DEVELOPMENT OF EFFICIENT SAMPLING METHOD USING BARK OF *Cassia fistula* AS BIOINDICATOR AND APPLICATION FOR ASSESSING ATMOSPHERIC HEAVY METALS ACCUMULATION FROM TRAFFIC EMISSION

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DOCTOR OF PHILOSOPHY
IN ENVIRONMENTAL SCIENCE

GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCE

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Rungruang Janta

หัวข้อดุษฎีนิพนธ์	การพัฒนาวิธีการเก็บตัวอย่างที่มีประสิทธิภาพโดยใช้เปลือกต้นราชพฤกษ์เป็นตัวบ่งชี้ทางชีวภาพและการประยุกต์ในการประเมินการสะสมโลหะหนักในอากาศที่ปล่อยจากการจราจร
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บทคัดย่อ

เมืองใหญ่หลายแห่งรวมถึงเชียงใหม่ ประเทศไทย มีการเจริญเติบโตอย่างรวดเร็วควบคู่ไปกับการเพิ่มขึ้นของจำนวนยานพาหนะ ซึ่งสัมพันธ์กับมลพิษทางอากาศ เช่น โลหะและสารมลพิษอื่น ๆ การตรวจติดตามมลพิษจึงมีความจำเป็นเพื่อควบคุมและจัดการมลพิษทางอากาศในอนาคต การใช้ต้นไม้เป็นตัวบ่งชี้ทางชีวภาพของมลพิษทางอากาศมีการใช้ในหลายพื้นที่ แต่วิธีการนี้ยังไม่แพร่หลายในเอเชียตะวันออกเฉียงใต้ งานวิจัยนี้มีวัตถุประสงค์เพื่อนำเสนอความรู้ใหม่ในการนำต้นไม้ท้องถิ่นมาเป็นตัวบ่งชี้ทางชีวภาพสำหรับโลหะที่ปล่อยจากการจราจร ซึ่งงานนี้ได้เลือกใช้ต้นราชพฤกษ์เนื่องจากเป็นต้นไม้พื้นถิ่นในเอเชียตะวันออกเฉียงใต้ได้แก่ ประเทศพม่า ไทย ลาว และอินโดนีเซีย โดยทำการทดสอบปัจจัยที่มีผลต่อการสะสมโลหะบนเปลือกไม้และการหาวิธีการเก็บตัวอย่างที่มีประสิทธิภาพสูง ทำได้ง่าย ราคาถูก และไม่ทำให้ต้นไม้เสียหาย พร้อมทั้งทำการประเมินระดับการสะสมของโลหะจากอากาศบนเปลือกไม้ในเมืองเชียงใหม่

เปลือกของต้นราชพฤกษ์แยกได้เป็น 4 ชั้น จากชั้นนอกสุดไปถึงชั้นในสุดตามลักษณะที่แตกต่างกัน คือ คอร์ก (สีเทา) คลอเรนคิมา (สีเขียว) โฟลเอ็ม (สีแดง) และชั้นรวมของทั้งโฟลเอ็มและวาสคิวลาร์แคมเบียม (น้ำตาลอ่อน) และทำการวิเคราะห์โลหะ 7 ชนิดได้แก่ อะลูมิเนียม โครเมียม ทองแดง เหล็ก นิกเกิล ตะกั่วและสังกะสี โดยชั้นนอกพบความเข้มข้นของโลหะสูงที่สุด จากนั้นความ

เข้มข้นจะค่อยๆลดลงตามลำดับชั้นและสูงขึ้นอีกครั้งในชั้นในสุด ซึ่งแสดงให้เห็นว่าโลหะที่สะสมในชั้นนอกมาจากมลพิษทางอากาศ ส่วนโลหะที่สะสมในชั้นสุดมาจากซึมผ่านจากเนื้อเยื่อลำเลียง ส่วนปัจจัยที่มีผลต่อการสะสมโลหะบนเปลือกไม้นั้นพบว่าความเข้มข้นของโลหะบนเปลือกของต้นไม้ที่เส้นผ่านศูนย์กลางที่ความสูงระดับอก (ดีบีเอช) ในช่วง 5 – 30 เซนติเมตร (อายุประมาณ 4 – 20 ปี) ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) ส่วนการทดสอบผลของทิศทางของลำต้นที่หันสู่ถนนโดยตรงเทียบกับลำต้นด้านตรงข้าม ก็ไม่พบความแตกต่างของปริมาณโลหะบนเปลือกไม้เช่นเดียวกัน ซึ่งอาจจะเนื่องด้วยตำแหน่งของต้นไม้ที่เลือกใช้อยู่ใกล้แหล่งกำเนิดจากการจราจร การทดสอบวิธีการเก็บตัวอย่างเปลือกไม้ โดยเปรียบเทียบวิธีการขูดต้นไม้และวิธีการแปะติดโดยใช้เรซิน พบว่าวิธีการขูดเปลือกไม้มีข้อดีมากกว่า เนื่องจากสามารถใช้ได้กับกรณีความเข้มข้นของสารต่ำได้ มีค่าความผันแปรในกลุ่มตัวอย่างน้อย และมีประสิทธิภาพสูง

ทำการเก็บตัวอย่างด้วยวิธีขูดเปลือกไม้และประเมินการสะสมโลหะจากการจราจรในเมืองเชียงใหม่ โดยเก็บตัวอย่างเปลือกต้นราชพฤกษ์ ที่ปลูกบริเวณริมถนนในอำเภอเมือง เชียงใหม่ จำนวน 115 ต้น และที่อำเภอพร้าว จำนวน 4 ต้น เพื่อเป็นค่าพื้นฐาน โดยทำการเก็บ 2 ครั้งในฤดูแล้งและฤดูฝน ความเข้มข้นเฉลี่ย (ไมโครกรัมต่อตารางเซนติเมตร) ของโลหะที่สะสมบนเปลือกไม้เรียงตามลำดับจากมากไปน้อยได้ดังนี้ อลูมิเนียม (1,238) > เหล็ก (707) > สังกะสี (162) >> ทองแดง (21.1) >> ตะกั่ว (6.37) > โครเมียม (2.14) > นิกเกิล (1.10) ซึ่งปริมาณโลหะที่ตรวจพบไม่มีความแตกต่างระหว่างฤดูอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) ยกเว้น อลูมิเนียมและเหล็ก ที่มีปริมาณสูงในฤดูฝน เนื่องจากได้รับอิทธิพลมาจากฝุ่นดินที่ติดบนถนนหรือล้อรถ ค่าปัจจัยการเพิ่มความเข้มข้นของโลหะแต่ละชนิดมีความสัมพันธ์กันสูง ($r > 0.6$) (โดยการเปรียบเทียบความเข้มข้นของโลหะบนเปลือกไม้กับความเข้มข้นของโลหะชนิดเดียวกันในพื้นที่มลพิษต่ำหรือเทียบกับความเข้มข้นในดิน) หมายถึงโลหะทุกตัวน่าจะมีแหล่งกำเนิดมาจากแหล่งเดียวกัน โดยอลูมิเนียมและเหล็กมาจากฝุ่นดินที่ฟุ้งกระจายในขณะที่ โครเมียม ทองแดง นิกเกิล ตะกั่ว และสังกะสี มีการปลดปล่อยมาจากยานพาหนะ

จากปริมาณโลหะที่สะสมบนเปลือกไม้นำมาคำนวณดัชนีชี้วัดมลพิษ และสร้างแผนที่คุณภาพอากาศของเมืองเชียงใหม่ ซึ่งบ่งชี้ว่าพื้นที่ที่มีมลพิษสูงในเมืองเชียงใหม่ คือ 1) พื้นที่ทางทิศตะวันออกเฉียงเหนือของตัวเมืองซึ่งเป็นสี่แยกขนาดใหญ่ซึ่งใกล้กับสถานีขนส่งเชียงใหม่ และ 2) พื้นที่ทางทิศตะวันตกเฉียงเหนือของตัวเมืองเชียงใหม่ บริเวณสี่แยกใกล้กับคูเมืองเชียงใหม่ ทั้งนี้พื้นที่ทั้งสองมีปริมาณการจราจรและสิ่งก่อสร้างหนาแน่น เนื่องจากเป็นที่ตั้งของโรงพยาบาลและโรงเรียน และพบว่าระดับของมลพิษที่แสดงในแต่ละพื้นที่ได้รับอิทธิพลมาจากทั้งปริมาณการจราจรและความหนาแน่นของสิ่งก่อสร้างซึ่งสัมพันธ์กับศักยภาพของการระบายอากาศ

Dissertation Title	Development of Efficient Sampling Method Using Bark of *Cassia fistula* as Bioindicator and Application for Assessing Atmospheric Heavy Metals Accumulation from Traffic Emission	
Author	Ms. Rungruang Janta	
Degree	Doctor of Philosophy (Environmental Science)	
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ABSTRACT

Many cities including Chiang Mai, Thailand are rapidly growing along with number of vehicles on road. It was related to level of air pollutants such as metals and other toxic substances. Therefore, monitoring of those pollutants is required for further air pollution management and control. Use of trees as air pollution bioindicator is implemented in many areas. However, it is not much used in Southeast Asia. This research aims to provide a new knowledge on using a native tree species as bioindicator for metals emitted from road traffic. *Cassia fistula* is selected due to its common in Southeast Asia including Myanmar, Thailand, Laos and Indonesia. Various, factors affecting on metals accumulation on tree bark were tested. An effective bark collecting method with high efficiency, simplicity, low cost and less damage on trees was investigated and employed. Degree of atmospheric metals accumulation on tree bark in the city of Chiang Mai was also evaluated to assess air quality.

Bark of *Cassia fistula* is separated into 4 layers from outermost to innermost layers by color differentiation; cork (gray layer), chlorenchyma (green layer), phloem (red layer) and a mix of phloem and vascular cambium (white brown layer). Seven species of metals including Al, Cr, Cu, Fe, Ni, Pb and Zn were analyzed. The highest

metal concentrations were observed at the outermost layer. Metal concentration decreased in a deeper layer and increased again in the innermost layer. It was revealed that metals contained in the outermost layer came from atmospheric pollutants, while those in the inner one came from translocation by the vascular tissue. Concentrations of metals found in tree barks with diameters at breast height (DBH) in the range of 5 – 30 cm (age around 4 - 20 year old), were not significantly different ($p > 0.05$). Testing of effect from exposure directions of traffic sources on tree bark sampling, revealed that metal concentration was not affected by tree exposed direction to the road. This might be due to the location of sampling tree was closed to the traffic source. Scrape and adhesive methods were compared for tree bark collecting. It was found that the scrape method provided good results i.e. low detectable level, low variation among replications and high efficiency.

The sampling criteria and the scrape method were applied to assess metals emitted from traffic in the city of Chiang Mai. *Cassia fistula* bark samples (n = 115) were collected from roadside in the city and in Phrao District (n = 4) as background value two times in dry and wet seasons. The mean concentrations (ng/cm^2) of metals accumulated on the bark in descending order were Al (1,238) > Fe (707) > Zn (162) >> Cu (21.1) >> Pb (6.37) > Cr (2.14) > Ni (1.10). Concentrations of metals found were not significantly different ($p > 0.05$) between seasons. The exceptions were Al and Fe, which were high in wet season, because they were bound with soil particulates attached on road surface and vehicle wheels. Relatively strong correlations ($r > 0.6$) were observed among enrichment factors of metals (ratio between metal concentration in bark and that in bark or in soil at the background site), meaning that they probably originated from the same sources. Al and Fe were generated from soil resuspension, while Cr, Cu, Ni, Pb and Zn were from vehicle emission.

Various pollution indices were calculated based on metals accumulation on tree barks. The indices were applied to generate air quality maps of the city. The maps illustrated two high polluted areas in the city; 1) the area in the northeastern side around a big junction closed to Chiang Mai bus terminal and 2) the area in the northwestern side around the intersection near Chiang Mai Moat. Both areas have high traffic volume and dense buildings including hospitals and schools. The degree of pollution was influenced by both road traffic volume and building density in relation to air ventilation capacity.

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LIST OF ABBREVIATIONS

CA	Cluster analysis
cm	centimeter
DBH	Diameter at breast height
cm	centimeter
CRM	Certificated reference material
CF	Contamination factor
EFs	Enrichment factors
EF_B	Enrichment factors of metal concentrations from the ratios between tree barks from study area and background area
EF_{TS}	Enrichment factors of metal concentrations from the ratios between tree barks and soils
GIS	Geographic information system
GPS	Global positioning system
hr	hours
ICP-OES	Inductively coupled plasma optical emission spectrometry
I_{geo}	Geoaccumulation index
$I_{GEO-tot}$	Total geoaccumulation index
LOD	Limit of detection
LOQ	Limit of Quantification
mm	millimeter
ng/cm^2	nano-gram per square centimeters
ND	Not detect
PC	PC
PCA	Principal component analysis
PLI	Potential load index
µm	micrometer
µg/ml	microgram per milliliter

µg/kg	microgram per kilogram
µg/g	microgram per gram

ข้อความแห่งการริเริ่ม

วิทยานิพนธ์นี้ได้นำเสนอความรู้ใหม่สรุปได้ดังนี้

1) เกณฑ์ในการคัดเลือกต้นราชพฤกษ์เพื่อเป็นตัวบ่งชี้ทางชีวภาพสำหรับโลหะที่ถูกปลดปล่อยมาจากการจราจร ซึ่งเกณฑ์ที่ได้สามาถนำไปใช้ในการคัดเลือกต้นไม้พื้นถิ่นเพื่อใช้ในการตรวจติดตามมลพิษทางอากาศในพื้นที่เขตร้อนชื้น

2) วิธีการเก็บตัวอย่างที่เหมาะสมสำหรับต้นไม้ที่มีเปลือกเรียบ ซึ่งการศึกษานี้จะได้วิธีการเก็บตัวอย่าง ซึ่งมีความง่าย ประหยัด และทำให้เกิดผลเสียต่อต้นไม้น้อย เมื่อเปรียบเทียบกับวิธีการเก็บตัวอย่างแบบดั้งเดิม

3) แผนที่คุณภาพอากาศของอำเภอเมืองเชียงใหม่ซึ่งสร้างโดยนำผลของดัชนีชีวัดมลภาวะมาใช้ประกอบกับระบบสารสนเทศภูมิศาสตร์ โดยแผนที่ที่ได้จากงานวิจัยนี้สามารแสดงให้เห็นคุณภาพอากาศตั้งแต่อากาศบริสุทธิ์จนถึงมีการปนเปื้อนจากมลพิษ ข้อมูลที่ได้อาจจะเป็นประโยชน์ต่อต่อหน่วยงานและผู้ที่เกี่ยวข้องในการจัดการคุณภาพอากาศในอำเภอเมืองเชียงใหม่

STATEMENTS OF ORIGINALITY

This study has provided new knowledge as listed

1) Criterias for selection of *Cassia fistula* as bioindicator for metals emitted from road traffic. The criterias can be implemented for selection of native tree species for air pollution monitoring in tropical area.

2) An appropriate collecting method for smooth surface bark. This study provides tree bark collecting method, which is easy, cheap and less damage to the tree comparing to the traditional collecting method.

3) Air quality (AQ) map of the city of Chiang Mai generated by integrating of pollutant indices together with geographic information system (GIS). The AQ map obtained from this research can distinguishing of air quality from clean to polluted. This information might be useful for authorized organizations and decision makers for air quality management in the city.

CHAPTER 1

INTRODUCTION

1.1 Air pollution and air pollutants

The United States Environmental Protection Agency (USEPA) definite the meaning of air pollution in term of environment is the presence of contaminants or pollutant substances in the air that interfere with human health or welfare, or produce other harmful environmental effects. In term of greenhouse gas emission definited meaning of air pollution as one or more chemicals or substances in the air which has high enough concentration would be harmful to humans, other animals, vegetation, or materials. Such chemicals or physical conditions (such as excess heat or noise) are called air pollutants (USEPA, 2013). Therefore, these definitions are concluded that air pollution is a mixture of solid particles or gases in the ambient, while the definition of contaminants is something like to corrupt by contract or association or to make inferior, impure or unfit. The contaminants which are directly released from sources are called primary pollutants, while the result of a combination of primary pollutants is called secondary pollutants. The environmental contaminants are harmful to human, ecosystems, plant, material or anything those society values, when they are contracted. Moreover, they make resource less valuable or less fit to perform their useful purposes. Additionally in term of air quality, the contamination is concentrated of chemical contaminant. It is often related to human health. contaminates also impact to structures and other engineered systems, including historically and culturally important monuments and icons, such as the acid contaminants in rainfall that render it more corrosive than would normally be expected (Vallero, 2008).

Table 1.1 shows the three categories of pollutants in municipal community which are described from their sources and the impacts of those chemicals, including acidifying chemicals, atmospheric oxidants and atmospheric toxic substances.

Table 1.1 The municipal community pollutants categories (Mcdonald, 2012)

Pollutant categories	Dominant chemicals	The example sources
Acidifying chemicals	Sulfur dioxide (SO_2), Nitrogen oxides (NO_x), Ammonia (NH_3)	Fossil fuel production and using
Atmospheric oxidants	Ozone (O_3), Particulate matter (PM), Carbon monoxide (CO), Radicals (OH*, ROO*)	Primary emission and Secondary formation
Atmospheric toxics	Volatile organic compounds (VOCs), Hazardous air, Pollutants (HAPs), Metals (Hg, Pb, Cr)	Specific chemical production and using

Air pollution is a result of both natural events and human activity. Natural events are including volcanic eruptions, wind erosion, pollen dispersal, evaporation of organic compounds and natural radioactivity. However, those natural sources are scantily occurrences when compares to anthropogenic source. Whereas, human activities are involved to air pollution including waste incinerators, manufacturing industries, power plants, automobile engines, household and farming chemicals. The urban atmosphere is the result of pollutants released from the complexity of emissions from anthropogenic activities (Mcdonald, 2012). In urban areas, transportation is a major contributor to local air pollution. Whereas, residential and industrial are contributed to poor air quality either local and long distances area (Citeair, 2007). Vehicles including cars, trucks, boats, buses, motorcycles and other motorized vehicles are the majority of the pollutant emission for both developed and developing countries. Acidifying gases, particulate matter, VOCs and trace toxic chemicals are product of diesel and gasoline combustion (Lewtas, 2007) Emission of gases and particles from transportation are related with

traffic volume. Hence, people who live in area with high traffic density should expose to large amount of traffic pollutants (Brugge *et al*., 2007).

Air pollution would increase in very part of the world especially in eastern China, northern India, the Middle East, and North Africa are considered to have the world poorest air quality in the future. Under the study of business as usual scenario, East Asia will be exposed to high levels of pollutants in 2025 and 2050 (ENN, 2015). Air pollution in Asia is composed of a mixture of pollutants, including particles and gases emitted in large quantities from many different combustion sources, including cars and industries. The rapid of industrialization, urbanization, and motorization are cause of deterioration of air quality in Asia countries. The Clean Air Initiative-Asia Network presents the average air quality levels in mega city of Asian countries between 2000 and 2003 (Figure 1.1), pollutants level in almost of Asian cities are not comply with the WHO air quality guidelines or USEPA but except for developed countries such as Singapore, Taiwan, and Japan. Several Asian cities in China, India, and Vietnam have the highest levels of outdoor air pollution in the world (Su *et al*., 2011).

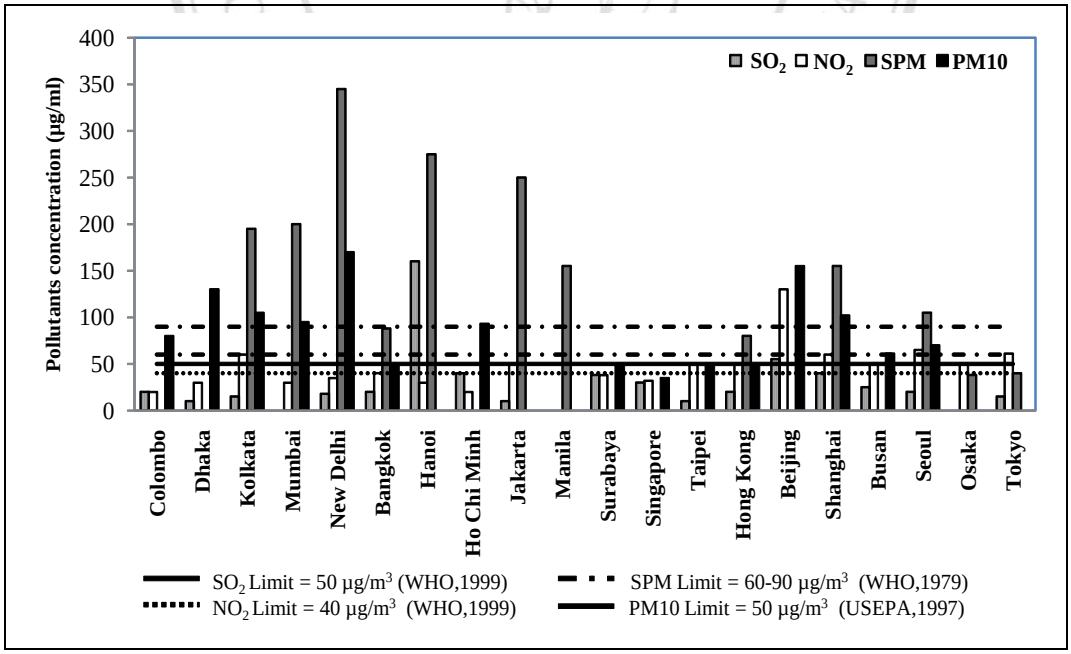


Figure 1.1 The average ambient air pollution levels in Asian cities between 2000 and 2003. (Su *et al*., 2011)

Environmental deterioration in Asia region is from rapid growth of population and increasing of industrial activities. Transportation, fuel combustion and open burning of

biomass and solid waste materials are important anthropogenic sources contributed to large amount of air pollutants emission in Asia counties. Sources of air pollutant in this region are specific and different from those in Europe or North America. Coal is a major energy source using in Asia countries. Therefore, high level of predominant pollutants in coal smoke including suspended particulate matter, SO_2 and NO_2 are observed in this area. Moreover, the burning of biomass, including agricultural wastes, garbage and forest fire is also important source of urban air pollution in South and Southeast Asia because almost countries in this region are agricultural city. Therefore the products of agricultural waste burning including CO, CO_2, nitrogen, methane, nonmethane hydrocarbons, methyl chloride, and PM in various size classes, are contributed directly to urban air pollution. In addition, the rapidly increasing of motor vehicles volume in the region, which it accelerates the emissions of other ambient air pollutants such as VOCs, CO, NO_x, O_3 and metals. The traffic emission in Asian countries is from motorcycle especially in South Asia which has the highest number of motorcycle using in the world. Exposure of traffic pollutants in Asian countries is getting worse because traffic congestion is observed in many countries such as China, India, Indonesia, Pakistan, Pilippines and Thailand (Su *et al*., 2011).

