Spribille T and Tonsberg T (2008) *Myochroidea*, a new genus of corticolous, crustose lichens to accommodate the *Lecidea leprosula* group. *Journal of The Lichenologist*, 40, 195-207.

Puckett KJ (1976) The effect of heavy metals on some aspects of lichen physiology. *Journal of Canadian of Botany*, 54, 2695-2703.

Poblet A, Andrede S, Scagoliola M, Vodopivez C, Curtosi A, Pucci A and Marcovecchio J (1997) The use of epilithic antarctic lichen (Usnea aurantiacoatra and U. antraca) to determine deposition patterns of heavy metals in the Shetland Islands, Antarctica. *Journal of the Science Total Environment*, 207, 187-194.

Phanwit. Panteethai [online]. 2009 July 3. Available from: hptt://www.panteethai.com [cite 2012 Oct 2].

Řezanka T and Guschina IA (2000) Glycosidic compounds of murolic, protocon-stipatic and allo-murolic acids from lichens of central Asia. *Journal of Phytochemistry*, 54, 635-645.

Rogers PC and Ryel RJ (2007) Lichen community change in response to succession in aspen forests of the southern rocky mountains. *Journal of Forest Ecology and Management*, 256, 1760-1770.

Riddella J, Nash TH and Padgett P (2007) The effect of $HNO_3$ gas on the lichen ramalina menziesi. *Journal of Flora*, 203, 47-54.

Rosenfeld JM (2004) *Sample preparation for hyphenated analytical techniques*. USA: Great Britain.

Rowsell WL, Griffith GS and Jones E (1989) Minerals, applied science for food studies. USA: Longman Group, 97.

Ramel G (2000) *The Earth Life Web* (Online). Retrieve from http://www.Earth Life.Net.

Sensen M and Richardson DHS (2001) Mercury levels in lichens from different host trees around a chloralkali plant in new Brunswick, Canada. *Journal of The Science of The Total Environment*, 293, 31-45.

Schmull M and Hauck M (2002) Extraction methods for assessing the availability of cations for epiphytic lichens from bark. *Journal of Environmental and Experimental Botany*, 49, 273-283.

Simonetti A, Gariepy C and Carignan J (2002) Tracing sources of atmospheric pollution in western Canada using the Pb isotopic composition and heavy metal abundances of epiphytic lichens. *Journal of Atmospheric Environment*, 37, 2853-2865.

Saipunkaew W, Wolseley P and Chimonides JP (2005) Epiphytic lichens as indicators of environmental health in the vicinity of Chiang Mai city. Thailand: Chiang Mai University, 1-12.

Seaward MRD (1996) *Lichens and the environment*. UK: Cambridge University Press.

Sanchez ML (2008) Causes and effects of heavy metal. USA: Nova Science Publishers.

Sarı A, Tuzen M, Uluözlü OD and Soylak M (2007) Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass. *Journal of Biochemical Engineering*, 37, 151-1518.

Shiel AE, Weis D and Orians KJ ( 2010) Evaluation of zinc, cadmium and lead isotope fractionation during smelting and refining. *Journal of Science of the Total Environment*, 408, 2357-2368.

Szczepaniak K and Biziuk M (2003) Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Journal of Environmental Research*, 93, 221-230.

Sooylaki M, Tuzen M, Narin I and Sari H (2004) Comparison of microwave, dry and wet digestion procedures for the determination of trace metal contents in spice samples produced in Turkey. *Journal of Food and Drug Analysis*,12, 254-258.

Sastre J, Sahuquillo A, Vidal M and Rauret G (2002) Determination of Cd, Cu, Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Journal of Analytica Chimica Acta*, 462, 59-72.

Toppia LS, Skowronska PB, Vurro E, Vattuone Z, Kalinowska R, Restivo FM, Musetti R and Skowron T (2007) First and second line mechanisms of cadmium detoxification in the lichen photobiont *Trebouxia* impressa (chlorophyta). *Journal of Environmental Pollution*, 151, 280-286.

Tehler A (1996) *Systematic, Phylogeny and classification.* UK: Cambridge University Press.

Tehler A and Wedin M (2008) *Systematics of lichenized fungi*. USA, Cambridge University Press.

Thosaikham W, Phansi P, Chantiratikul P, Maneechote U and Chantiratikul A (2009) Determination of selenium content in watermeal (Wolffia globosa) supple mental sodium selenate using ICP-MS technique. Pure and apply chemistry. international conference. 14 the January 2009. Naresuan University, Phitsanulok, Thailand. pp. 151-152.