Air pollutants are not only a problem on local region but also problem to neighboring countries because of long-range transport, for example biomass burning episode in Indonesia impacted to all the downwind countries including Malaysia, Singapore, Brunei and southern Thailand. Moreover, the Southeast Asia aerosols may long distances transport due to the mid-latitude wave cyclones which possibly impact on climate and weather patterns. (Vadrevu *et al*., 2014).

1.2 Heavy metals and its toxicity

Metals is defined as elements with metallic luster, the capacity to lose electrons to form positive ions and the ability to conduct heat and electricity. Heavy metal has been known as a group name of metals and metalloids which is related to contamination and potential toxicity or ecotoxicity. According to the review of Duffus (2002), many meaning are definited for heavy metal depending on term of usage. The definition in term of density indicates that it is metal having a density greater than 4 or 5 g/cm^3. Atomic weight definition, it is group of metals that atomic weight is greater than that of

sodium while the atomic weights greater than 40 are also preferred from some report. Moreover, any metals with have atomic number over 20 is classified by atomic number. In biology, heavy metal is referred to a series of metals and also metalloids that can be toxic to both plants and animals at very low concentration (Rascio and Izzo, 2011). Heavy metals have long persistent time in environment especially atmospheric heavy metals. It is able to travel over long distances because there cannot be degraded and destroyed. Moreover, small size heavy metals enters to the body system by intake via food chains, inhalation from ambient air contamination and drinking contaminated water (Lenntech, 2004; WHO, 2007).

Toxicity of substance on the living organism or system is always dependent on the exposure concentration by cells and no substances is always toxic. Therefore, heavy metal toxicity is presented when its concentration is over the critical concentration. Some heavy metals are required at low concentration for cell metabolisms in animals, plants and many micro-organisms but they are toxic at high concentrations. The heavy metals which show those characteristic are indicated as micronutrients or essential element such as Co, Cu, Fe, Mn, Mo, Ni and Zn. (Appenroth, 2010). Normally, heavy metals are dangerous because there can be accumulated in a biological organism and increased in the concentration over time. Their compounds accumulate in living things any time. They are taken up and stored faster than they are metabolized or excreted. Toxicity of heavy metals is evaluated by using quantitative dose–response relationships of substances, which the relationship trend is similar for both plant and animal.

Figure 1.2 presents typical dose-response curves for essential and non-essential trace elements in crops. Essential trace element presents both positive and negative affecting on plant yield. It has benefit on the yield when its concentration lowers than lower critical concentration. However, it is a toxic element when its concentration higher than upper critical concentration. In case of non-essential element, it presents only toxicity when the concentration is over the critical concentration. The maximum concentrations of heavy metal which can contaminate in air, soil and water with no toxicity to the living were presented in Table 1.2.

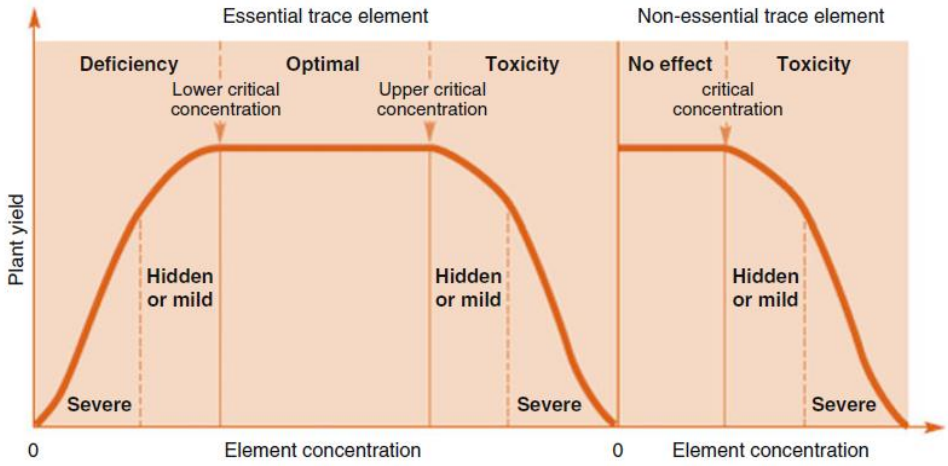


Figure 1.2 Dose-response curves for essential and non-essential trace elements (Appenroth, 2010 referred to Alloway, 2008)

Table 1.2 Maximum level of heavy metals concentration in environment (Duruibe *et al*., 2007)

Heavy metal	Max conc. in air (mg/m^3)	Max conc. in sludge (soil) (mg/Kg or ppm)	Max conc. in drinking water (mg/l)	Max conc. in H_2O supporting aquatic life (mg/l or ppm)
Cd	0.1 -0.2	85	0.005	0.008[3]
Pb	--	420	0.01[1] (0.0)	0.0058[3]
Zn[2]	1,5 [4]	7500	5	0.0766[3]
Hg	--	<1	0.002	0.05
Ca	5	Tolerable	50	Tolerable >50
Ag	0.01	--	0	0.1
As	--	--	0.01	--

Value in bracket is the desirable limit; [1]WHO ; [2] EPA, July 1992; [3]USEPA, 1987; Georgia Code, 1993; Florida Code, 1993; Washington Code, 1992; Texas Code, 1991; North Carolina, 1991; [4]1 for chloride fume, 5 for oxide fume; - - no guideline available.

1.2.1 Role of heavy metals on plants

Heavy metals are essential as a micronutrient for plants in physiological functions including biosynthesis, metabolism, stress resistance and integrity of membranes (Rengel, 2004) because they are constituent in many enzymes and other proteins. However, heavy metal phytotoxicity is occurred when their concentration rises to supra-optimal values of plant. The toxicity of heavy metals on physiological

processes at cellular/molecular level are inactivating enzymes, blocking functional groups of metabolically important molecules, disturbing the metabolism of essential elements and disrupting membrane integrity. A commonly poisoning of heavy metal is extension of reactive oxygen species (ROS) production due to it disrupts the electron transport process in photosynthesis and respiration activities. High concentration of ROS in cells is lead to lipid peroxidation, biological macromolecule deterioration, membrane dismantling, ion leakage, and DNA-strand cleavage (Rascio and Izzo, 2011). It also inhibits seed germination and seedling growth in plant (Li *et al*, 2005). Moreover, Appenroth (2010) presented the descending order of heavy metals toxicity on growth inhibition of *Lemna minor* (the higher plant) as the following, $Ag^{+} > Cd^{2+} > Hg^{2+} > T1^{+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{6+} > Cr^{6+} > As^{3+} > As^{5+}$.

1.2.2 Role of heavy metals on human and animal

Biological essential heavy metals, such as Cu, Ni, Fe and Zn, are required by an organism for healthy growth, DNA and protein synthesis, male and female reproduction, neurological function and immune function because there are constituent and/or activators of enzyme. Moreover, Fe is an essential component of haemoglobin which is oxygen carrier (in the red blood corpuscles) from the longs to other tissues. Although, the essential heavy metals are necessary for health at low concentration but they become toxic at high concentrations. In cause of non-essential heavy metals, such as Pb, Hg, Cd and Sn, are tolerated by human body at low concentration but they are a toxic at elevated concentration (Alloway, 2013). Heavy metals are potentially harmful to exposure such as damaged gill and liver cell lines in fish and many effects in human such as kidney damaged, nervous system damaged, coronary heart disease and bone marrow depression (Nriagu, 1996; Montaser *et al*, 2010). Toxicity of heavy metals on plants, human and animal is influent from several factors including the dose, chemical species, chemical properties such as structure or form, oxidation state and solubility, concentration as well as rout and time of exposure.

1.3 Sources and source identification of heavy metal in environment

1.3.1 Sources of heavy metals in environment

Heavy metals in environment can be originated from both natural and anthropogenic sources. Weathering process of earth crust is one of natural source of heavy metals. Composition and concentration of heavy metals from this process are depended on geologic parent material and environmental condition such as the geologic plant materials generally have high concentrations of Cr, Mn, Co, Ni, Cu, In, Cd, Sn, Hg and Pb (Sharma and Agrawal, 2005). Another source is volcanic gas emission such as Na, K, As, Zn, In, As, Se, and Au which the heavy metals are emitted along with toxic and harmful gases (Gostyns *et al*, 1997). In case of anthropogenic sources, heavy metal is generated from various source including agriculture, industry, traffic, house hold, and energy supply. Summary of anthropogenic source of heavy metal in environment is shown in Table 1.3.

1.3.2 Atmospheric metals from traffic emission source

In atmosphere, air borne metal is mainly bound to particulate matter, while only a small fraction (<5%) is observed in vapor phase (Reddy et al., 2002). Therefore, atmospheric metal is the greatest concern for human health because of its quantity involved, the widespread dispersion and potential for exposure that often exposes (Järup, 2003).

In urban area, heavy metals are released into atmosphere through many sources such as industry, transportation and incineration, especially traffic emission is the main cause of rise up of heavy metals contaminant in metropolitan city. Heavy metal emission sources are from both exhaust and non-exhaust part of vehicle. During engine combustion, fuel and small amounts of engine oil are being burned together then byproduct and heavy metals are emitted to ambient air via exhaust pipe or catalytic converter. Table 1.4 presents concentration of heavy metal in diesel and gasoline fuel which Zn, Cr, Hg and Cu are main component for both types of fuel. For engine oil, heavy metals are added into the oil for component of viscosity index improvers, corrosion inhibitors as well as detergents and dispersants. Sources of heavy metals in a gasoline or diesel engine oil are shown in Table 1.5.

Table 1.3 Anthropogenic sources of metals in environment (Gimeno-García *et al*. 1996; Sharma and Agrawal, 2005; Winther and Slentø, 2010)

Source of Heavy metals	Cd	Cu	Pb	Zn	Ni	Mn	Fe	Hg	Se	As
Industry										
Ore outcrops	+	+	+	+	+					
Metal smelters	+	+	+	+	+					
Blast furnaces				+		+	+			
Electrolysis								+		
Traffic density										
Fuel		+	+	+	+			+		
Tires wear		+	+	+	+				+	
Brake wear		+	+	+			+			
Car body		+	+	+	+		+			
House hold										
Waste	+	+	+	+	+					
Sewage sludge	+	+	+	+	+					
Energy supply										
Coal burning	+								+	
Petroleum combustion					+					
High tension lines		+		+						
Agriculture										
Food additives		+		+						
Phosphate fertilizers	+	+		+						
Pesticides	+	+						+		+

Note; + emitted heavy metal

Table 1.4 Heavy metal content in Danish road transport fuels (Winther and Slentø, 2010).

Emission component		Concentration (µg/kg fuel)	
		Diesel	Gasoline
Arsenic	As	0.1	0.3
Cadmium	Cd	0.05	0.2
Chromium	Cr	8.5	6.3
Copper	Cu	5.7	4.5
Mercury	Hg	5.3	8.7
Nickel	Ni	0.2	2.3
Lead	Pb	0.5	1.6
Selenium	Se	0.1	0.2
Zinc	Zn	18	33

In case of non-exhaust part, heavy metals are content in many parts of vehicle such as tire, break, clutch, paint and body. Zinc content in tire material is in zinc oxide form (vulcanizing agent) which it is contained 1.2% and 2.1% of car tires and truck tires, respectively (Winther and Slentø, 2010). Moreover, other heavy metals are also observed in the tire such as Cu, Cr, Co, Fe, Ni, Pb, Ti (Lindgren, 1996; Luhana *et al*., 2004; Apeagyei *et al*., 2011). Brakes are an important source of Ba, Cd, Cu, Cr, Fe, Ni, Pb, Sb, Ti and Zn (Westerlund, 2001; Folkeson, 2005; Apeagyei *et al*., 2011) which those metals are emitted from friction between metal parts during braking.

Many reports had shown that elevated level of heavy metals is related to traffic volume (e.g. Odukoya *et al*. (2000), Amusan *et al*. (2003), Fakayode *et al*. (2003) and Popescu (2011)). The study of Winther and Slentø (2010) presents calculation result of amounts of heavy metals emitted from each source of vehicle in Denmark in 2007 (Table 1.6). It is found that brake wear is the largest source of heavy metal emission. Therefore, Cu, Zn and Pb which are high composition in vehicle, are major heavy metal species emitted to atmosphere from vehicle. However, amount of heavy metals emitted from transportation is influenced from many factors such as traffic volume, material used, vehicle characteristics (vehicle weight, engine power), road surface characteristics and vehicle operation.

Table 1.5 Common sources of metals in a gasoline or diesel engine oil. (Blackstone Laboratories, 2006)

Elements	Sources
Ag	Trace element
Al	Pistons, bearings, cases (heads and blocks)
B	Detergent/dispersant additive, antifreeze inhibitors
Br	Detergent/dispersant additive
Ca	Detergent/dispersant additive
Cr	Rings, a trace element in steel
Cu	Brass or bronze parts, copper bushings, bearing, oil coolers, also an additive in some gasoline engine oils
Fe	Cylinders, rotating shafts, the valve train, and any steel part sharing the oil
K	Antifreeze inhibitor, additive in some oil types
Mg	Detergent/dispersant additive
Mn	Trace element, additive in gasoline
Mo	Anti-wear additive, coating on some new rings (washes off as break-in occurs)
Na	Antifreeze inhibitors, additive in some gasoline engine oils
Ni	Trace element in steel
P	Anti-wear additive
Pb	Bearings
Si	Airborne dirt, sealers, gaskets, antifreeze inhibitors
Sn	Bearings, bronze parts, piston coatings
Ti	Trace element
Zn	Anti-wear additive

Table 1.6 Total heavy metal emissions by source category for Denmark in 2007.

Emission component	Heavy metal emission (kg)					
	Exhaust		Non-exhaust			Total
	Fuel	Engine oil	Tire wear	Brake wear	Road abrasion	
As	0.8	-	0.8	6.5	-	8.0
Cd	0.5	39.4	2.5	5.2	0.1	47.6
Cr	31.6	65.0	3.4	74.5	22.7	197.2
Cu	21.7	106.5	14.8	51,624.6	11.4	51,779.0
Hg	28.0	-	-	-	0.1	28.1
Ni	4.5	39.4	24.2	72.1	18.2	158.4
Pb	3.3	173.5	76.3	6,682.2	53.8	6,989.1
Se	0.6	-	18.9	13.0	-	32.6
Zn	101.0	7,876.2	9491.7	11,000.9	86.5	28,556.3

1.3.3 Source identification of metals

Regarding heavy metals can be generated from various sources, therefore source estimation of heavy metal is very useful for environmental improvement. Many multivariate statistical models had been used for source identification which principal component analysis (PCA) and Cluster analysis (CA) are effective method for commonalities identification of metal distribution (Facchinelli *et al*., 2001; Davis *et al*., 2009; Wang *et al*., 2015). PCA is a statistical technique that mostly used to reduce the multiple dimensions associated to multivariate analyses and creates new variables (principal components (PCs)) which the variation of the original data is descending order by number of PC (the first PC is the largest fraction). Abril *et al*. (2014) used PCA to classify source of heavy metal from *Tillandsia capillaris* biomonitors which indicated that cement plant, waste dumping site fires, brick kilns, vehicular traffic and soil re-suspension are important source of atmospheric heavy metal in Córdoba, Argentina. Moreover, Ferreira *et al*., (2012) also applied PCA for indentify source of metals accumulation on tree bark, the result demonstrated that cement plants had substantial environmental impacts in Sergipe State, Brazil. On the other hand, CA is well-known method used to classify the data into group or clusters (CL) which objects in same

cluster are same and different from the objects classified into another cluster. The number of clusters is commonly presented though a dendrogram graphical (Pires *et al*., 2008). Normally, PCA and CA are used together for data reduction, there are useful to clearly distinguish from complexity emission source. Lee *et al*. (2006) applied both PCA and CA to present the distinctly difference of elemental association and clustering patterns among metals in urban, suburban and country park soil of Hong Kong which Heavy metal (Cd, Co, Cr, Cu, Ni, Pb and Zn) in country park soil presented closely association with major element in natural material, urban soil indicated anthropogenic source, while suburban soil associated with both anthropogenic and natural input. In case of urban are of China, the result of PCA and CA interpreted that two main sources were identified: (1) Ba, Cu, Pb, Cr and Zn represented industry sources, coal combustion as well as traffic; (2) Mn, V, Co, As, Ni were mainly originated from natural source (Li and Feng, 2012)

In recent years, continuous increase of vehicle volume on the road has been observed in all the mega cities, and large amount of heavy metals has been emitted from parts of automobile. For instance, Zn has been emitted from tire wear, Cu from break lining ware, Fe from brake pads and Pb from gasoline and diesel fuel (Denier van der Gon *et al*., 2007; Kummer *et al*., 2009; Apeagyei *et al*., 2011). Consequently toxic metals which were emitted from road traffic had become to receive more attention worldwide for atmospheric pollution monitoring. Pb, Cu and Zn were common elements, which had been used as potential indicators for road traffic. Assessment of another elements such as Al, Ba, Cr, Fe, Ni, Mn and Mg, had been measured together with those common elements (Odukoya *et al*., 2000; Olajire and Ayodele, 2003; MacFarlane *et al*., 2003; Majolagbe *et al*., 2010; Ukpebor *et al*., 2010; Fujiwara *et al*., 2011).

1.4 Bioindicators and biomonitoring

Current air quality monitoring can be done by both active and/or passive methods, but many commercial samplers involve high costs and need technical support which were limiting factor for sampling and monitoring in big cities and heavily industrialized. Thus, biomonitoring including algae (Topcuoğlu *et al*., 2003), moss (Ogunfowokan *et*

al., 2004), mussel (Giarratano and Amin, 2010), grass (Fatoki, 2000) lichen and tree parts (Pacheco *et al*., 2008; Serbula *et al*., 2012) is interested worldwide.

Bioindicator or biomonitor is using of organism or community of organism to present a situation of environment and how it change over time. Bioindicator is always used to detect changes in the natural environment and monitor for the presence of pollutant and its effects on the ecosystem. The bioindicator has particular requirements for physical and chemical variables such as changes in presence/absence, numbers, physicology, morphology or behavior of interested species. Mostly, bioindicator is used to access anthropogenical effects on ecosystem and minority used for natural effects (Holt and Miller, 2010; Gerhardt, 2015). Advantages of bioindicator are including, it can be indicated the environmental factor which the past are reconstructed such as climatic change, it can be used in the indicated factor is difficult to measure and it can applied for the environmental factor is easy to measure but difficult to summarize such as wheatear the observed changes have ecological significance.

Figure 1.3 present a comparison of environmental tolerances of bioindicator, rare and ubiquitous species. Red areas represent portions of an environmental gradient such as substrate, light, temperature, competition (x-axis) which living organisms or community, has fitness or abundance greater (y-axis) than zero. The dashed line represents the peak performance along this particular environmental gradient, while yellow boxes are presented of tolerance zone. The bioindicator species is moderate tolerance to environmental variability therefore there are effective indicator to environmental change. Rare species has a narrow tolerance zone which meaning is too sensitive to the change, while ubiquitous species (very broad tolerance zone) are less sensitive or nothing disturbs the rest of the community. Using of many species (entire communities) as bioindictor, which there are encompassing a broad range of environmental tolerances, can represent multiple sources of data to assess environmental condition in a "biotic index" or "multimetric" approach.

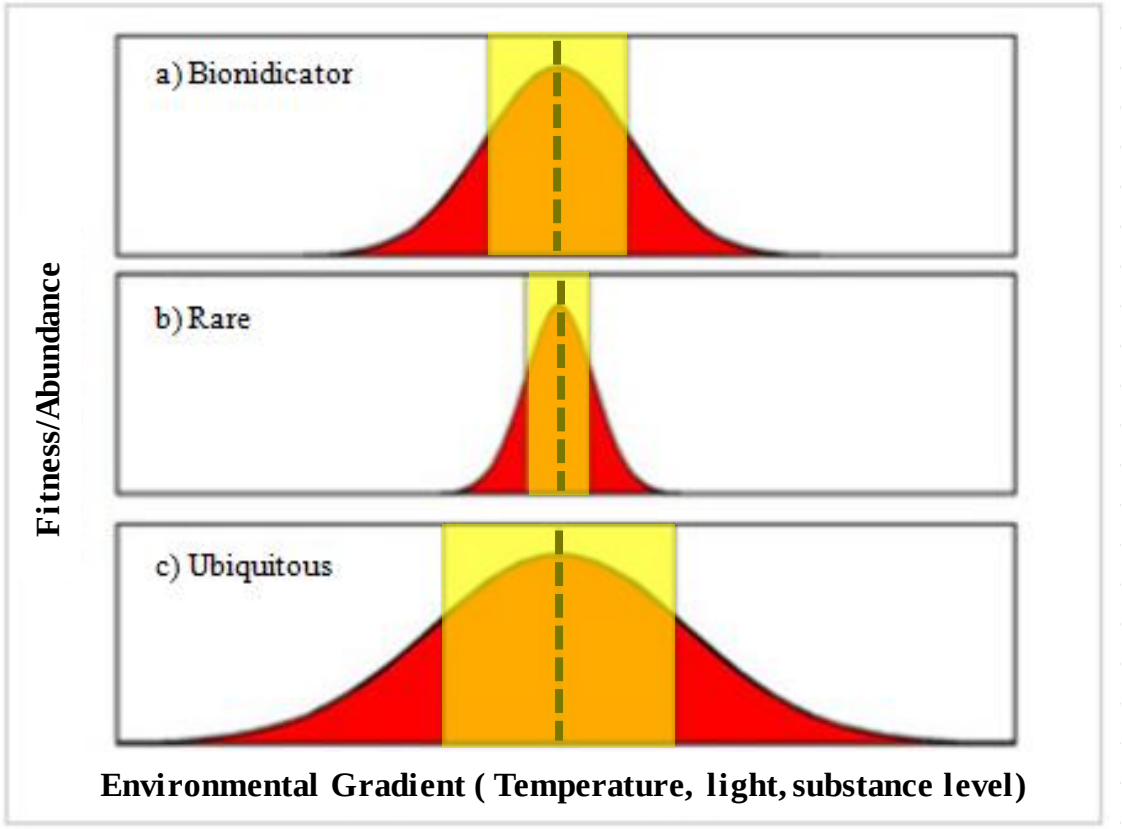


Figure 1.3 Environmental tolerances comparison of each species type, a) bioindicator species, b) rare species, c) ubiquitous species (Holt and Miller, 2010)

The widespread development and application of bioindicators has occurred primarily since the 1960s, there have been applied in studying all types of environments for both aquatic and terrestrial. There are many types of bioindicators including;

*Microbial indicator*s; using of microorganisms as indicators of environmental health, which they can be use for either aquatic or terrestrial ecosystem. Microorganisms are easier to sample than other organisms because there are found in large quantities. Some microorganisms will produce stress proteins (new proteins) which are produced when they are exposed to contaminants such as cadmium and benzene. These proteins are useful to present pollutant level.

Animal indicators; ecosystem change can increase or decrease population of some animal species, therefore there use for environmental indicator. Moreover, other mechanisms of animal including the concentration of toxins in animal tissues, malformation in animal populations and animal behavior, can be used for monitoring.

*Plant indicator*s, there are using of the presence or absence of certain plant or other vegetative life, or contaminants level in/on plant in an ecosystem to indicate the health of the environment. Several types of plant biomonitors, including mosses, lichens, tree bark, bark pockets, tree rings, leaves, and fungi have been applied for environmental monitoring.

1.4.1 Tree as bioindicator for air pollution

Using tree as bioindicator is a popular for air pollution because it is easy to collect the sample and variety of tree species can be used. Moreover, the age of tree is longer than other indicators. Therefore, the tree is useful for long time pollutant monitoring such as bark pockets. Mechanism of atmospheric pollutants accumulation on the bark and tree ring is shown in Figure 1.4. In the first, pollutants emitted from source in the form both dry deposition and wet deposition are transferred to accumulate on surface of outer bark while some pollutants (both soluble and insoluble) are deposited on soil, then the soluble pollutants are dispersed from the soil to root reach the annual ring, which this process may take for several years.

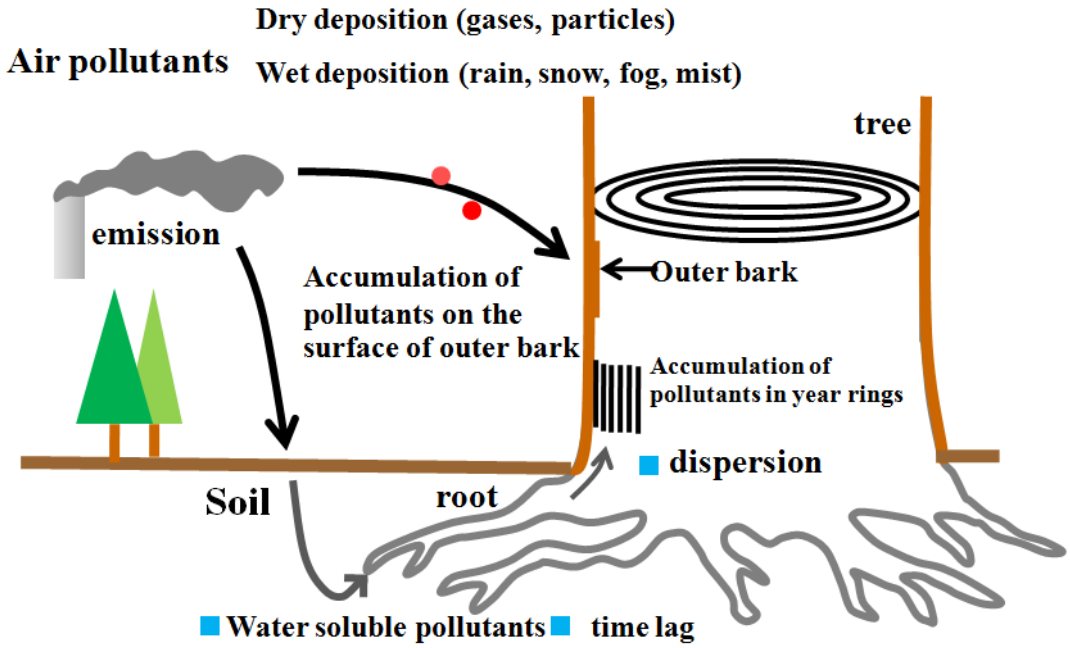


Figure 1.4 Mechanism of pollutants accumulation on the trees and in annual rings (Satake, 2011)

Some tree species have been used successfully to monitor the levels of aerial heavy metals including *Azadirachta indica*, *Casuarina equisetifolia*, *Delonix regia*, *Fraxinus pennsylvanica* and *Pinus massoniana* (Odukoya *et al*., 2000; Kuang *et al*., 2007; Ukpebor *et al*., 2010; Fujiwara *et al*., 2012). Besides, *Cedrus deodara*, *Mangifera indica* and *Pinus sylvestris* were used for PCDD/Fs, PCBs and PAHs monitoring (Chantara, 2000; Orecchio *et al*., 2008; Guguen *et al*., 2011). Sawidis *et al*., (2011) mentioned that tree bark was more effective accumulator for heavy metals (Cr, Cu, Fe and Pb) and better bioindicator of urban pollution than tree leaf because the heavy metal content in tree bark was higher than that in leaves. The tree bark can be used to assess the impact of Cr(VI) as a pollutant in the specific geographical area and it reflected more reliably for heavy metal content, since the bark has high accumulating ability of heavy metals from both atmospheric aerosols and dust for long period (Mandiwana *et al*., 2006). Accumulation of atmospheric pollutants on the bark occurs from both dry deposition and wet deposition (Satake, 2001). The efficiency of heavy metal accumulation of tree bark was different among tree species, and the major effects depended on the bark quality such as surface structure, stand throughfall and stemflow (Ukpebor *et al*., 2010). The accumulation process of airborne substances was started after the external bark sheet had been rejected and a new bark layer (sheets growing from inside) had been exposed to outside (Schulz *et al*., 1999). In study of Odukoya *et al*. (2000), it was showed that using smooth & tough bark as bioindicator was as good as rough & hard bark. More importantly, the native and common tree species, widely cultivated in this study site, had been suggested for study tree section from all literatures.

Accumulation of airborne substances on tree bark takes place on the outer porous layer of bark surface. The gradient of elements concentration such as Fe, S and Ca was significantly observed at outer bark layer between 0.5 and 1.5 mm bark depth and very low concentration levels at deeper layers (Schulz *et al.*, 1999). Therefore, 1.5 mm tree bark depth from the outer bark surface should be adequate to accumulate all pollutants taken up from the air. In previous researches, about 4 – 10 mm in depth with a size of 3 – 10 cm^2 of the outer bark were collected from trunk at 1.2 – 2.0 m above ground level by using a knife (El-Hasan *et al*., 2002; Suzuki, 2006; Berlizov *et al*., 2007; Belivermis *et al*., 2010; Fujiwara *et al*., 2011). The sample height over 1.5 m

from ground level can prevent a result of soil splash by heavy rain (Kilic, 2012). Skrbic *et al*. (2012) evaluated the concentration level of heavy metal such as Pb, Ni, Cu, Cr, Fe, Hg, Zn and Co on linden tree bark (*Tilia* sp.) at 0.5 m and 100 m distance far from roadside in Banja Luka, Bosnia and Herzegovina. The concentration of Cu, Fe, Pb and Zn at 0.5 m distance was about 4 – 10 times higher than that at 100 m distance. Contaminate level from plant decreased exponentially with distance from edge of the road and dropped to the background level at the 10 m, and there were high in downwind direction (Jaradat and Moman, 1999). Then, the distance less than 10 m between study tree and roadside should be proper for traffic pollution monitoring. The age of tree is also an important parameter for pollutant accumulation on tree bark because it related to exposure time of pollutant.

In general, this parameter was correlated with tree diameter, and amount of pollutant accumulation on tree bark was also correlated with tree diameter. However, the correlation was limited by equilibrium of pollutants accumulation on tree bark which depended characteristic of tree bark such as surface type and thickness (Schulz *et al*., 1999). Sawidis *et al*. (2011) reported that amount of metals accumulated in rough surface bark was higher than that in plane surface bark because of high efficiency of air particle trapping and accumulation in rough surface bark tree.

In the traditional sampling method, the bark sample is collected by using a knife for directly taking off tree bark, but this method caused damage on tree bark. Therefore, a new sampling method which is simple, quick and less damage on the tree, was proposed by Hokura *et al*. (2009). Heavy metals deposited on tree bark were collected by using synthetic resin adhesive. This resin is known as epoxy or polyepoxide, a thermosetting polymer formed from reaction of an epoxide with polyamine. Because of excellent adhesion property, it is chosen for heavy metals deposited removing. The resin is applied on tree bark and leaved for hardening and then peeled off. The deposit on only outer layer is removed. In this method, the sampled tree should have smooth surface bark because the resin and deposits are removed easier and clearer than rough surface bark. Traffic pollutants such as Cu, Zn, and Pb are well collected by this method. V, Cd, Sb and W are also effectively collected. The purity of the resin is an important factor for analysis result. The analyzed element should not be contaminated in the resin.

1.5 Use of pollution indices for heavy metal contamination assessment

Environmental quality indices are a powerful tool for evaluation and converging raw environmental information to present level of contamination. Many quality indices are applied in order to assess enrichment of sample. The most common quality indices are the index of enrichment factor (EF), contamination factor (CF), geo-accumulation (I_{geo}) and pollution load index (PLI). Enrichment factor is always used for measurement of potential contamination and the origin assessment of the analysts accumulated in many types of sample such as sediment, soil, mosses, lichen or tree. EF calculation in order to reduce variability of metal content in originated crust, and is an effective tool for plotting geochemical trends across large geographic areas, which have high variations of the original metal content.

In biomonitoring by using tree bark, EF is compared the relative concentration of an analyst accumulated in sample to that in the bark collected from background area (EF_B) or surrounding soil composition (EF_{TS}) in order to investigate accumulation rate and indicate originated source, respectively (Abril *et al*., 2014). Moreover, before comparison the interest element is normalized with crust element in order to reduce regional variation of interest elements before compare with reference material. The normalizing element should be rarely entering to the atmospheric aerosols from anthropogenic sources material (Klos *et al*., 2011). Moreover, Reimann and de Caritat (2005) indicated conditions for normalizing element selection which there should be low in natural regional variation, distributed by geology factor controlling and effected from natural process such as weathering.

The elements, including Sc, Ti, Zr and Al are widely used for EF calculation because there are small variability contain in environment and minute effected from weathering and pedogenesis (Zhang *et al*., 2014). Deely and Fergusson (1994) accepted for using Fe as normalizing element because its distribution was not related to other heavy metals. However, Niencheski *et al*. (1994) indicated that Fe concentration was very high in natural composition therefore it was not expected to be substantially enriched from anthropogenic sources in estuarine sediments (referred by Abrahim and Parker, 2008). Mn is appropriate normalizing element for establishing the enrichment factor and assessing anthropogenic contribution to soil total Hg (Zhang *et al*., 2014). Additionally, Ferreira *et al*. (2012) applied both Fe and Mn for normalization heavy

metal in bark sample in order to identification of anthropogenic origin from cement plant in Brazil. EF_{TS} values of Ca, Cu, S, and Zn were greater than 10 which were indicative of anthropogenic influence. While, EF_{TS} of heavy mental emitted from cement plant Córdoba, Argentina indicated that only Cd and Cu were originated from anthropogenic source (Abril *et al*, 2014). However, the ranges of EFs critical value are quite rough (> 10 indicate anthropogenic source) and not clear (>1 indicate either natural or/and anthropogenic sources) in term of source identification for low polluted area. Therefore, The EFs should be use together with other method such as PCA and CA.

The I_{geo} has been widely utilized for pollution measurement in freshwater sediment (Singh *et al*., 1997), while the PLI represents the number of times by which the heavy metal concentrations in the sediment is higher than that in the background, and presents the overall level of heavy metal toxicity in a particular sample (Priju and Narayana, 2006). Normally, I_{geo} and PLI are applied from soil and sediment polluted. Boszke *et al*. (2004) used geoaccumulation index (I_{geo}) to indicate metals distribution in sediments of Odra (second largest) river in Poland, the result shown that this river was polluted by various metals, especially mercury, cadmium and zinc. Moreover, I_{geo} can be used together with other useful indices including contamination factor (CF), pollution load index (PLI) and enrichment factor (EF) for sediment pollution assessment (Varol, 2011; Godwin *et al*., 2015), not only sediment but other material including soil (Yaqin *et al*., 2008; Mmolawa *et al*., 2011; Jafaru *et al*., 2015), sawash (rice field soil) (Aflizar *et al*., 2015), lichen (Boamponsem *et al*., 2010) and tree (Gueguen *et al*., 2012) are also applied indices to access their degree of contamination. Moreover, Gueguen *et al*. (2012) used new index ($I_{GEO\text{-}tot}$), which is integration of I_{geo} and PLI indices, to classify the degree of pollution for urban environment in the Rhine valley (French–German boundary) and elaborated $I_{GEO\text{-}tot}$ indices map in order to recognize the areas which suffered from pollution.

1.6 Background of this study

Chiang Mai-Lumphun basin is the largest plain area in the Northern of Thailand that rapidly growth of economic to urbanization, especially for Chiang Mai province. Many kinds of pollutants have been introduced through various anthropogenic activities

including biomass burning, cremation, garbage burning, forest fire, industrial process and especially vehicle emission to the atmosphere. Department of transportation reports that the volume of vehicle in Chiang Mai province has been increased over than 2,500 vehicles/year for both motorcycle and car&truck since 2010, especially after the first - car tax scheme project had been started during Oct 2011 – Dec 2012 which number of new registered car are higher than that in new registered motorcycle (Figure 1.5). Therefore, heavy metal pollutant emitted from road traffic in this basin should be increase. Besides, as this province is surrounding by mountains. Thus, all ambient air pollutants still remain inside the basin and causes of health problem of villagers.

According to the data of lung cancer in Chiang Mai Province, the incidence rate is much higher than that in other province in Thailand. The worst air quality in Chiang Mai Province always observed in dry season due to meteorological condition together with anthropogenic activities. The maximum daily average of PM10 in Chiang Mai during 2007-2010 exceeded the national ambient air quality standards (120 $\mu g/m^3$ for 24-h) in all monitoring stations during dry season from February to March (PCD, 2012). Then, pollutant monitoring and pollution mapping is necessary for this area in order to caution and admonish the people about how to practice when rise up of pollutants and need to go through high pollutant area. This study will focus on determination heavy metals from traffic in Chiang Mai Province because of rapidly increase of traffic volume in this area.

Many methods for monitoring of toxic metals contaminated in environment have been applied but the biomonitoring of tree bark is significant interest because it is cheap, easy and there are many choices of tree species. The use of trees bark as bioindicator is well accepted worldwide, but only a few researches was conducted in Thailand. Moreover, finding of suitable sampling methods which give less damage on tree is challenging subject.

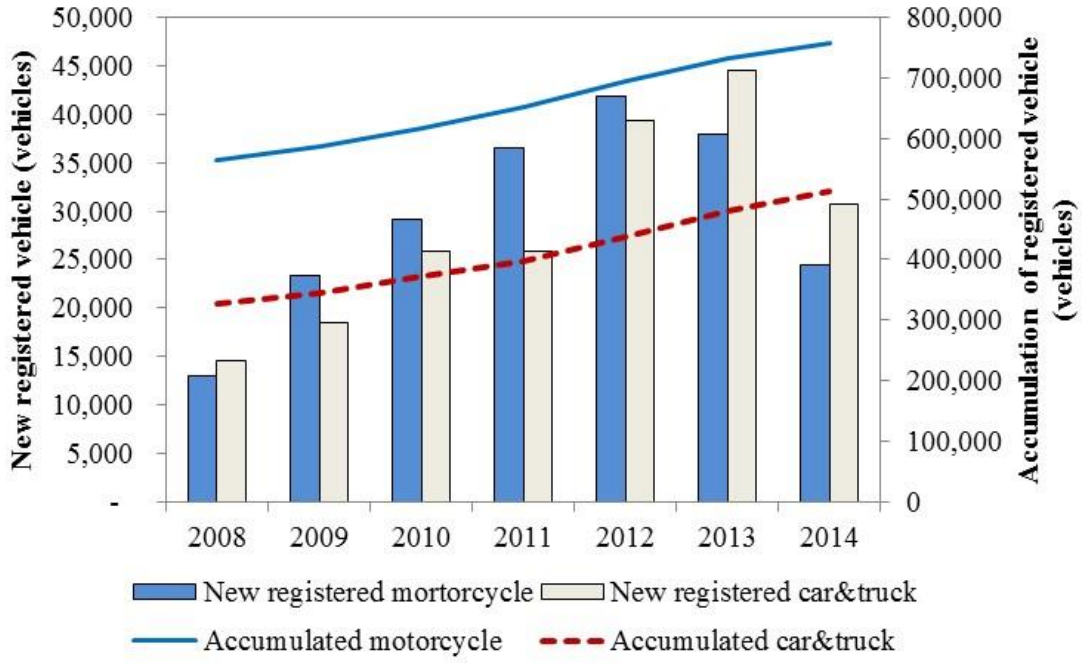


Figure 1.5 Registered number of vehicles in Chiang Mai period 2008- 2014.

Pollutant monitoring by using tree bark is generally applied to the same tree species for prevention of differences of bark features between tree species. Therefore, the selected tree should be extensively found in study area. Although many native tree species are planted in Chiang Mai such as purple bauhinia (*Bauhinia purpurea*), Thai copper pod (*Cassia siamea*), bastard teak (*Butea monosperma*), mango (*Mangifera indica*), bullet wood (*Mimusops elengi*), cananga (*Cananga odorata*), Queen's crape myrtle (*Lagerstroemia speciosa*), tropical almond (*Terminalia catappa*) and devil tree (*Alstonia scholaris*), most of them are improper for environmental monitoring by synthetic resin adhesive sampling method because they have rough surface bark or not exist near roadside.

Using *Cassia fistula* (golden shower tree) is interested in this study because this tree species is a popular ornamental and an herbal medical plant. Moreover it is commonly planted along the road both inside and outside *Cassia fistula* could serve as indicator of air pollution and it was identified as moderately tolerant to air pollution. Moreover, this tree have smooth surface, which is suitable to use of synthetic resin adhesive (less damage sampling method to the tree) for sampling the deposited on bark. Thus, *Cassia fistula* should be an appropriate native tree for heavy metals assessment in Chiang Mai Province. However, the sampling method using the adhesive resin was

proposed by Japanese researchers, and the resin is the Japanese commercial brand which is not available worldwide. Therefore, using of domestic material is more favorable for local or regional environmental monitoring.

Pollutant concentration mapping is the interest application of the pollutants assessment in order to understand characteristic of the pollutant distribution in study area and indicate risky area which may have effect to neighborhood. Moreover, data obtained from the mapping is also useful for local and governmental organizations in terms of environmental management, especially to improve air quality.

1.7 Research Objectives

1.7.1 To find optimum sampling criteria for using bark of *Cassia fistula* as bioindicator by testing factors affecting heavy metal accumulation of tree bark.

1.7.2 To test and find effective sampling methods for heavy metals accumulated on tree bark.

1.7.3 To determine atmospheric heavy metals emitted from road traffic in Chiang Mai by use of *Cassia fistula* bark as bioindicator.

CHAPTER 2

EXPERIMENTAL

2.1 Apparatus, Instruments and Chemical

2.1.1 Apparatus

1) 0.5 mm mesh size nylon sieved
2) 10, 25, 100, 1000 ml volumetric flask, Duran, Germany
3) 10 ml plastic Syringe, Norm-Ject, Germany
4) 1000 µl micropipette, Brand, Germany
5) 20 mm x 80 mm polyethylene spatula for scape method
6) 18 mm x 18 mm glass cover Slip, Sail Brand, China
7) 25.4 mm x 76.2 mm clear glass microscope slides, Sail Brand, China
8) 20 mm chisel for wood
9) 30 ml polyethylene bottle
10) 45 µm, 13 mm Nylon membrane filter, Agela Technologies, USA
11) 450 g rubber mallet, Stanley, USA
12) 25 mm (diameter) core soil sampler
13) 63 mm x 102 mm, 125 mm x 200 mm Polyethylene bag
14) Razor blade, Gillette, Thailand

2.1.2 Equipment/ instruments

1) Double Teflon digestion (50 mm diameter)
2) Analytical balance AB-304S, Mettler Toledo, Switzerland
3) Hot air oven, UE 400, Memmert, Germany
4) Hot plate, C-MAG HS4, IKA, Germany

5) Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Optima 3000, Perkin Elmer, Germany
6) Light Microscopes, CH30, Olympus, Japan
7) Ultrasonicator, 690D, Crest, Malaysia
8) Ultrasonicator, P300H, Elma, Germany

2.1.3 Chemicals

1) Standard reference material (SRM), Clay soil 051, NIST, USA
2) Standard reference material (SRM), Apple Leaves 1515, NIST, USA
3) Standard reference material (SRM), Pepperbush No.1, NIES, Japan
4) Hydrochloric acid (HCl), PH EUR grade, 37%, Merck, Germany
5) Nitric acid (HNO_3), Extra pure grade, 65%, Merck, Germany
6) Hydrofluoric acid (HF), AR grade, 40 – 45%, Sigma- Aldrich, USA
7) Aluminum (Al), standard solution, 1,000 µg/ml, BDH, England
8) Chromium (Cr), standard solution, 1,000 µg/ml, BDH, England
9) Copper (Cu), standard solution, 1,000 µg/ml, BDH, England
10) Iron (Fe), standard solution, 1,000 µg/ml, BDH, England
11) Manganese (Mn), standard solution, 1,000 µg/ml, BDH, England
12) Nickel (Ni), standard solution, 1,000 µg/ml, BDH, England
13) Lead (Pb), standard solution, 1,000 µg/ml, BDH, England
14) Zinc (Zn), standard solution, 1,000 µg/ml, BDH, England
15) Deionized water (conductivity < 0.15 mS/m), Chemistry Department, Faculty of Science, Chiang Mai University
16) Milli Q water, Chemistry Department, Chiang Mai University
17) Powdered Precision Cleaner, Alconox, USA
18) Epoxy adhesive, Cemedin, Japan
19) Epoxy adhesive, Duro, Germany
20) Epoxy adhesive, X'traseal, Malaysia

2.2 Experimental framework

Native tree species (*Cassia fistula*) was selected and use as bioindicator accumulated of metals emitted from traffic. The study is consisted of 2 sections including 1) sampling method testing and 2) criterias for tree bark sampling and analysis of metals accumulated in tree barks. Experimental framework of both sections are presented in Figure 2.1-2.2.