Thomas R (2008) *Practical quite to ICP-MS*. USA: Acid-Free Paper.

Tinggi U, Reillyb C and Pattersod C (1997) Determination of manganese and chromium in foods by atomic absorption spectrometry after wet digestion. *Journal of Food Chemistry*, 60,123-128.

Takeshi KSM, Takashi MRKM and Toshihiko ONK (2003) Accumulation mechanisms of uranium, copper and iron by lichen *Trapelia involuta*. *Journal of Bioresource Technology*, 9, 292-298.

Ulshöfer J and Rosne HJ (2001) GIS-based analysis of lichen mappings and air pollution in the area of Reutlingen (Baden-Wurttemberg, Germany). *Journal of Meteorologische Zeitschrift*, 10, 261-265.

Underwood EJ (1971) *Trace elements in human and animal nutrition*. USA: Academic Press.

Uluozlu OD, Sari A, Tuzen M and Soylak M (2008) Biosorption of Pb(II) and Cr(III) from aqueous solution by lichen (*Parmelina tiliaceae*) biomass. *Journal of Bioresource Technology*, 99, 2972-2980.

Unala D, Isikb NO and Sukatara A (2010) Effects of chromium VI stress on photosynthesis, chlorophyll integrity, cell viability, and proline accumulation in lichen *Ramalina farinacea*. *Journal of Plant Physiology*, 57, 664-669.

Vriesmanna LC, Teófilob RF and Petkowicza CL (2011) Optimization of nitric acid-mediated extraction of pectin from cacao pod husks (Theobroma cacao.) using response surface methodology. *Journal of Carbohydrate Polymers*, 84, 1230-1236

Valladaress F, Sancho L and Ascaso C (1996) Functional analysis of the intrathalline and intracellular chlorophyll concentrations in the lichen family *Umbili-cariaceae*. *Journal of Annals of Botany,* 78, 471-477.

Wang WX and Robert CHD (2005) Metal stoichiometry in predicting Cd and Cu toxicity to a freshwater green alga chlamydomonas reinhardtii. *Journal of Environmental Pollution*, 15, 280-285.

Woess ET, Hua1 G, Gärtner G and Hesse M (2005) Observations in hemichloris antarctica tschermak woess and friedmann (Chlorophyceae) and the occurrence of a second hemichloris species, hemichloris polyspora. *Journal of Plant Systematics of Evolution*, 258, 27-37.

Zotz G and Schleicher T (2003) Growth and survival of the foliose lichen parmotrema endosulphureum in the lowland tropicals of panama. *Journal of Tropical Ecology*, 39-44.

Zhanga ZH, Chaia ZF, Maoa XY and Chen JB (2002) Biomonitoring trace element atmospheric deposition using lichens in China. *Journal of Environmental Pollution*, 120, 157-161.

# BIOGRAPHY

# BIOGRAPHY

| | |
|---|---|
| **NAME** | Mr. Sopha Keo inpaeng |
| **DATE OF BIRTH** | April 9, 1982 |
| **PLACE OF BIRTH** | Luangnamtha Province, Lao PDR |
| **INSTITUTIONS ATTENDED** | National University of Laos 2001-2006: Bachelor of Science degree in Chemistry, Mahasarakham University 2010-2012: Master of Science degree in Chemistry |
| **SCHOLARSHIP** | Asean-European University Network (ASEA-UNINET) |
| **HOME ADDRESS** | Dong Dok Village, Xythany District, Vientiane Capital, Lao PDR |

**OUTPOUT OF THE RESEARCH**

**PUBLICATION**

Keo inpaeng, S., Chantiratikul, P., Papong, K., Sittiput, R. (2011) Determination of iron and zinc in some lichens in Nongsoung District, Mukdahan Province. Proceeding of the 7th Mahasarakham University Research Conference. 8th-9th September 2011, Mahasarakham, Thailand, p 69-73.

**CONFERENCE**

Keo inpaeng, S., Chantiratikul, P., Papong, K. (2012) Determination of iron and zinc in some lichens in Nongsoung District, Mukdahan Province. Proceeding of the 7th Symposium of Lichen from genome to ecosystems in a changing world. 9th-13th January 2012, Bangkok, Thailand, 147.