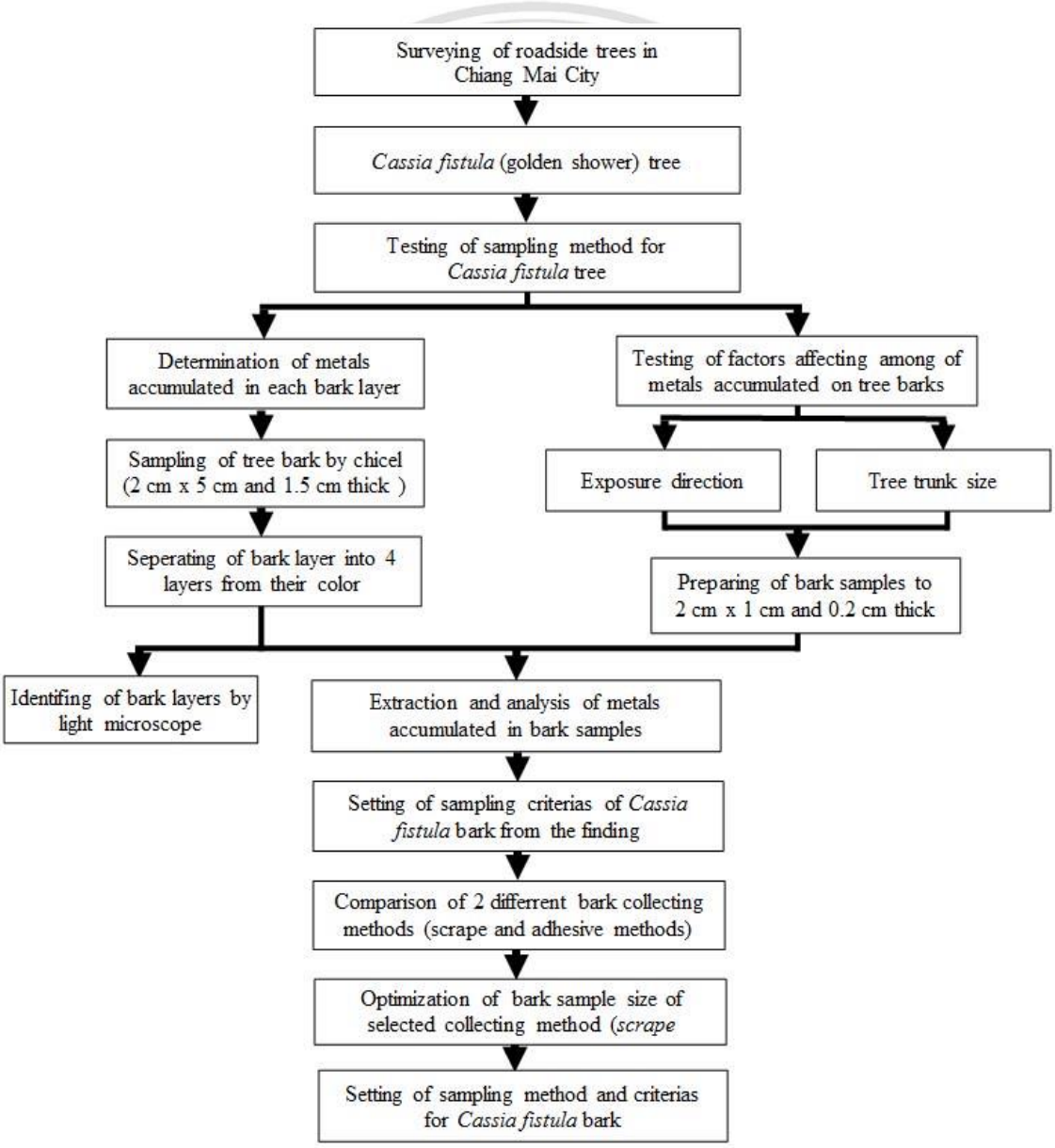


Figure 2.1 Diagram of experimental framework for sampling method testing

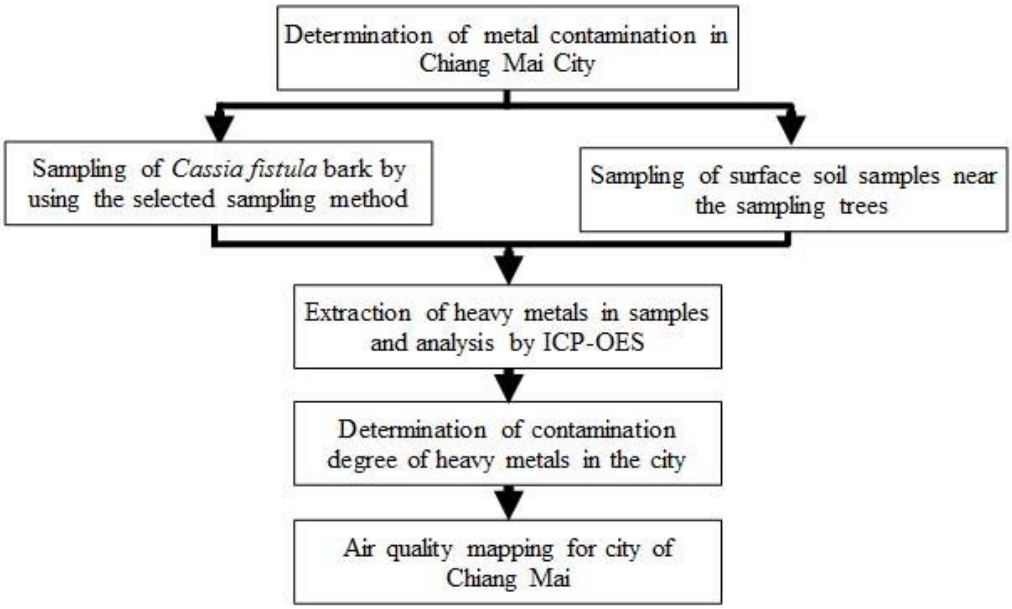


Figure 2.2 Framework of using *Cassia fistula* as bioindicator for metals accumulation

2.3 Sampling tree

The concentrations of metals were measured from roadside tree. Golden shower tree (*Cassia fistula*) is selected for this study because it is the common species in Chiang Mai city. This species is commonly grown along the road in Chiang Mai province in order to decoration and shading (Figure 2.3).

Figure 2.3 Planting of *Cassia fistula* on road side

Cassia fistula is deciduous plant which is native in Southeast Asia and distributed across India, Indochina, and Malaysia. It has also been introduced to the tropical regions of Africa and America. The scientific classification of this tree is showed in Table 2.1. The *Cassia fistula* is fast-growing and medium-sized tree. It can grow up to 15 m in height and 60 cm diameter at breast height (DBH). Its trunk is straight, and the open crown consists of horizontal and extended branches with sparse foliage. The trunk consists of hard reddish wood. Stem bark is pale grey, smooth and slender when young and it is changed to dark brown and rough when old. It has compound leaves with 4-8 pairs of opposite leaflets (12 - 28 cm long). This tree always shed leaves for a short period of time and quickly replaced with new leaves in summer. During shed out of leaves, it produces yellow flowers and hang in has been decorating with yellow flowers and hang in clusters up to 40 cm long, although individual flower length is 2 – 4 cm. This tree produces a dark brown bean-pod 30 - 60 cm long filled with a sticky pulp and 40-100 hard brown seeds (Rocas, 2002; Staples and Herbst, 2005; Gilman and Watson, 2014). *Cassia fistula* is used for ornamental and shading around houses, road edge, parks, and gardens. The DBH (~1.5 m above ground level) of almost tree on the road edge of Chiang Mai is 5 – 30 cm and the bark is smooth.

Table 2.1 The scientific classification of *Cassia fistula*

Scientific Classification	Golden shower tree
Kingdom	Plantae-Plants
Subkingdom	Tracheobionta-Vascular plants
Superdivision	Spermatophyta-Seed plants
Division	Magnoliophyta-Flowering plants
Class	Dicotyledonae
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Cassia*
Species	*fistula*

Source: Staples and Herbst (2005)

2.4 Study area in Chiang Mai Province

2.4.1 Geographical background, population and economic structure

Chiang Mai province is located in Northern Thailand, 700 km north of Bangkok (capital city). It is the second largest city in Thailand. Its coordinates are between 17°15′ - 20°06′ N (latitude) and 98°05′ - 99°21′ E (longitude) at elevation 310 meters above sea level. This province is situated in Chiang Mai –Lamphun besin on the coast of the river Ping. Figure 1.4 presents landuse map of Chaing Mai province, the province covers an area of 12,566,911 rai, which consists of 10,380,924 rai (82.6%) of forest, 1,830,686 rai (14.6%) of agricultural land and 355,300 rai (2.8%) of residential and other land (National Statistical Office, 2012).

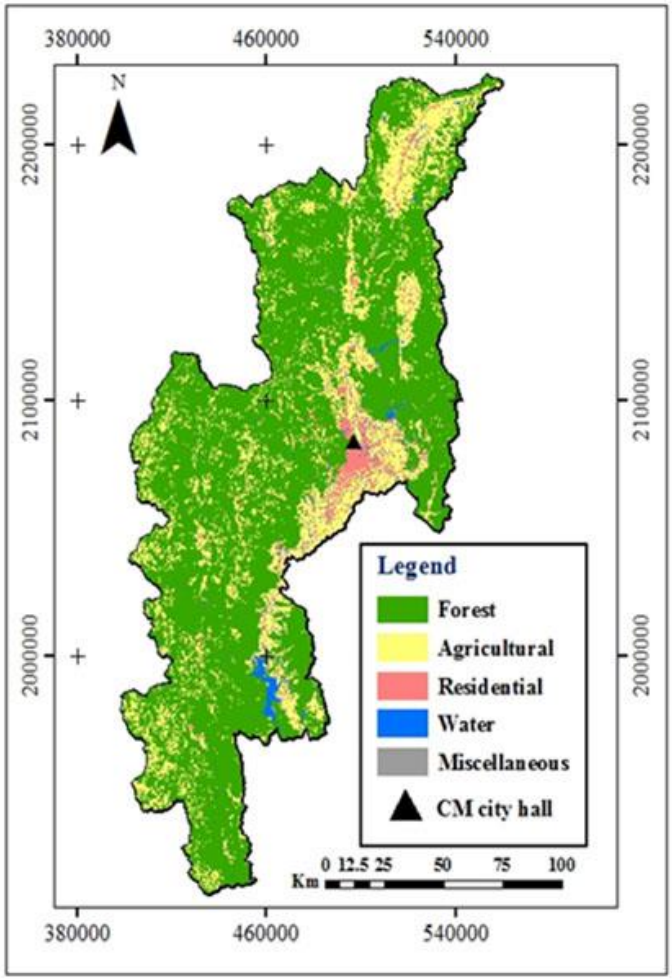


Figure 2.4 Landuse map of Chiang Mai Province

Chiang Mai Province consists of 25 districts. National Statistical Office (2010) reports total population in this province is 1,640,479 people. The population density of whole area is 81.6 people per km^2. Chiang Mai City or Muang district, which is central administrative district of the province, covering an area about 152.4 km^2, has a population of about 238,332 people and the population density averages 1,563 people per km^2 (National Statistical Office, 2010) which is the highest density among districts in Chiang Mai province.

The weather of Chiang Mai is characterized by monsoons. The northeast monsoon brings cold dry air from the anticyclone in China mainland over major parts of Thailand (most prevalent in December-January), it presents cool season and the southwest monsoon brings stream of warm moist air from the Indian Ocean towards Thailand (most prevalent in July-August) causing abundant rain (rainy season). The period from mid-February until the end of May is the transition period between both monsoons which this period have no influent from monsoons and intensive thermal low therefore the hottest weather is observed in this period. (Thailand Meteorological Department, 2014; One stops Chiang Mai, 2015). The annual average temperature is 27.0 ºC. The highest temperature is 39.2 ºC in April and the lowest is 11.0 ºC in January. The annual average humidity is 71.3 %. An annual number of rainy days are 122 days, while the total rainfall is 1,074.9 mm. (Northern Meteorological Center, Thailand, 2015).

Chiang Mai is one of two tourist destinations in Thailand because it has both cultural and natural tourist attraction. There are around 7 million visitors annually and 54 billion baht revenues from tourism, which the period from December to the end of February is a travel season for this area (Chiang Mai provincial Office of Tourism and Sports, 2015). Consequently, this province is rapidly growing to urbanization, especially in the city of Chiang Mai.

2.4.2 Sampling Sites

The sampling areas were located in Chiang Mai province (Figure 2.5a), Thailand. The high pollutant sampling site was located in the city of Chiang Mai, while Phrao district was selected to present a low pollutant sampling site. The geological of Chiang Mai City and Phrao sampling site is shown in Figure 2.5b). The sampling area

consists of whole area of Mueang Chiang Mai district and the border area of Mae Rim, Sansai, Doi Saket, San Kamphaeng, Saraphi and Hang Dong district. The area covers approximately 272 km^2 in which the major use areas are 64% community area and 23% forest area. Traffic volume on main roads in Chiang Mai city is ranging from 3,000 – 80,000 vehicles/day (Figure 2.6).

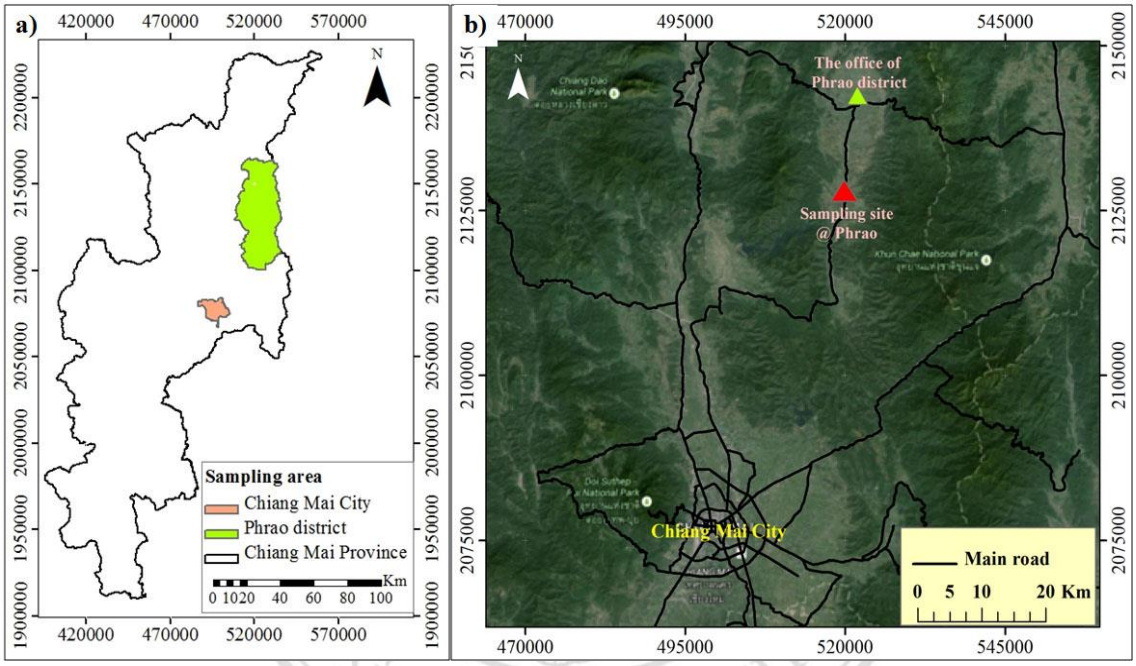


Figure 2.5 Sampling sites a) Location of sampling sites in Chiang Mai province, b) satellite map of Chaing Mai City and Phrao sampling sites

Figure 2.6 Road characteristic at Chiang Mai city sampling site a) Sri Poom road, b) Huay Kaew road

Normally, *Cassia fistula* found in Thailand are mainly from human cultivation for decoration and shading purpose. The tree is rarely found in natural area with no interference from human activity. Therefore, the low pollutant sampling sites were chosen from Chiang Mai-Phrao road at Mae Pang municipality, Phrao District. It is around 90 km from Chiang Mai city and 13 km from Phrao district office. The used areas within radius 5 km around the sampling site are agricultural area (52%) and forest (34%). The traffic volume is relative low compared to the city of Chiang Mai, Phrao sampling site is shown in Figure 2.7

Figure 2.7 Road characteristic at Phrao sampling site

2.5 Sampling method of tree barks

The *Cassia fistula* were selected based on the distance from the road (< 3 m). Their bark skin must be smooth in order to prevent different of ability in metal trapping and accumulation due to various types bark surface (Sawidis *et al*., 2015). The bark sample was collected at 1.5 - 2.0 m above ground level in order to prevent contamination from soil sprat. However, knowledge in using *Cassia fistula* as bioindicator is limited. Therefore, the study was separated into 3 parts including criteria for determination sampling of *Cassia fistula* bark, bark sampling method and use of *Cassia fistula* bark for metals and estimation of pollution degree in Chiang Mai City.

2.5.1 Criteria for sampling barks of *Cassia fistula*

In order to set criteria for sampling the *Cassia fistula* bark, characteristics of the bark including sequence of bark layers and their metal content were determined. Moreover, factors affecting of heavy metal accumulated in the bark including exposed direction to source and size of tree trunk were also tested.

1) Characteristics of bark layer

Five bark samples were collected from five *Cassia fistula* planted along Manee Nopparat Road (18.796°N 98.981°E). The selected trees must have a measured at 1.5 m above ground level (diameter at breast height (DBH)) of tree trunk ranging from 21 – 30 cm (Figure 2.8). This sampling area is in the city center and the road has high level of traffic density (~ 60,000 vehicles/day). Bark sample with an area of approximately 10 cm^2 (2 cm x 5 cm) and 15 mm depth were collected by a chisel from the tree side facing the road (Figure 2.9).

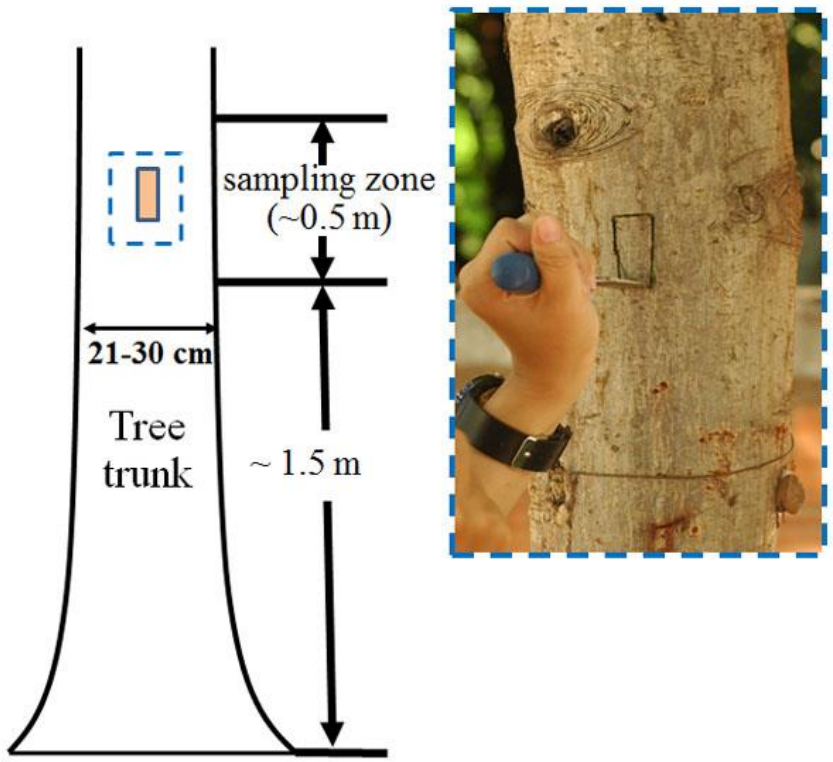


Figure 2.8 Diagram and picture of tree bark sampling from a tree trunk by a chisel

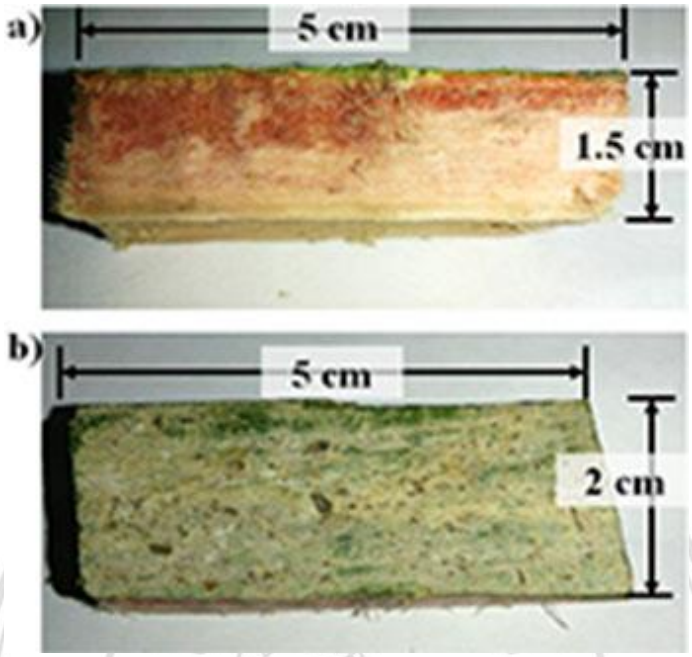


Figure 2.9 *Cassia fistula* bark sample for bark layer determination a) side view, b) top view

2) Affecting factors of metal accumulation on bark

The affecting factors of metal accumulation on bark are 1) the exposed direction to source and 2) size of the tree trunk selected as sampling trees. Bark samples were collected by a chisel with the same method as mentioned in a previous section.

2.1) Tree exposed direction to traffic source

The bark samples were taken from both direction, which were 1) tree bark directly exposed to road side (direct exposed side) and 2) the opposite sides of the same tree (indirect exposed side) (Figure 2.10). Five roadside trees on Manee Nopparat Road with DBH ~ 21 – 30 cm were selected and 5 bark samples were collected from each side of the trees.

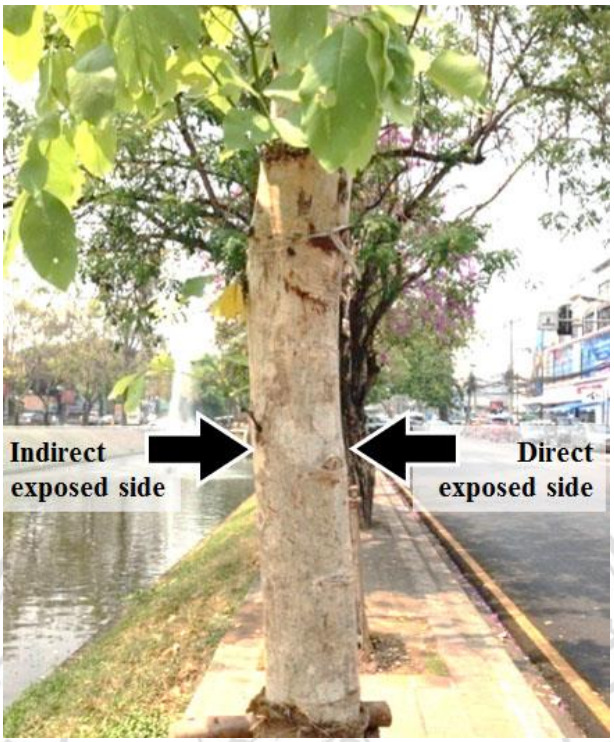


Figure 2.10 Exposure direction of tree to road side

2.2) Size of tree trunks

The *Cassia fistula* planted along Huay Kaew Road (18.805°N, 98.962°E) were selected. Bark samples were taken from the side that directly exposed to the road. Three sizes of trees based on their DBH (5– 10 cm, 11– 20 cm and 21– 30 cm) were selected for this experiment. Each DBH size range was collected from 5 trees. In total 15 bark samples were collected.

2.5.2 Tree bark sampling methods

Two collecting methods including adhesive and scrape methods were compared. This method is less damage for trees after bark sample removal. Different types of adhesive resin (2 Thai brands and 1 Japanese brand) were used in this study.

1) Scrape method

Approximately 2 cm x 5 cm block area was made on the tree bark by using a flat plastic spatula (polyethylene material with a size of 8 cm x 2 cm). The outer layer of the bark was scraped into polyethylene bag. Only gray color was taken in bark scraping. The sampling scrape direction was done from the right to the left side of the block in order to prevent contamination (Figure 2.11).

Figure 2.11 Steps of bark sampling by scrape method a) making sampling area on bark surface, b) collecting bark sample, c) tree bark surface after collecting

2) Adhesive method

This method is using synthetic resin adhesive material, which is the sticky material, to sampling the outermost layer of tree (Hokura *et al*., 2009) The adhesive material consists of 2 components which are epoxy resin part and hardener part. Both components (1:1 v/v) were mixed on a plastic plate. The mixed material were pasted on the bark with in a block (2 cm x 5 cm) and leaved for 4 hrs for hardening. After that it was peeled off and kept in a polyethylene bag for further analysis (Figure 2.12).

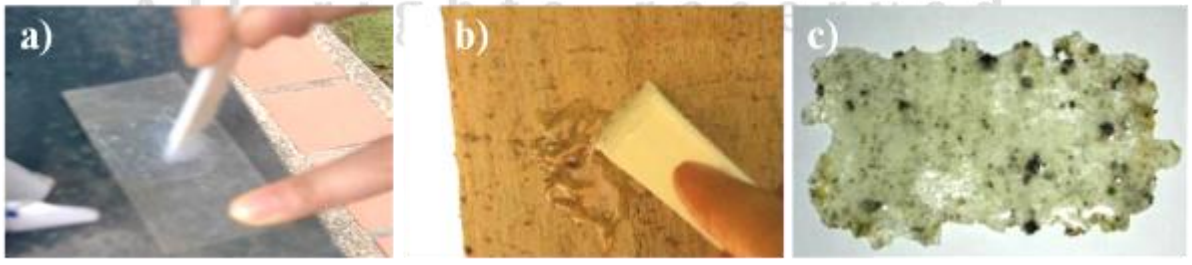


Figure 2.12 Steps of bark sampling by adhesive method a) mixing of adhesive materials, b) pasting resin on tree bark, c) adhesive sample after peeling

Both collecting methods were tested on the same tree in order to prevent any variation caused by different trees. Figure 2.13 shows positions of collecting methods on the tree. The selected trees were from 2 study sites; Manee Noparat Road in Chiang Mai City represented high pollution area (n = 5) and Chiang Mai-Phrao road illustrated low pollution area (n = 5).

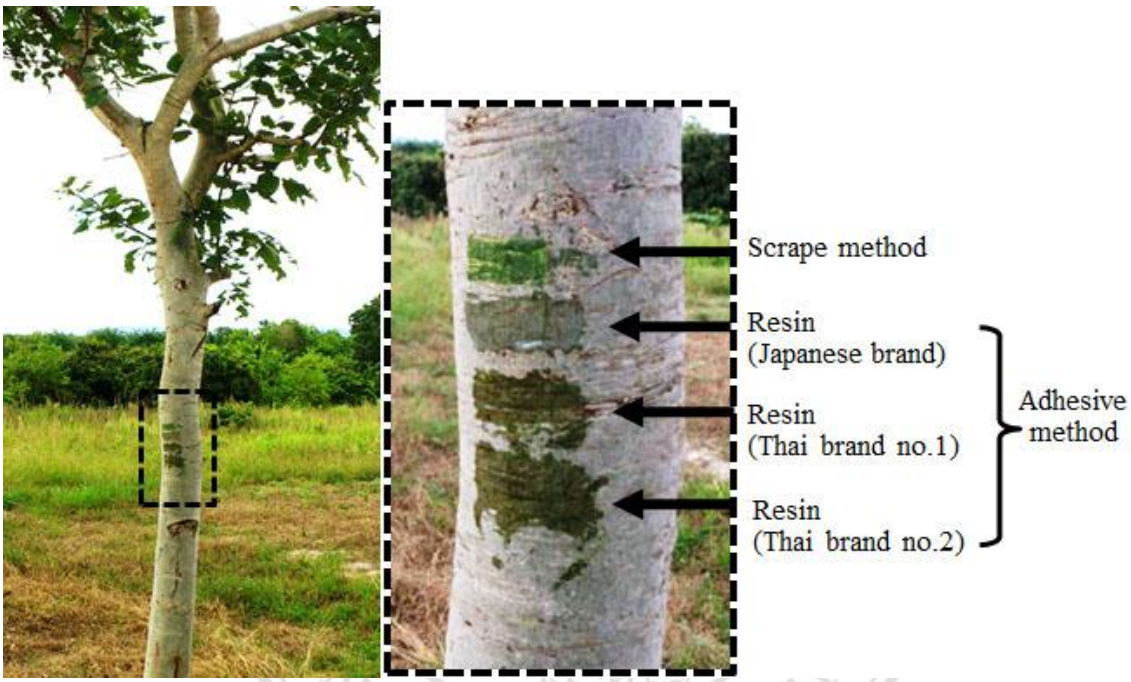


Figure 2.13 Position of different collecting methods on *Cassia fistula* trunk

2.5.3 Optimization of bark sample size

In this study, a scrape method was preferable for *Cassia fistula* bark collecting. However, surface area of bark sample was optimized in order to improve analysis efficiency for analysis of metals accumulation on the bark. Two sizes of bark samples; 10 cm^2 (2 cm x 5 cm) and 20 cm^2 (4 cm x 5 cm) surface area were collected from the same tree (Figure 2.14).

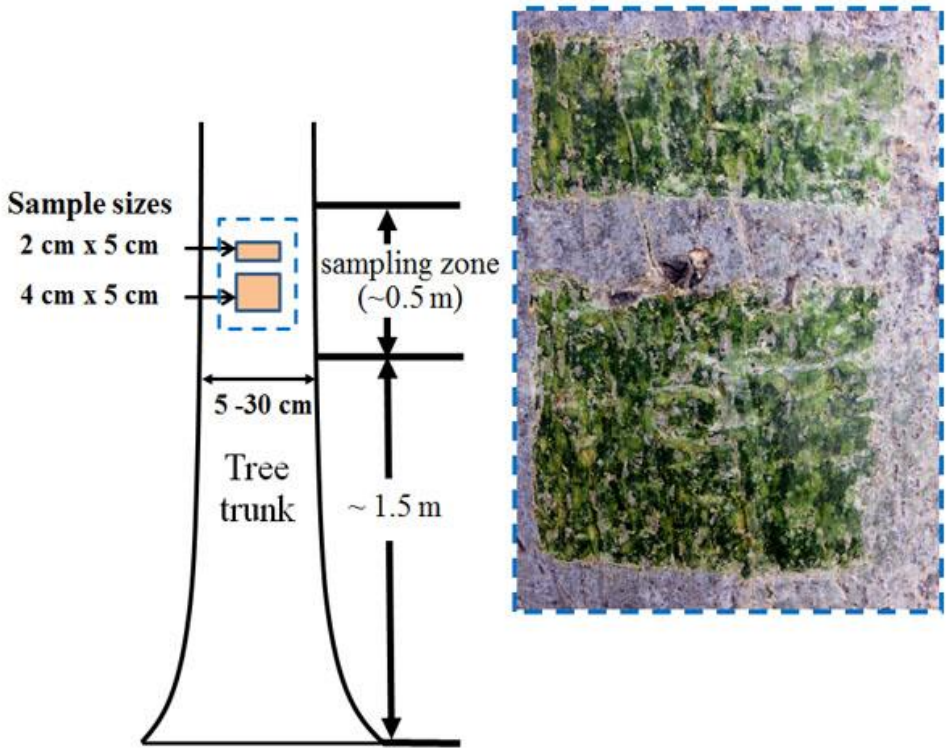


Figure 2.14 Collecting areas of bark samples on the same tree trunk

2.5.4 Sampling of bark samples from *Cassia fistula* in Chiang Mai City

After testing all mentioned factors affecting tree bark samples, the sampling criteria, the collecting method and the suitable sample size were set. Bark samples were collected from *Cassia fistula* with DBH (~1.5 m above ground level) between 5 – 30 cm by scrape method. Approximately 20 cm^2 (4 cm x 5 cm) surface area of bark was collected from the tree trunk either from the direct exposed side to the road or the opposite side. Sampling locations in the city were divided into urban and sub-urban areas based on population density and human activities (Bootdee *et al.*, 2012). Sampling position are shown in Figure 2.15a). The urban area (the inner pink circle) is categorized as 5 km radius from the city center. It consists of residential area, schools and business buildings. The sub-urban area (the outer part of the pink circle) comprises of residential area, agricultural area and park. The mean volume of vehicle on main roads in urban area (~ 24,000 vehicles/day) was lower than that in sub-urban area (~ 29,000 vehicles/day) because main roads in urban area are normally short and narrow, while those in sub-urban area are wider and longer.

Bark samples of *Cassia fistula* were collected 2 times in different seasons to investigate leaching impact from rain. The 1st sampling (yellow dots) was carried out in January 2014 (dry season and high tourist season) and the 2nd sampling (black triangles) was in September 2014 (wet season). In the first sampling, 79 bark samples were collected from the trees in urban area, while another 36 samples were collected in sub-urban areas. In the second sampling, only 26 trees (urban) and 14 trees (sub-urban) from the first sampling were selected (Five trees/direction for each 8 directions from city center). Position of the sampling points on tree trunk from the 1st and the 2nd samplings are at the same level and close to one another as shown in Figure 2.16.

Moreover, four samples of tree barks of *Cassia fistula* located near Chiang Mai-Phrao road were collected in September 2014 as background values (Figure 2.15b). This sampling site was located ~ 15 km far away from Phrao District office in South direction. It was surrounded by agricultural and forest areas. Therefore, this sampling site was selected for background area.

All bark samples were kept in polyethylene bags and stored in a refrigerator at 4^{o}C until analysis.

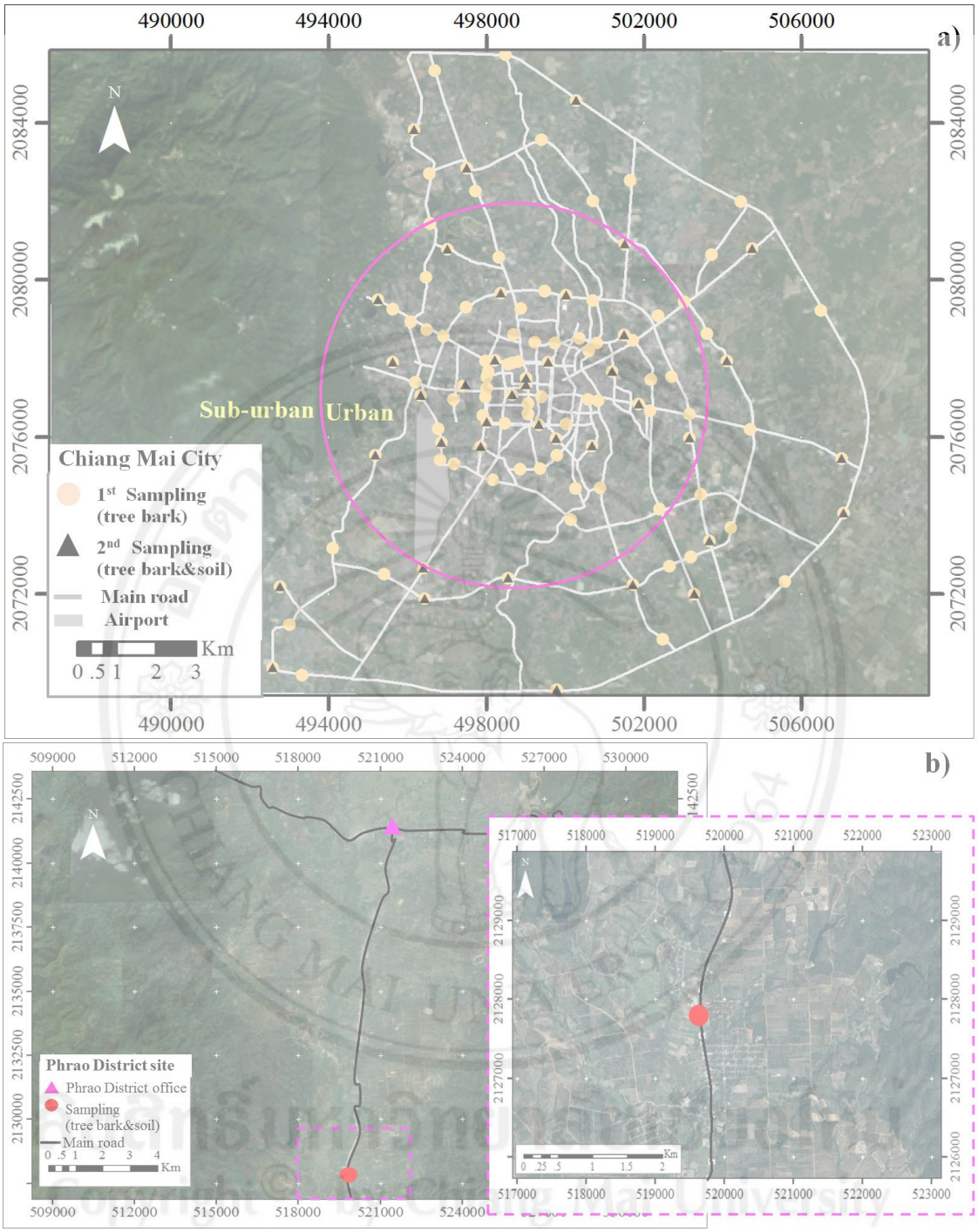


Figure 2.15 Map of sampling tree (*Cassia fistula*): a) Chiang Mai City, b) Phrao District

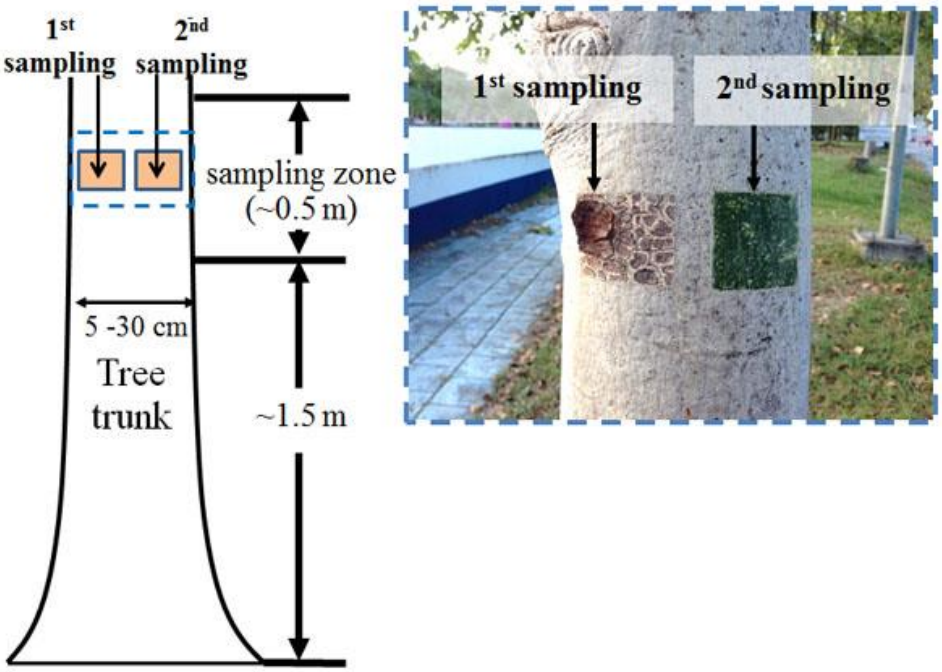


Figure 2.16 The 1st and 2nd sampling points on *Cassia fistula*

2.6 Sample preparation of tree barks

After sample collection, bark samples were prepared by different methods for each experiment i.e. characteristic of tree bark and factors affecting metals accumulated on tree bark.

2.6.1 Bark sample preparation for determination of tree bark characteristics

The bark samples of *Cassia fistula* were determined for metals accumulation in each layer and for identification of bark layers.

1) Metals accumulation on *Cassia fistula* bark

Bark samples were separated into 4 layers by using stainless knife from the outermost layer to the innermost layer by color differentiation, starting from gray (1st layer), green (2nd layer), red (3rd layer) and white brown (4th layer), which layers of bark sample are shown in Figure 2.17.

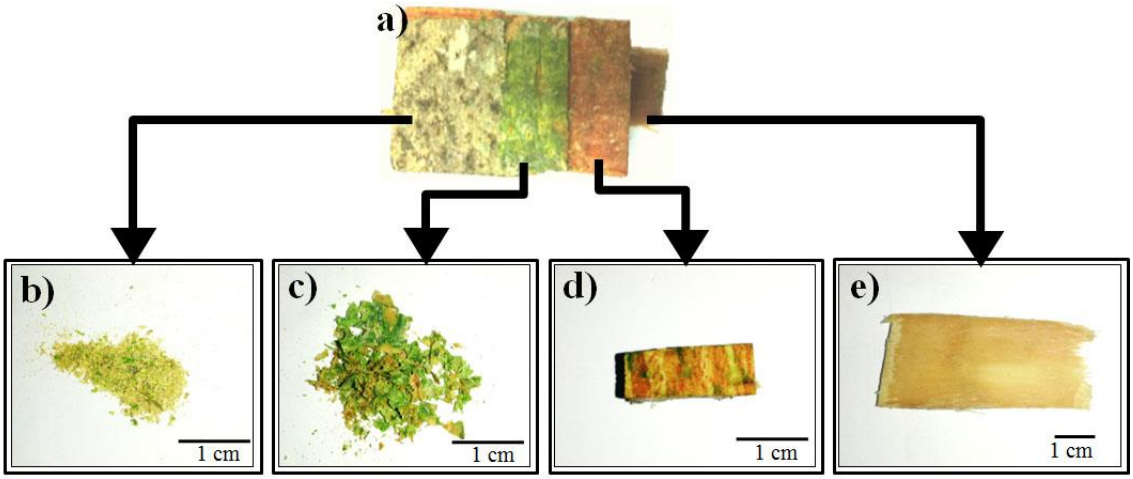


Figure 2.17 Layers of *Cassia fistula* bark a) bark sample, b) 1st layer, c) 2nd layer, d) 3rd layer, e) 4th layer

2) Bark layer identification

Each tree bark sample was slided by fresh free-hand tangential sections into 4 layers. The bark layer samples were prepared with water on clear glass microscope slides and covered with cover slip (Figure 2.18). Light microscope was used to assess the morphological characterizations for each layer. The bark layers were identified from cell characteristics and the tissue position.

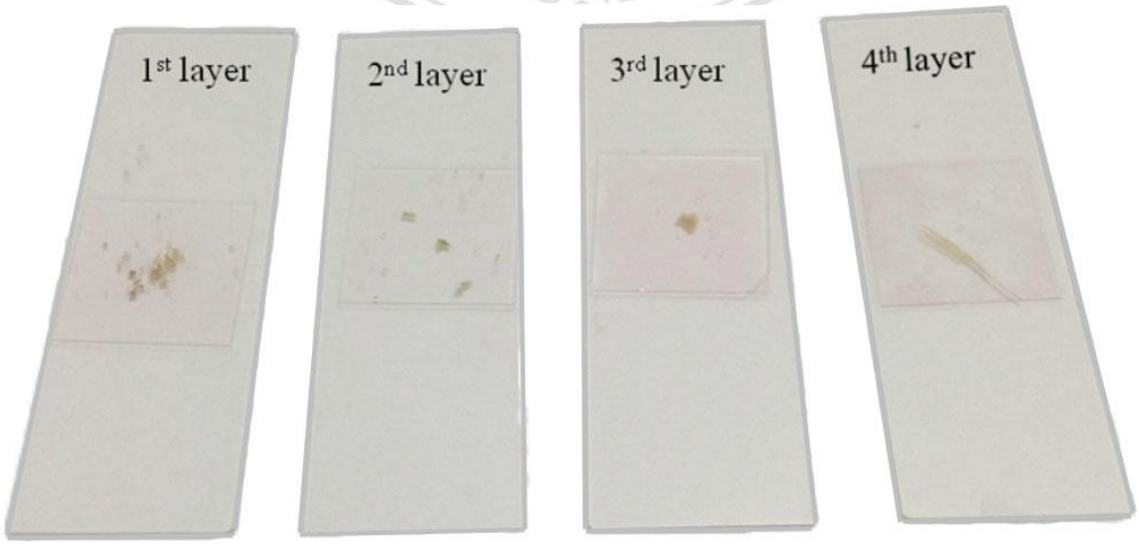


Figure 2.18 The sample of bark layers for optical microscope

2.6.2 Bark sample preparation for factors affecting on heavy meals accumulated on tree bark

The collected bark samples were prepared to approximately 2 cm x 1 cm area and 2 mm thick prior metal analysis (Figure 2.19). All bark samples were kept in polyethylene bags and stored in a refrigerator at 4^{o}C until analysis.

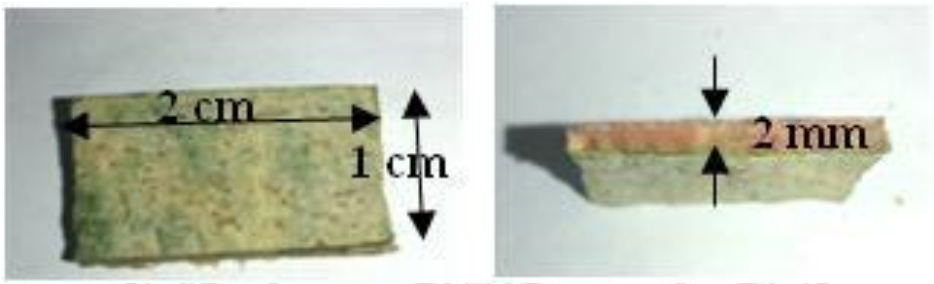


Figure 2.19 The bark sample prepared for testing of factors affecting on heavy metals accumulated on the bark

2.7 Sampling method and sample preparation for surface soil sample

Forty surface soil samples were collected in September 2014 from the same location of the sampling trees in the 2nd sampling (Figure 2.15). A core soil sampler (2.5 cm diameter) was used to collect approximately 0 – 10 cm depth soil samples (Figure 2.20 a). Three random points within 1 m radius around the sampling tree were collected and mixed to get 1 sample. The samples were kept in plastic bags and transferred to the lab. Soil samples were dried in a hot air oven at 80 ^{o}C for 24 hr. After cool-down, they were grinded in a mortar and sieved through a of 0.5 mm mesh size nylon sieve. Pictures of samples before and after sieving are shown in Figure 2.20 b) – c). The samples were kept in polyethylene bags until analysis.

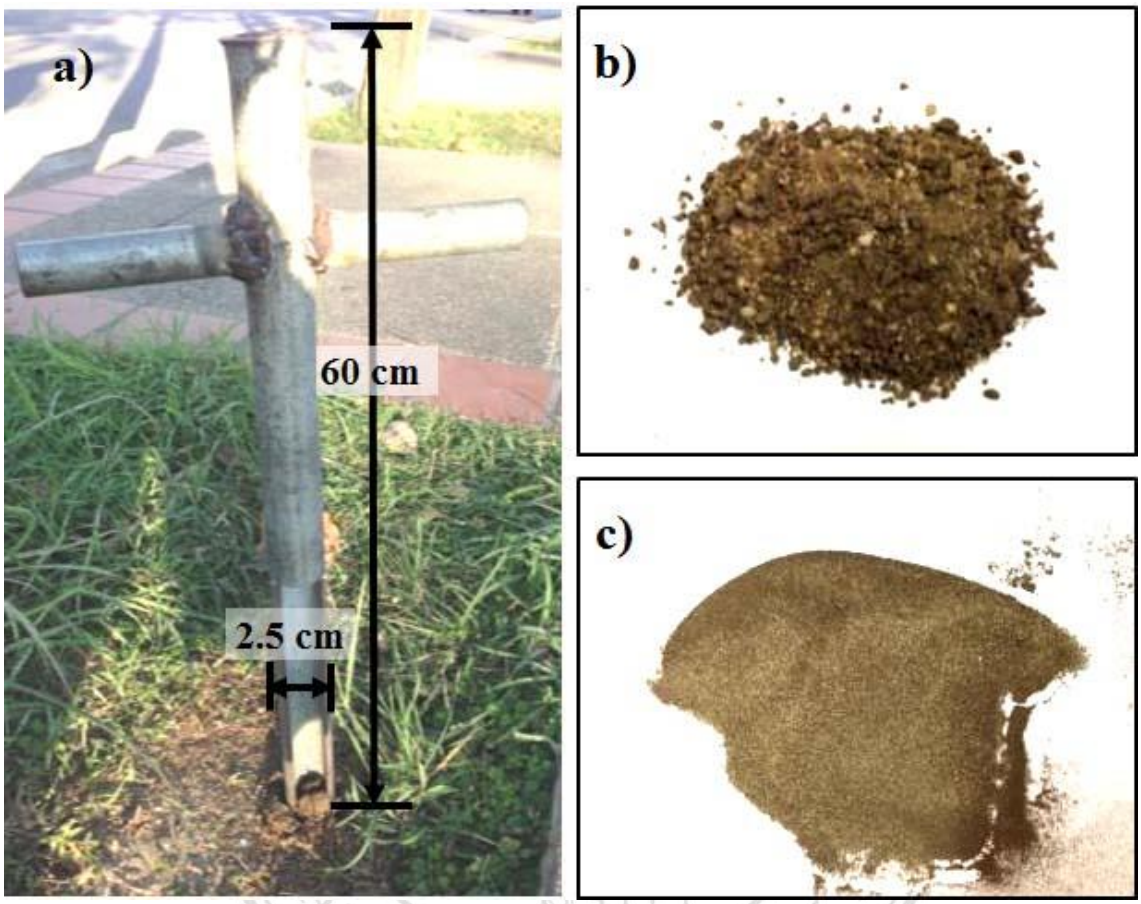


Figure 2.20 Surface soil sampling a) core soil sampler, b) soil sample before sieving, c) soil sample after sieving

2.8 Sample preparation and extraction

2.8.1 Preparation and extraction of tree bark sample extraction

Bark samples were dried at 60°C for 2 hrs and then cool down to room temperature in hot air oven. Dried samples were weighed by a 4 digits balance. In case of affecting factors testing, the weight of whole sample sheet was around 500 - 800 mg. The bark sample sheet was grounded into small pieces. Approximately 300 mg of the sample was taken for extraction. In case of scrape sampling method, weight of the bark samples was in a range of 50 to 270 mg. Therefore, the whole sample was used in sample extraction. For digestion, the sample was put into the inner layer of a double layer Teflon digestion device (Figure 2.21), which contained 5 ml high purity concentrated HNO_3 in the outer layer (Figure 2.23 b). It was covered by the lid and put

in the oven. Samples were digested for 4 hrs at 140 ^{o}C. After that the residue was diluted to 10 ml in volume flask with 0.1 M HNO_3 (modified from Qiuquan *et al.* (2003) and Hokura *et al.*, (2009)). The sample solutions were filtered through a 0.45 µm nylon-membrane syringe filter before analysis by ICP-OES.

2.8.2 Preparation and extraction of adhesive resin samples

The adhesive resin samples (2 cm x 5 cm) were cut into small pieces (1 cm x 1 cm) and 6 pieces were randomly picked up for 6 pieces to get 1 sample. Based on optimum extraction condition for bark, the maximum weight of sample was 350 mg which was the maximum weight for sample extraction (based on optimum condition for bark extraction). The sample put into the inner layer of a double layer Teflon digestor (Figure 2.22 b)). The digestion method was the same as for bark sample (section 2.8.1).

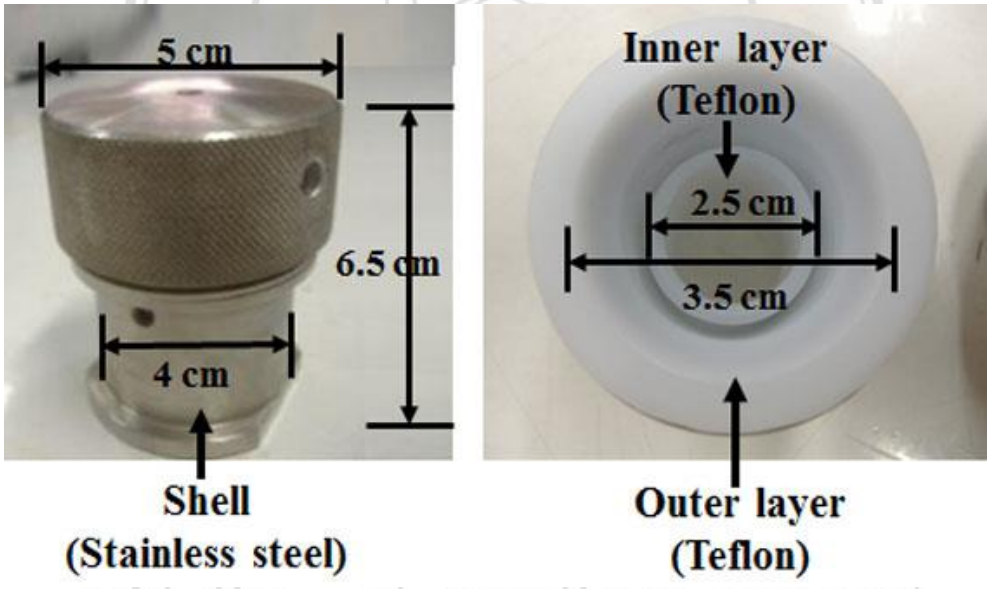


Figure 2.21 Double layer Teflon digestion device

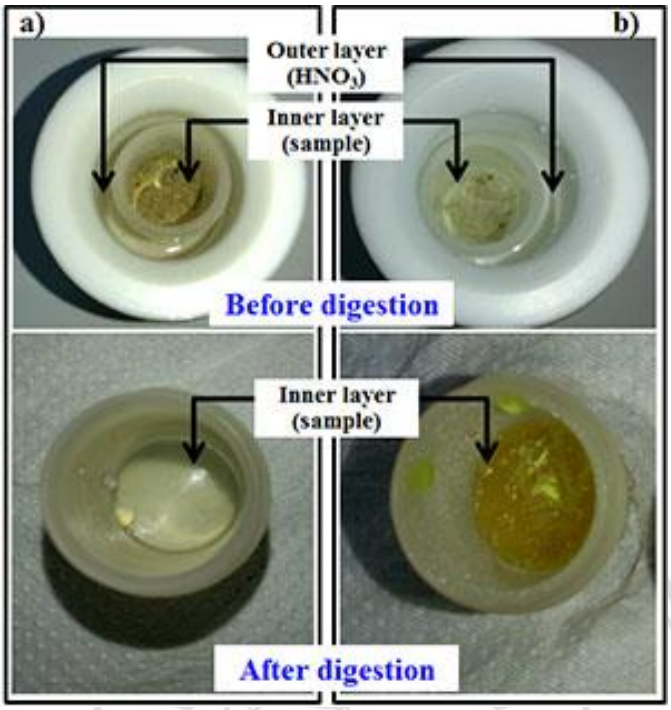


Figure 2.22 Sample digestions a) bark sample digestion, b) adhesive sample digestion

2.8.3 Preparation and extraction of soil sample

The two extraction conditions were employed in this study in order to get high efficiency of metals extraction.

1) HCl: HNO_3 (3:1 v/v)

A mixed acid solution of aqua regia was used to extract six metals composite including Cr, Cu, Mn, Ni, Pb and Zn (Khamkaew *et al.*, 2016). Approximately 100 mg sieved soil sample were weighed and put into the inner layer of Teflon digestion device (Figure 2.23). Four ml of aqua regia solution (HCl: HNO_3, 3:1 (v/v)) was added to mix with the sample. Unlike the bark extraction (the sample was not directly contract with the acid), soil sample was directly contract with the acid. The devices containing samples were put into the oven and heated at 180 ˚C for 5 hrs. The residue was adjusted to 25 ml with 0.3 M HNO_3 in a volumetric flask.

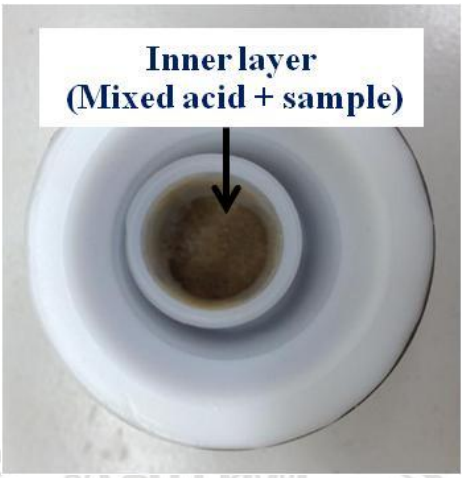


Figure 2.23 Soil sample digestion by double layer Teflon digestion device

2) HF: HNO_3 (2:3 v/v)

Mixture of HF and HNO_3 (2:3, v/v) was used for digestion of Al and Fe in the soil samples (developed from US EPA, 3052). Approximately 100 mg of soil sample was weighed and transferred to inner layer Teflon digestion device. Five ml of the mixed acid was added into the inner layer containing soil sample. They were heated at 180^{o}C for 5 hrs in the oven. After digestion, the residue (inner layer) was again heated at 60 – 65 ^{o}C for 20 minute by hot plate for HF removal then it was adjusted to 25 ml with deionized water.

The solutions were filtrated through 0.45 µm nylon filter and transferred polyethylene bottle and stored at 4^{o}C until metal analysis by ICP-OES.

2.9 Analysis of metals by ICP-OES

2.9.1 Preparation of standards and chemicals

1) Metal standard solution

1.1) Metal standard stock solution

A 0.5 ml of 1,000 µg/ml individual standard solutions (Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were pipetted to volumetric flask and then the mixed solution was adjusted the volume to 25 ml with 0.1 M HNO_3. Each metal content in the stock solution was 20 µg/ml.

1.2) Metal standard working solution

Various concentrations including 10, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 and 0.005 µg/ml of mixed standard solutions were prepared by serial dilution method. The concentration of 10, 5, 2.5, 0.5, 0.25, 0.05 and 0.025 µg/ml were prepared by pipetting 12.5 ml of 20, 10, 1, 0.5, 0.1, 0.05 and 0.01 µg/ml to volumetric flask while 10 ml of 2.5, 0.25 and 0.025 µg/ml concentration were transferred to prepare 1, 0.1, and 0.01 µg/ml concentration. The mixed standard solution in volumetric flask were adjusted the volume to 25 ml with 0.1 M HNO_3. The standard solutions were stored at 4^{o}C in refrigerator and those solutions were monthly renewed.

1.3) Preparation of standard calibration curve

A standard calibration curve of individual metal species was constructed for qualitative analysis. The calibration curve was plotted between metal concentrations (x axis) versus emission intensity obtained from ICP-OES (y axis). The standard concentrations were measured in the range between 0.005 - 20 µg/ml (6 - 9 concentrations). If the sample concentration was higher than the maximum concentration of calibration curve, the sample was diluted to obtain a concentration within the range of calibration. The dilution factor was multiplied afterwards to gain the sample concentration.

2) Nitric solution

2.1) 0.1 M HNO_3 solution

A 6.9 ml of 65% concentrated HNO_3 was pipette into 500 ml of deionized water, and then the volume was adjusted with deionzed water to 1,000 ml in a volumetric flask.

2.2) 0.3 M HNO_3 solution

A 20.0 ml of 65% concentrated HNO_3 was pipette into 500 ml of deionized water, and then the volume was adjusted with deionzed water to 1,000 ml in a volumetric flask.

2.9.2 Condition of ICP-OES for metal analysis

Inductively coupled plasma optical emission spectrometry (ICP- OES Optima 3000, Perkin Elmer, Germany) used for determination of metals such as Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The analysis conditions of ICP-OES are detailed in Table 2.2

Table 2.2 Condition of ICP-OES for metal analysis

Analysis item	Conditions
RF generator	1300 W
Plasma flow rate	15 L/min
Auxiliary flow rate	0.5 L/min
Nebulizer flow rate	0.80 L/min
Gas types	
- Plasma gas	High Pure Argon
- Purge spectrometer	Ultra High Pure Nitrogen
Gas pressure	
- Argon	80 Psi
- Nitrogen	60 Psi
Sample flow rate	1.50 ml/min
Delay time	50 second
Measurement	2 replicates

2.9.3 Analytical characteristics of ICP-OES for metal analysis

1) Limit of Detection (LOD) and Limit of Quantification (LOQ) of ICP-OES for metal analysis

The LOD and LOQ values of ICP-OES for all analysis were determined by 7 times measurement of the lowest standard concentration (0.1 µg/ml)

used for calibration curve construction. The LOD and LOQ were obtained from 3 times and 10 times of standard deviation of those 7 measurement values (US EPA, 2010).

2) Quality control of elemental analysis

2.1) Accuracy of the analysis

Two types of botanical certificated reference material (CRM), including apple leave 1515 from National Institute of Standard & Technology (NIST) and pepperbush no.1 from the National Institute for Environmental Studies (NIES), and one type soil CRM (clay soil 051 from R.T. Corporation (RTC)), were analyzed in order to check the analysis method efficiency. Percent recovery of each metal was calculated by using Equation 2.1.

$$\%\,\mathrm{Recovery} = \frac{MV}{CV} x100 \qquad \text{Eq. 2.1}$$

Where MV is a measured value (mg/kg)

CV is a certified value (mg/kg)

2.2) Precision of the analysis

Precision of elemental analysis was tested in term of repeatability and repeatability.

2.2.1) Repeatability

Repeatability of measurement which is known as an error due to measurement process, refers to the variation in repeating measurement on the same sample under identical condition and in a short period of time (Bartlett and Frost, 2008).

Repeatability of ICP-OES measurement was obtained from 7 times continuous measurement of 0.25 µg/ml mixed standard solution (Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn) by ICP-OES under the optimum condition.

2.2.2) Reproducibility

Reproducibility refers to the variation in measurements on a sample under changing conditions such as different in sample preparation, measurement methods and instruments being used (Bartlett and Frost, 2008).

The reproducibility of ICP-OES was measured by measurement at 0.25 µg/ml mixed standard solution by ICP-OES under the optimum condition ones a week for 7 weeks.

The values of repeatability and reproducibility were calculated in terms of by standard deviation and relative standard deviation (RSD) of those 7 measurement using Equation 2.2

$$\%RSD = \frac{SD}{\overline{X}} x100 \qquad \text{Eq. 2.2}$$

Where % RSD is a percentage relative standard deviation

SD is a standard deviation of measured concentration

$\overline{X}$ is a mean value of measured concentration

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Method efficiency and analytical characteristic of ICP-OES for metal analysis

Metal content of the samples was analyzed by ICP-OES. The results of method validation are discussed in the following sections.

3.1.1 Standard calibration curves for quantitative analysis of metal concentration

Standard calibrations of metals were employed from measurement of six to nine concentrations (0.005 - 20 µg/ml) of the mixed standard solutions by ICP-OES. Concentrations of each metal standard, which were in comparable range with its amount contained in sample, were selected to plot versus the emitted light intensities in order to conduct the calibration curve for each metal species. Linear calibration curves and equations for all metal species are shown in Figure 3.1 and Table 3.1. The values of variation coefficient (R^2) were in a range between 0.998 – 0.999.

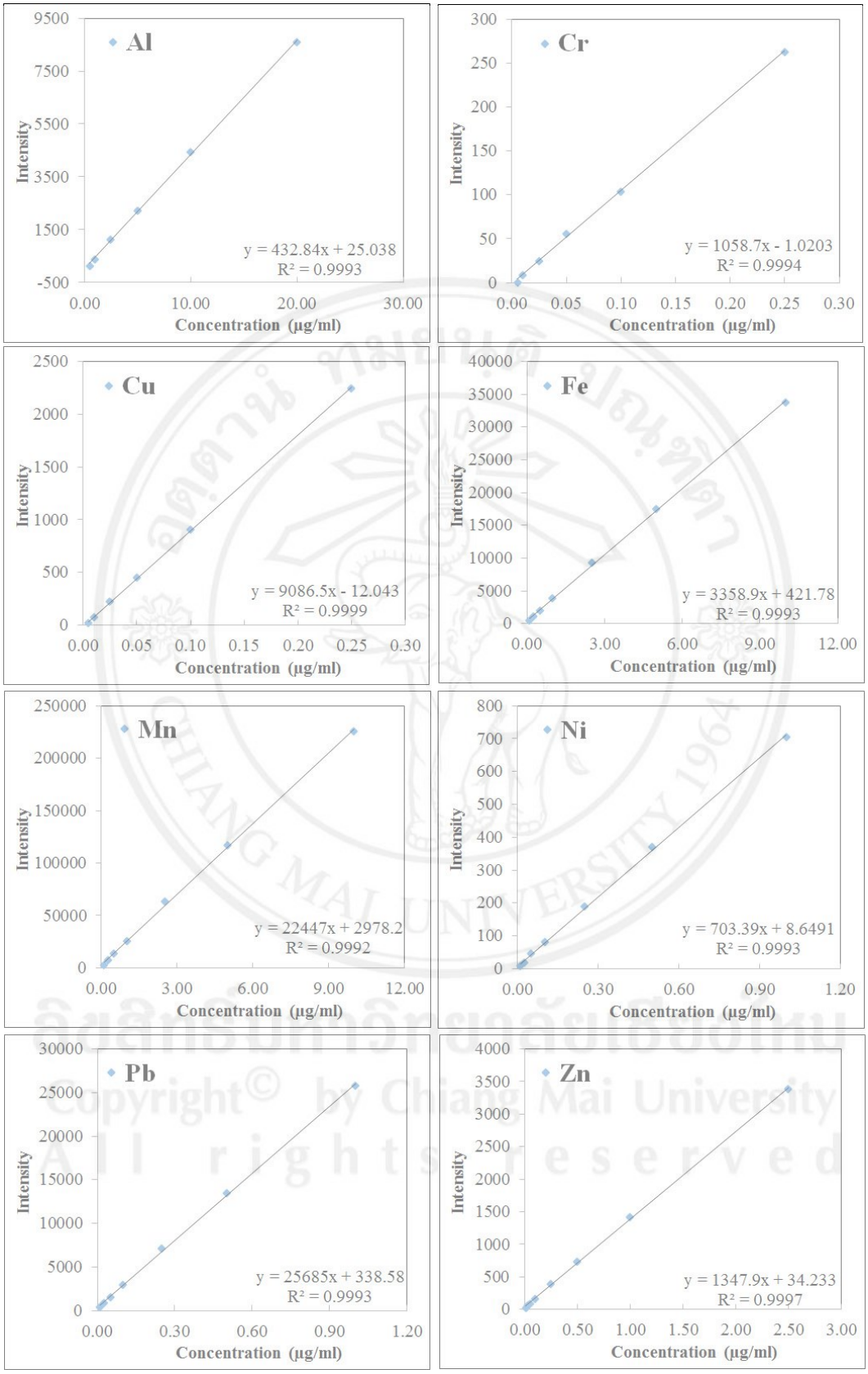


Figure 3.1 Calibration curves of individual metal species measured by ICP-OES

Table 3.1 Calibration equations and coefficients of metal determinations

Metals	Wavelengths (nm)	Linear equations	Variation coefficient (R^2)
Al	308	y = 432.84x + 25.038	0.9993
Cr	205	y = 1058.7x - 1.0203	0.9994
Cu	324	y = 9086.5x - 12.043	0.9999
Fe	238	y = 3358.9x + 421.78	0.9993
Mn	257	y = 703.39x + 8.6491	0.9993
Ni	231	y = 22447x + 2978.2	0.9992
Pb	220	y = 25685x + 338.58	0.9993
Zn	206	y = 1347.9x + 34.233	0.9997

3.1.2 Analytical characteristic of ICP-OES

1) Limit of detection (LOD) and limit of qualification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) of ICP-OES were obtained from 3 and 10 times of standard deviation (SD) of 7 measurement of 0.1 μg/ml mixed metal standard, respectively. LOD values of metals were in the range between 0.003-0.032 μg/ml, while those of LOQ were 0.009-0.108 μg/ml (Table 3.2).

Table 3.2 Values of limit of detection and limit of quantification of ICP-OES for metal analysis

Metals	Concentration (µg/ml)	
	Limit of detection (LOD) (3SD)	Limit of qualification (LOQ) (10SD)
Al	0.032	0.108
Cr	0.005	0.015
Cu	0.004	0.015
Fe	0.003	0.009
Mn	0.003	0.011
Ni	0.014	0.047
Pb	0.023	0.077
Zn	0.005	0.016

2) Quality control and metal analysis

2.1) Accuracy of metal analysis by ICP-OES

Five replications of certificate reference materials (CRMs) of botanical and soil materials were used for optimization of metal extraction condition based on recovery values.

2.1.1) Botanical CRMs

Two types of botanical CRMs including apple leaves (NIST 1515) and pepperbush leaves (NIES no.1) were analyzed. The apple leaves CRM was the leaves of the Golden Delicious and Rome varieties, while pepperbush leaves CRM was the leaves of pepperbush tree planted in Mikouchi in the Ashio district, Japan. Metals (Al, Cr, Cu, Fe, Mn, Ni, Pb, Zn) from tree leaves CRMs were extracted by concentrated HNO_3 acid and analyzed by ICP-OES. Even though, the measured value of most of metals were significantly difference from their certificate values. However, the recoveries obtained from those metal were in a good range of recoveries

(79 – 97%) (Table 3.3). Exception for metal species with low concentration i.e. Cr presented high variation of detected concentration (115±18 %).

Table 3.3 Recovery of elements obtained from apple leaves (NIST 1515) and pepperbush leaves (NIES no.1)

Metals	Certified value (µg/kg)		Measured values (n=5) (µg/kg)		% Recovery ± SD (n=5)	
	Apple	Pepperbush	Apple	Pepperbush	Apple	Pepperbush
Al	286	-	277±4*	-	97±1	-
Cr	-	1.3	-	1.5±0.2	-	115±18
Cu	5.64	12	4.87±0.02*	11 ± 1*	86±1	92±4
Fe	83	205	72±4*	168±10*	87±5	82±5
Mn	54	2,030	51+1*	1888±96*	94±1	93±5
Ni	0.91	8.7	ND	7.3±0.5*	ND	84±6
Pb	0.47	5.5	ND	4.4±0.1*	ND	79±3
Zn	12.5	340	11.7+0.9	304±12*	94±7	89±4

Note: **significant difference ($p<0.05$) from the certificate value (t-test); - no certificated value; ND concentration lower than limit of detection*

2.1.2) Clay soil CRM

In order to verify the effectiveness of soil extraction with mixed acid reagent, clay soil (RTC 051) was employed for metal analysis. Metals including Cr, Cu, Mn, Ni, Pb and Zn in the soil CRM were extracted by aqua regia reagent, while mixture of HF and HNO_3 (2:3, v/v) was used for Al and Fe digestion. The soil CRM is naturally contaminated soil from a site located in the western United States. Measured values of the eight metals extracted were compared with the certificated values. Percent recoveries of all metals are shown in Table 3.4. Recoveries of all metals were in a range 83 – 115%.

Table 3.4 Recovery of elements obtained from clay soil CRM (RTC 051)

Metals	Certificated value (µg/kg)	Measured value (n=5) (µg/kg)	% Recovery ± SD (n=5)
Al	5530	5396±742	98±13
Cr	246	283±12*	115±5
Cu	58.5	53.5±2.8*	91±5
Fe	4520	3853±221*	85±5
Mn	757	669±37*	88±5
Ni	96.8	102.0±4.2	105±4
Pb	44.1	36.6±3.2*	83±7
Zn	44.0	45.8±2.3	104±5

Note: **significant difference ($p<0.05$) from the certificate value (t-test)*

2.2) Precision of ICP-OES for metal analysis

The precision of each individual metal was estimated from relative standard deviation (%RSD). Repeatability and reproducibility of metals determined by ICP-OES was measured from 7 measurements of a 0.25 µg/ml mixed standard solution by ICP-OES. High precision values were obtained for all metals with 0.3 to 2.3 % RSD as repeatability and 1.4 – 4.9% RSD as reproducibility (Table 3.5).

Table 3.5 Repeatability and reproducibility of 7 measurements by ICP-OES

Metals	Repeatability (n = 7)			Reproducibility (n = 7)		
	Mean	SD	%RSD	Mean	SD	%RSD
Al	0.254	0.006	2.189	0.254	0.005	2.166
Cr	0.255	0.001	0.552	0.253	0.003	1.376
Cu	0.254	0.001	0.296	0.253	0.004	1.422
Fe	0.255	0.001	0.385	0.252	0.004	1.704
Mn	0.253	0.001	0.426	0.253	0.004	1.601
Ni	0.244	0.004	1.790	0.252	0.007	2.869
Pb	0.246	0.006	2.297	0.251	0.012	4.857
Zn	0.250	0.001	0.560	0.251	0.008	3.149

3.2 Characteristic of *Cassia fistula* barks and factors affecting metal accumulation

Characteristic of the bark including sequence of the bark layers and metal content in each layers, and factor affecting on metal accumulation in the bark were estimated in order to set criteria for sampling barks of *Cassia fistula* to be used as bioindicator for metal accumulation from road vehicles.

3.2.1 Sequence *Cassia fistula* bark layers and their metal content

1) Identification of *Cassia fistula* bark layers

Bark layers of *Cassia fistula* were identified by their position from the outer part and characteristic of cells measured under optical. Figure 3.2 presents cross section of all layers of *Cassia fistula* bark.

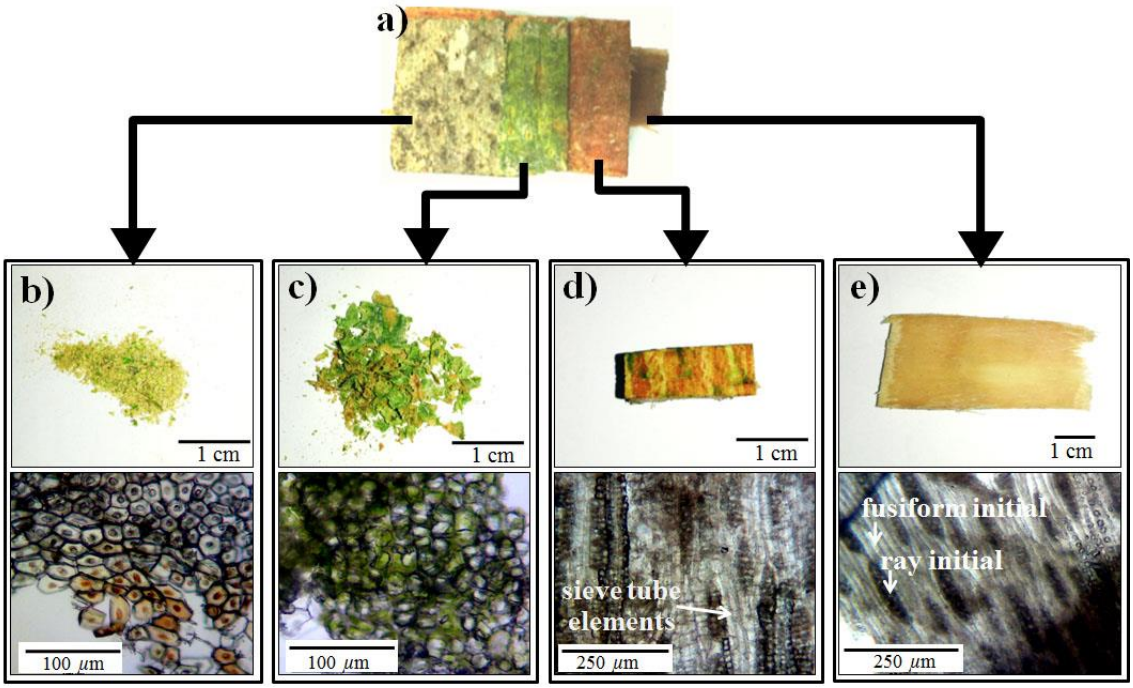


Figure 3.2. Characteristic of *Cassia fistula* bark layers under optical microscope (longitudinal section) a) bark, b) cork, c) chlorenchyma, d) phloem, e) phloem&cambium

The outermost layer (the 1st layer) is gray powder. It is about 50 -150 µm thick (Figure 3.3). It is easily removed by scratching on the bark surface. Cell in this layer have a thick cell wall and high density of cell arrangement. This layer was classified as cork layer. The next inner layer (2nd layer) is green. It was classified as chlorencyma because cells in this layer were similar to those found in the cork layer, but chlorophyll pigments were observed inside the cell. The chlorenchyma layer is about 200 – 300 µm thick (Figure 3.3). The 3nd layer is red and approximately 0.7 – 12 cm thick. Even though several type of cells were found in this layer but sieve tube elements which are a specialised type of elongated cell in the phloem tissue, were observed (Figure 3.2d)). Therefore, this layer was classified as phloem layer. The innermost layer (4th layer) was about 1 mm thick with light brown color.

The result from microscope was found that two type of initial cells which were basically presented in vascular cambium tissue, were observed in this layer (Figure 3.2e)). Ray initials (short and rounded cells) give origin to the ray cells (elements of the transverse or ray system of the xylem and the phloem), while fusiform initials (elongated cells and tapering end) form the longitudinal or axial system of xylem and phloem (Esau, 1965). Based on the stem structure of tree, vascular cambium tissue is generally composted of one to a few layers of cell with approximately 50 – 100 µm thick (Lachaud *et al*., 1999; Frankenstein *et al*., 2005; Elo *et al*.,2009; Beck, 2010) thus this layer should be contained both vascular cambium and secondary phloem tissue.

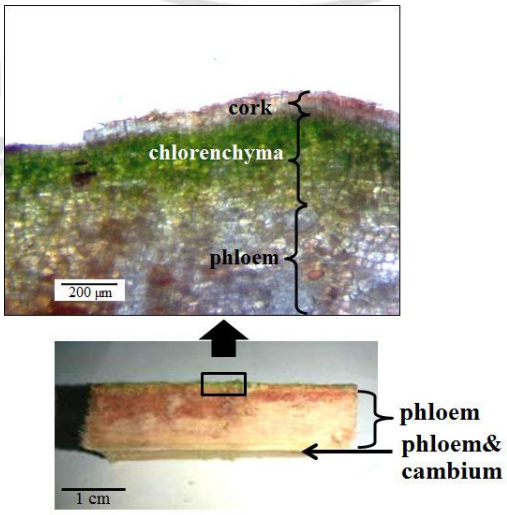


Figure 3.3. Characteristic of bark layer from cross-section

2) Metal content in bark layers

Each bark layer was extracted and analyzed for its metal content. The total metal content in barks were calculated from summation of the multiplied between metal concentration in each layer with the weight of layer (Figure 3.4). Zn (16.5±1.2 µg/g) was the metal with highest concentration found in the bark followed by Fe (11.2±2.1µg/g), Cu (0.88±0.25 µg/g) and Cu (8.9±2.2 µg/g). It might be due to quantities of those metals were high in the environment. According to Apeagyei *et al*. (2011), Fe, Zn and Cu are major composite found in the brake pads and tires of vehicles. Therefore, large amount of these heavy metals were emitted to environment from degradation of part of vehicle during driving. Moreover, Al and Fe are abundant metal composite released from Earth's crust (Dong *et al*., 2002; Yongjie *et al*., 2009; Enamorado-Báez *et al*, 2015). Therefore, high accumulation of these metals on tree bark was observed. On the other hand, Cr, Ni and Pb were not detected in this study. Basically, amount of these heavy metals in the environment was very low. Thorpe and Harrison (2008) and Pulles *et al*. (2012) reported that emission of Cr and Ni from vehicle was approximately 20 – 30 times less than that of Zn and Cu. Pb was band to be used in gasoline in Thailand since 1996, but low quantities of this element still existed in part of vehicle i.e. tire and brake wear (Kummer *et al*., 2009) Furthermore, the uptake of these heavy metals was hindered such as the translocation of Cr from the roots to the shoots was intercepted by the cell wall (Adriano, 2001). Pb was found to be immobile and an easily formed organic metal complex in the soil (Prasad, 1999). There suggested reasons of very small amount of Cr, Ni and Pb accumulated on tree bark.

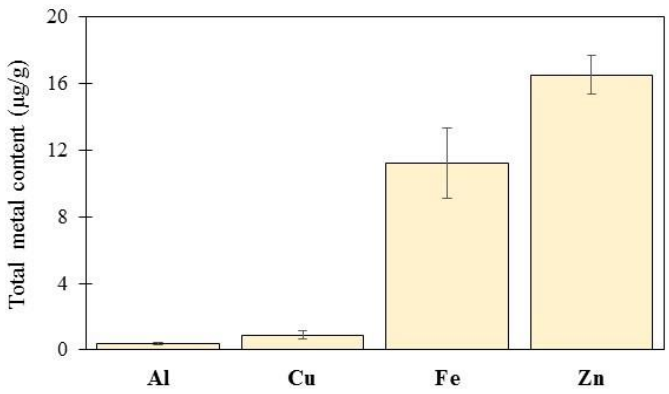


Figure 3.4. Total metal content in *Cassia fistula* bark (n = 5)

Comparison of metal content in each layer of *Cassia fistula* bark is shown in Table 3.6. It was found that trend of metals concentration (Al, Cu, Fe and Zn) in each layer of the bark was in the same pattern. The highest concentration with significant different ($p<0.05$) was observed in the cork layer and decreased in the chlorenchyma and phloem layers (inner layer) and increased again in the phloem&vascular cambium layers. Event though, the ANOVA analysis indicated no significant different of metal content in chlorenchyma and phloem&vascular cambium. It might be due to high variation in the phloem&vacular cambium. The trend well agreed with the results of metal content in *Crytomeria japonica* bark, in which concentration of Cu, Fe and Zn had been decreasing in deeper level from bark surface and the concentrations increased in inner bark layer and cambium layer. Unlike Al concentration, it was exponentially decreased from outside of the bark toward inside of the bark (Satake *et al*., 1993). Moreover, it was also related to the result of Liebergeld *et al*. (1996) (referred by Schulz *et al*., 1999), in which trend of the element content (S, Fe and Ca) in Pine bark decreased at 1.5 mm depth from the bark surface, then the trend stabilized in a range of 1.5 – 4 mm and then increased at the deeper levels. The sequence of metal distribution in each layer of bark sample indicated two routes of metal accumulation including airborne particles and soil by root uptake. High concentration of metals (Al, Cu, Fe, Zn) on cork layer (outermost layer) should be mainly influenced from atmospheric pollution (airborne and soil particle) because this layer is directly exposed to ambient air. In case of their concentration in phloem&vascular cambium layer, increasing of the concentration should be supplied from soil by roots. Normally, metals were uptake from soil to leave through xylem layer and the metal was translocated to remain in the vascular cambium layer because location of both layers is connected.

Table 3.6 Metal contain in *Cassia fistula* bark layers (n = 5)

Metals	Mean ± SD (µg/g)			
	Cork	Chlorenchyma	Phloem	Phloem&cambium
Al	68.7±16.1[a]	4.5±3.3[b]	ND	2.9±1.9[b]
Cr	ND	ND	ND	ND
Cu	5.09±1.39[a]	1.11±0.25[b]	0.84±0.25[b]	1.84±0.34[b]
Fe	73.4±22[a]	21.9±5.7[b]	10.3±2.1[c]	37.6±13.9[bc]
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Zn	52.2±13.6[a]	26.2±1.4[b]	16.1±1.3[c]	19.1±2.4[bc]

Note: *[a,b,c] significant different ($p < 0.05$) among bark layers (ANOVA test); ND: Not detected (LOD (µg/ml): 0.005 (Cr), 0.014 (Ni) and 0.23 (Pb))*

3.2.2 Factors affecting metal accumulation on tree bark

Factors affecting metal accumulation on tree bark including exposure direction of the trees to source and age of the tree, in which related to DBH size of tree trunk were determined in order to set criteria for *Cassia fistula* bark sampling.

1) Exposure direction of trees to traffic source

Tree bark samples collected from the tree trunk in two directions (directly exposure to the road and the opposite side) were analyzed for their metal content. Concentration of metals in bark samples are shown in Figure 3.5. Only Al, Cu, Fe and Zn concentrations were not significantly different between both sides of the tree by pair t-test at a 95% confidential level. Cr, Ni and Pb were not detected. It might be due to the reason that mobile source is majority sources in that area and therefore metals emitted from the source dispersed in ambient air. Moreover, the sampling trees were < 3 m from the road. Consequently, the amount of metals were equally distributed and accumulated on both side of the trees.

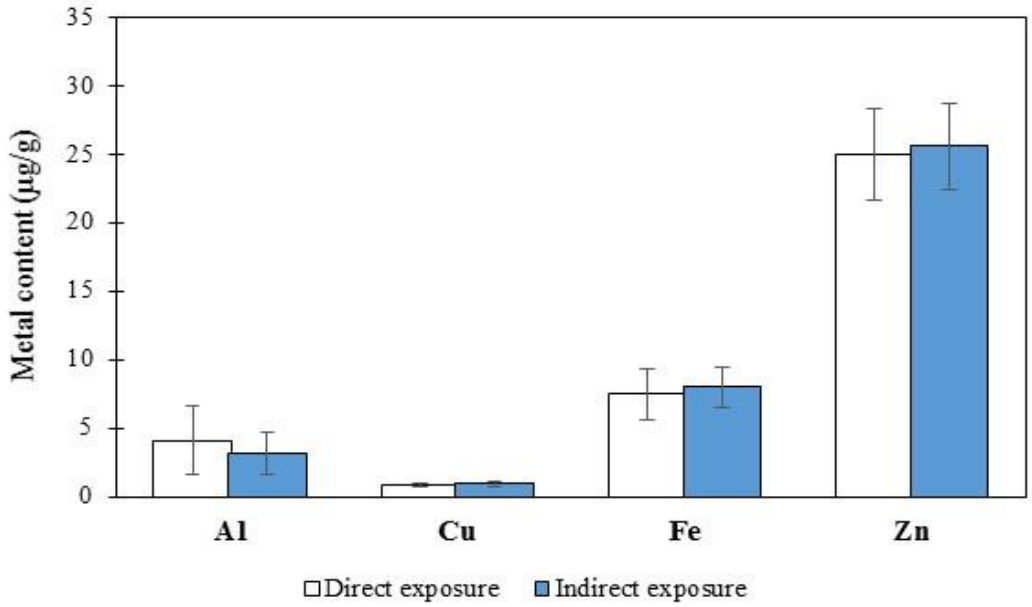


Figure 3.5. Comparison of metals concentration on bark at different exposed directions to source (n = 5)

2) Age of trees

Normally, the DBH size of a tree increases with its age thus the bigger DBH size, the older tree and the longer period of pollutant accumulation. *Cassia fistula* in the city of Chiang Mai have various DBH sizes due to various ages. However, the most common size is found in a range of 5 – 30 cm. Therefore, metal content in each DBH range was determined in order to find out variation pattern among trees with different sizes. *Cassia fistula* in this study were selected from the trees planted near road side of Huay Kaew road (same environment condition). Growth factors (i.e. rain amount, temperature, light intensity, nutrients) among trees in the site were identical, therefore DBH increasing (growth rate) of the trees should be also similar. The tree DBH sizes were divided into 3 groups including 5– 10, 11– 20 and 21– 30 cm. Five trees were selected for each group and their tree barks were sampling and analysis for metal content.

Generally, annual rings are the result of the change in growth speed of new xylem cells forming in each season due to different of temperature and rain amount. Numerous large cells with thin walls are formed in rainy season (lighter brown color ring), while small cells with thick wall were produced in dry season (dark

brown color ring). Hence, 2 rings are observed in circumference of tree. Even though, annual rings counting method is quick and accurate for determining age of tree, but the tree has to be down for its ring counting. Therefore, age of the sample trees were calculated (estimated) based on the samples found in rural area of the city. Five *Cassia fistula* for age estimation were selected from trees which had DBH size in the range of 5 – 30 cm and planted near the road in rural area of Chiang Mai Province. Those trees were cut down (~ 30 - 50 cm above ground level) due to road construction. Their ages were estimated by annual ring counting. Seven rings were observed from *Cassia fistula* tree at DBH size 10.50 cm, therefore the age of the tree should be around 7 years (Figure 3.6). Linear equation were obtained from the plot between DBH sizes versus tree age (Figure 3.7). After that ages of sampling tree were estimated by using the linear equation (Table 3.7). The result indicated that ages of trees selected in this study were in the range of 4 – 20 years. However, a difference of planting area condition, such as water availability, climate, soil condition and competition of light, definitely affected on size of trees and therefore can cause error on age estimation.

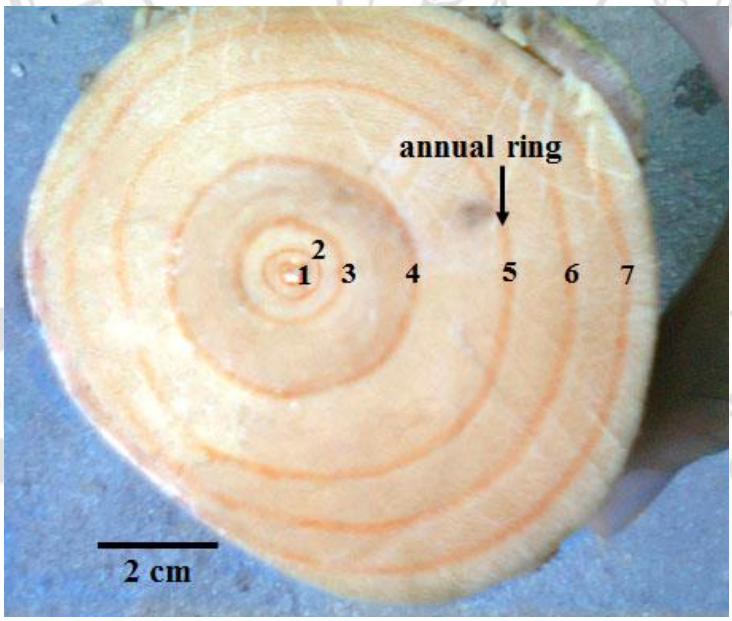


Figure 3.6. Annual rings of *Cassia fistula* (7 years)

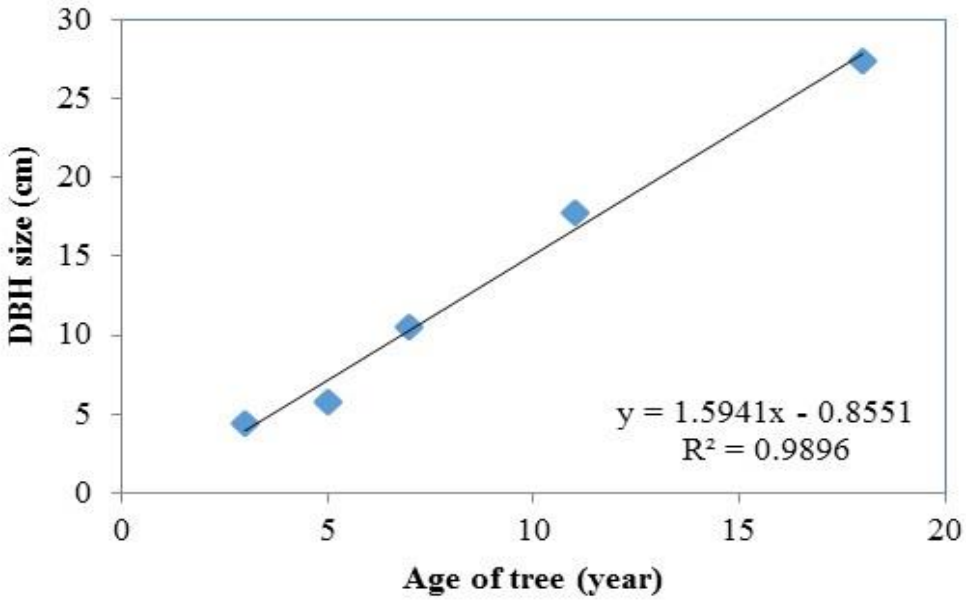


Figure 3.7. Relation of DBH sizes and ages of *Cassia fistula*

Table 3.7 DBH sizes and the estimated ages of *Cassia fistula*

Annual ring count		Linear equation	
DBH size (cm)	Ages of tree (year)	DBH ranges (cm)	Age of tree (year)
4.45	3	≤ 10	≤ 7
5.73	5		
10.50	7	11– 20	7 - 13
17.28	11		
27.36	18	21– 30	14 - 20

Table 3.8 presents metal concentrations (Al, Cu, Fe and Zn) on tree barks collected from trees with various DBH sizes. Those three groups of DBH sizes were not significantly different in terms of metal concentration accumulated on their tree barks, according to ANOVA test at a 95% confidential level. The result means that the age of *Cassia fistula* (smooth bark) in the range between 4 – 20 years was not relate to metals accumulation on tree barks. It might be due to the thickness of cork layer (the highest metal content layer) was not related to the age of the tree because of exfoliation of this layer.

Table 3.8 Metal content in tree bark samples with different DBH sizes (n =5)

Metals	Concentrations (mean± SD) (µg/g)		
	5 – 10 cm	11 – 20 cm	21 – 30 cm
Al	4.67±3.27[a]	3.68±2.02 [a]	3.81±0.99 [a]
Cr	ND	ND	ND
Cu	0.76±0.08 [a]	0.89±0.18 [a]	0.77±0.19 [a]
Fe	12.88±4.14 [a]	13.3±3.51 [a]	10.2±2.36 [a]
Ni	ND	ND	ND
Pb	ND	ND	ND
Zn	21.85±1.66 [a]	21.36±2.56 [a]	20.75±3.44 [a]

Note: *ND: Not detected (LOD (µg/ml): 0.005 (Cr), 0.014 (Ni) and 0.23 (Pb))*
[a] *no significant different from statistic result among DBH sizes (ANOVA test)*

3.3 Collecting method for *Cassia fistula*

Traditional method for bark sample collecting is using a knife to collect 1 – 5 mm depth from bark surface (El-Hasan *et al*., 2002; Patrick and Farmer, 2007; Guéguen *et al*., 2011), however damage on tree is observed after that (Figure 3.8). Hokura *et al*. (2009) released new sampling method which was simple, quick and cause less damage to the trees. The new method was using synthetic resin adhesive to remove metals deposited on tree bark. The resin used in this method is the Japanese commercial brand which is not available worldwide. Therefore, the two brands of resin which are domestic available were used instead and their efficiency for pollutant sampling was tested. According to result of metals content in bark layers (selection 3.2.1), most of metals are accumulated in cork layer and the deposits on only cork layer can be collected by using resin adhesive method. Besides, cork layer collecting with scrape method was also applied in this study in order to compare the sampling methods and select the appropriate one for local or regional environmental monitoring.

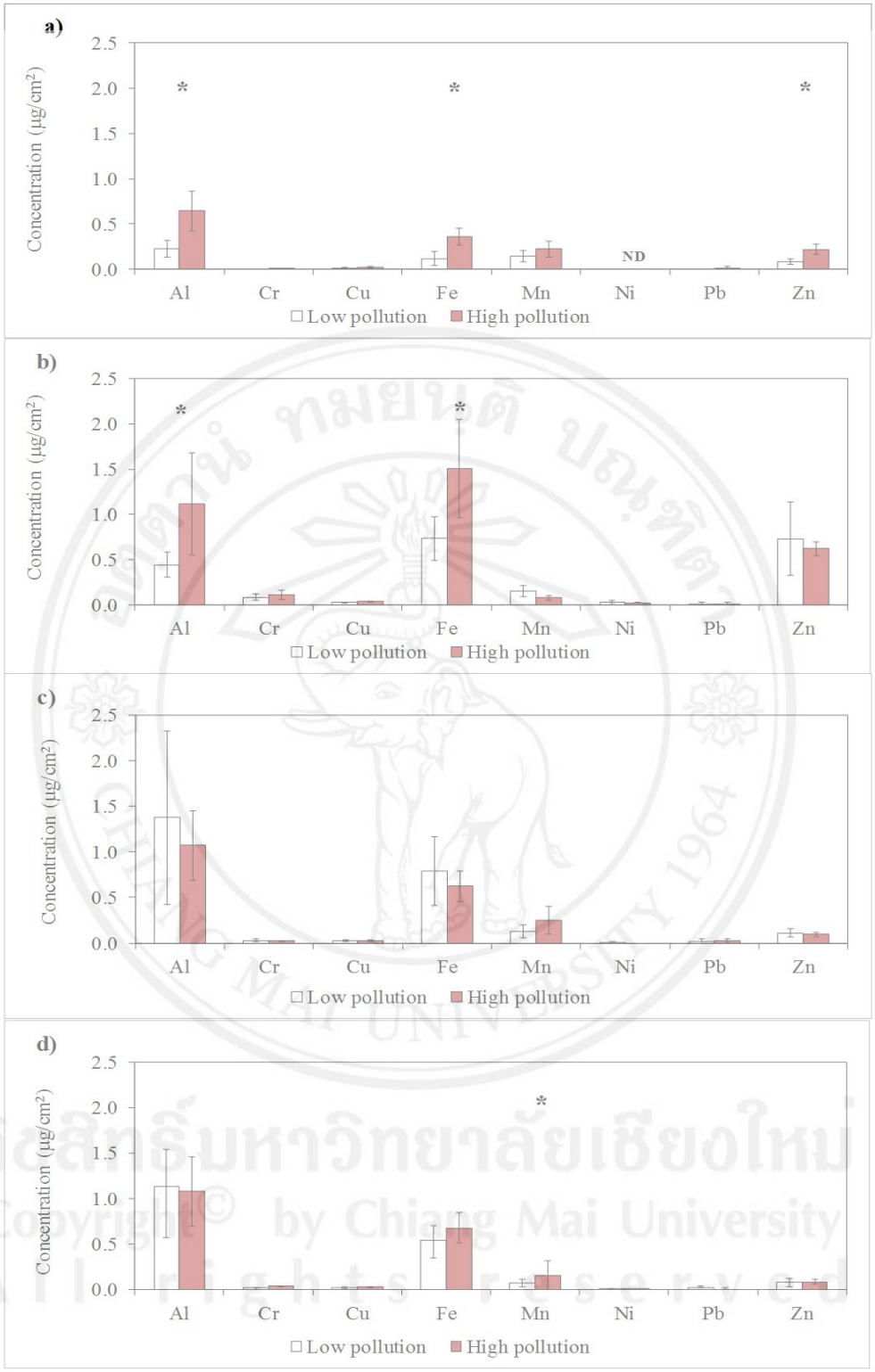
Figure 3.8 Tree bark damage after sample collecting

The *Cassia fistula* barks collected by scrap and adhesive methods from both Chiang Mai City (high pollution) and Phrao District (low pollution) were digested and analyzed for metals concentrations. Statistic t-test was used to indicate the difference of metal levels between areas. The results showed that concentration of metals deposited on *Cassia fistula* bark collected from scrape method was highest in Al (0.1 – 0.5 µg/cm^2: low polluted and 0.4 – 0.9 µg/cm^2: high polluted), while Ni was not detected for both areas (Figure 3.9 a)). The metal concentrations found in high pollution area were 1.5 – 3 times higher than those in low pollution area for scrape method. Significant difference ($p < 0.05$) between areas were observed from Al, Fe and Zn. In case of adhesive resin methods, Fe was the highest metal species found on the bark collected by using Japanese resin brand for both area (Figure 3.9 b)). Their mean concentrations were 0.73 µg/cm^2 and 1.51 µg/cm^2 for low and high polluted areas, respectively. Significant difference ($p < 0.05$) between areas were found on Al and Fe. However, the concentration of Mn, Ni and Zn found in low pollution areas were higher than those in high pollution area. It might be due to high variation of those metal concentration content in the sample.

For adhesive method by Thai resin brands, similar pattern of metal content in samples was found from both rain brands (Figure 3.9 c - d)). The variation of metal concentrations were high for both resin brands. Therefore, most of metal concentrations were not significantly different ($p > 0.05$) between low and high pollution areas. The result of adhesive method, it was indicated that high variation among samples was observed from all type of adhesive resin brands. There might be due to metal content in the resin itself. Therefore, all resins used were also analyzed for metal content. It was

found that all types of resins contained high amount of Al (0.12 – 0.22 μg/cm^2), Fe (0.05 – 0.10 μg/cm^2) and Zn (0.02 – 0.14 μg/cm^2) as shown in Figure 3.10. Additionally, the resins took long hardening time (3 – 4 hrs) due to high temperature in Thailand. The Thai resins took 4 hrs for hardening, while the Japanese one took 3 hrs. The main reason might be because their viscosity, in which the Thai resins was lower than that of the Japanese resin. Long hardening time cause the resin moved from the upper to the lower part due to gravity effect. Consequently, the sample size could not be control to be 10 cm^2, it was larger than the control size. Besides, uncertainty of resin sheet thickness was observed on the resin sample sheet. Normally, the thickness of the resin sheet is positively related to its metals content, therefore high thickness variation between sample sheets could cause high variation of metals concentration observed from adhesive method.

Metal concentrations obtained from in scrape method were about 2- 8 times lower than those from adhesive resin methods. However, the scrape method had low variation of metal concentration found on the bark samples from the same study area, which illustrated high precision on metals analysis. Moreover, it can detected and distinguished level of pollutants between areas based on the statistic result. Meaning that this method had high accuracy on metals analysis. In addition, collecting the tree bark sample by scrape method was less cost and time consuming during bark sampling. Therefore, scrape method was selected for sampling method in this study in order to assessment metals emitted from traffic in the city of Chiang Mai.



** Significant different ($p < 0.05$) between areas (t-test)*

Figure 3.9 Comparisons of metal concentrations (µg/cm^2) from difference sampling methods (n=5) a) scrape method, b) adhesive method (Japanese brand), c) adhesive method (Thai brand#1), d) adhesive method (Thai brand#2)

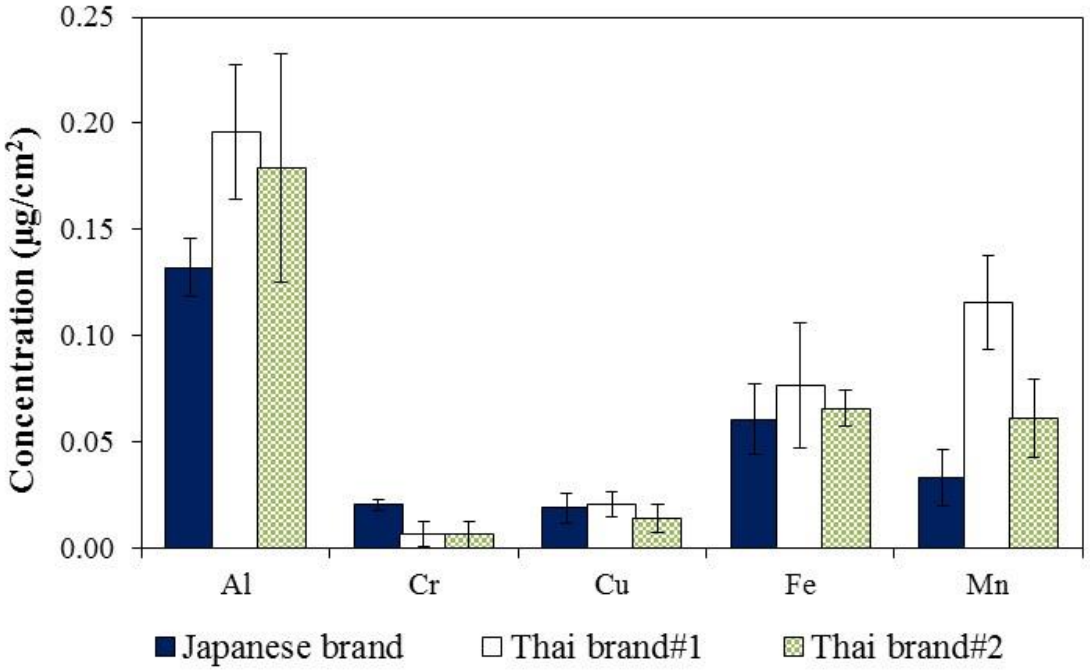


Figure 3.10 Metal composites in synthetic resin adhesive samples

3.4 Sampling size optimization

Due to low amount of metals found from scrap method, sample size was therefore increased from 10 cm^2 (2 cm x 5 cm) to 20 cm^2 (4 cm x 5 cm). Bark samples were collected from the background site (Phrao District) and 3 main roads in the city of Chiang Mai and analyze for their metal content. Concentration of metals are shown in Table 3.9. Al, Cu, Fe, Mn and Zn were found in high amount and no significant different was found between 10 cm^2 and 20 cm^2 sample sizes. However, standard deviation (SD) of 20 cm^2 was slightly lower than that of 10 cm^2 sample size. It can be concluded that sample size (area) has no effect on metal concentration in a unit of weight per area size. However, the bigger sample size gave benefit for low amount of pollutant. Therefore, Cr, Ni and Pb were mainly able to be analyzed at 20 cm^2 sample size only, while their concentrations were lower than limit of detection at 10 cm^2 sample size. It might be because amount of Cr, Ni and Pb were quite low on the bark, therefore increasing of sample size was also increased amount of metals in extracted solution and they can be detected by the instrument (higher than limit of detection of ICP-OES).

Regarding the result of sample size optimization, increasing of sample size were helpful for group of metals in which low amount contented on bark in order to increase

their concentration to archive the detection limit of ICP-OES. Even though, it seem to be unnecessary for group of metals with high concentration in the environment and accumulated on bark (ng/cm^2), however it was useful to reduce variation between samples. Moreover, the metal concentrations found in 10 cm^2 sample size were slightly higher than those in 20 cm^2 sample size. However, the metal concentrations (ng/cm^2) were not significant difference between sample sizes base on the result from statistic pair t-test. It might be due to sample loss during sample collection because weight of sample (particulate) was very light (hence wind is strongly responsible for sample loss). Moreover, time consuming during the sampling was also considered. It took about 20 mins to collect at 20 cm^2 area sample, which was 2 times longer than the small sample size. Therefore, sample loss and long time consuming during sampling should have more effect in the bigger sample size (> 20 cm^2).

In conclusion, the sample size of 20 cm^2 was selected for bark sample collecting using scrap method.

3.5 Metal accumulation in Chiang Mai City

A Total of 155 of bark samples were collected from different locations in the city of Chiang Mai (CM) in order to estimate contamination of metals generated from traffic activities such as fuel combustion exhaust, vehicle part corrosion and soil resuspension (vehicle wheels and road surface). Sampling locations in the city of Chiang Mai were classified into urban and sub-urban areas based on population density and human activities (Bootdee *et al*., 2012). The sampling trees were selected from 5 – 30 DBH size *Cassia fistula* planted near roadside (< 3 m distance from the road). Approximately 20 cm^2 of bark samples were collected from cork layer (only gray color layer) by using scrape method. Moreover, the four bark samples which were collected from Phrao District to be used as background samples.

Table 3.9 Comparisons of metal concentrations collected from different sample sizes

Metals		Concentration (ng/cm^2)							
		Background (n=5)		Ratanakosin road (n=4)		Hauykaew road (n=4)		Manee Nopparat road (n=5)	
		10 cm^2	20 cm^2	10 cm^2	20 cm^2	10 cm^2	20 cm^2	10 cm^2	20 cm^2
Al	Mean±SD	460±261^a	315±240^b	692±362^a	501±157^a	1,060±793^a	698±335^a	884±418^a	697±298^a
	Min - Max	220 - 889	138 - 730	454 - 1,230	341 - 711	389 - 2,185	239 - 1,034	451 - 1,451	344 - 1,042
Cr	Mean±SD	ND	ND	3.26±3.81	3.38±2.92	ND	2.11±1.42	ND	1.98±3.08
	Min - Max	-	-	ND - 7.28	ND - 6.74	-	ND - 3.08	-	ND - 7.03
Cu	Mean±SD	18.5±3.0^a	15.7±2.0^a	49.6±12.2^a	40.5±19.1^a	33.3±8.2^a	28.8±6.1^a	20.6±4.7^a	16.6±4.7^b
	Min - Max	13.9 - 22.0	13.8 - 18.5	32.1 - 59.5	20.1 - 63.4	23.1 - 42.1	23.4 - 36.0	16.7 - 27.3	12.3 - 22.1
Fe	Mean±SD	233±197^a	191±190^a	585±357^a	512±153^a	715±644^a	545±293^a	446±254^a	397±202^a
	Min - Max	51 - 558	55 - 517	357 - 1,118	416 - 741	142 - 1,619	116 - 778	186 - 810	175 - 657
Mn	Mean±SD	247±140^a	239±128^a	311±108^a	303±98^a	879±470^a	821±332^a	260±76^a	232±110^a
	Min - Max	164 - 495	153 - 462	217 - 462	193 - 424	455 - 1,290	455 - 1,129	152 - 336	129 - 404
Ni	Mean±SD	ND	ND	ND	2.44±4.88	ND	ND	ND	ND
	Min - Max	-	-	-	ND - 9.76	-	-	-	-
Pb	Mean±SD	ND	ND	ND	18.6±12.7^a	7.77±15.53^a	ND	ND	7.72±7.32^a
	Min - Max	-	-	-	ND - 27.79	ND - 31.07	-	-	ND - 16.10
Zn	Mean±SD	106±37^a	119±27^a	251±41^a	189±12^a	238±37^a	216±77^a	244±65^a	215±59^a
	Min - Max	66 - 156	94 - 160	201 - 302	178 - 203	193 - 282	125 - 306	202 - 358	154 - 300

Note: *ND = Not detected (<LOD); ^{a,b} significant different ($p < 0.05$) between sample sizes (pair t-test)*

3.5.1 Metal concentrations on *Cassia fistula* bark

Cassia fistula bark samples were extracted and analyzed for metals content by ICP-OES. Metal concentrations on the bark samples collected from the city of Chiang Mai and background sites are shown in Table 3.10. Al was the metal with highest concentration accumulated on tree bark at sampling sites. Ranges of its concentrations were 180 – 6,975 ng/cm^2 for Chiang Mai City and 53 – 186 ng/cm^2 for background site. Moreover, the concentration of Al was significantly higher (p<0.05) than other metal species. Meanwhile, Ni was the metal with lowest concentration. Its mean concentrations were 1.10 ng/cm^2 (CM city) and no-detected (<LOD) (background site). However, the concentration of Cr was not significantly different (p>0.05) from that of Pb, Cu and Zn.

Comparing of metal concentrations between sampling sites illustrated that the highest concentration of all metals were observed at urban area followed by sub-urban area and background site. Concentration of metals accumulated on bark samples in the city of Chiang Mai were not significantly different among urban area and sub-urban areas. They were approximately only 1.5 times difference from each others. However, the concentration in urban area were significantly higher than those in background site (2 – 27 times higher). Moreover, the concentrations of most of metals in sub-urban area was not significantly different (p>0.05) from that in background site. Exception was observed for Fe.

Concentrations (ng/cm^2) of Al, Cu, Pb and Zn in bark samples were approximately 10 – 40 times lower than those represent in Tokyo (Japan), in which bark of street trees (*Zelkova serrata*) were collected by using adhesive resin method (Hokura *et al*., 2009). The major reason be traffic volume, which is much higher in a metropolitan city such as Tokyo than in a small city like Chiang Mai. Moreover, concentrations (µg/g) of metals found in this study were compared with other studied (Table 3.11). Concentrations of Cr, Cu, Pb and Zn were similar to the level found in street trees of many cities including *Casuarina equisetifolia* L. (Oyo Town), *Ficus vopelli* (Benin City), *Pinus sylvestris* L. (Rösa), *Platanus orientalis* L (Thessaloniki) and *Quercus ilex* L (Genova) (Schulz *et al.,* 1999; Ukpebor *et al*., 2007; Majolagbe *et al*., 2010; Sawidis *et al*., 2011; Drava *et al.,* 2016).

Levels of metals emitted from traffic in the city of Chiang Mai are almost the same as found in many countries such as Greece, Germany, Italy and Nigeria but lower than those found in Herzegovina and Argentina (Fujiwara *et al.*, 2011; Skrbi´c *et al.*, 2012). However, metals accumulated on tree bark could be contributed by many factors such as vehicle types, fuel types, activities in the surrounding area and the tree itself i.e. tree species and bark types as well as collecting method.

Ukpebor *et al*. (2007) reported that metal concentrations found on bark of *Casuarina equisetifolia* L. (rough, porous, brittle and slightly thick bark) were 1.5 times lower than that those from *Delonix regia* (rough bark with a thin layer), in which planted in the same area with *C. equisetifolia* tree. This report was well agreed with the result of Majolagbe *et al*. (2010), in which accumulation of metals on the rough and hard bark tree *(Azadiractha indica*) was higher than that on the smooth and tough bark tree (*Ficus vopelli*) planted in the high traffic density area of Oyo Town (Nigeria). Nevertheless, both *Azadiractha indica* and *Ficus vopelli* trees were suggested as good bioindicators to indicate the level of atmospheric pollutants. Both studies illustrated that amount of metals accumulated on tree bark was influenced by tree species (different bark feature). Tree bark used to compare level of pollutants contamination between areas should be come from the same tree species. Moreover, using only level metal contaminations on tree bark was seem to be unsuitable for comparison degree of contamination in different areas because various tree species were used.

Therefore, use of ratio between metal concentrations in sample and in background area (EF_B) or in soil (EF_{TS}) and use of pollutant indices (I_{geo}, CF and PLI) might be more effective for air quality comparing between areas, than use only pollutant concentration.

Table 3.10 Comparison of metal concentrations among sampling sites

Sampling sites (Sample no.)		Metal concentration (ng/cm^2)						
		Al	Cr	Cu	Fe	Ni	Pb	Zn
Chiang Mai City Urban (n=79)	Mean	$1{,}312^{aA}$	2.38^{aB}	22.4^{aB}	734^{aC}	1.03^{aB}	8.53^{aB}	173^{aB}
	SD	1,245	4.32	12.0	587	3.05	17.60	95
	Min-Max	180-6,975	ND- 29.1	5.1-65.0	63-2,725	14.7 - ND	133-ND	39-609
Sub-urban (n=36)	Mean	$1{,}075^{abA}$	1.60^{aB}	18.4^{abB}	649^{aC}	1.26^{aB}	1.58^{aB}	137^{abB}
	SD	844	2.60	8.8	513	3.25	5.39	100
	Max -Min	190-4,044	ND -10.9	6.4-49.4	63-2,208	11.8 - ND	23-ND	31-644
Background site (n=4)	Mean	105^{bA}	ND^{aB}	9.0^{bB}	28^{bB}	ND^{aB}	ND^{aB}	82^{bA}
	SD	57		7.8	28			22
	Max -Min	53-186	ND	2.7-20.4	67-ND	ND	ND	66-113

Note: *ND = Not detected (<LOD);* $^{a,b\ and\ A,B,C}$ *significant different ($p < 0.05$) among sampling sites and metal species, respectively (ANOVA test)*

Table 3.11 Metal concentrations found in various cities (µg/g unit)

Locations	Tree species	Bark feature	Concentration (µg/g)							References
			Al	Cr	Cu	Fe	Ni	Pb	Zn	
Rösa, Germany	*Pinus sylvestris* L.	Rough, thick	1,040 – 1,140	6.7 – 7.5	-	3,280 – 3,700	-	19.6 - 22.2	-	Schulz *et al.*, 1999
Benin City, Nigeria	*Delonix regia*[1]	Rough, thin	-	-	4.00 – 16.0	-	-	20.0 - 700	27.0 - 65.0	Ukpebor *et al.*, 2007
	Casuarina equisetifolia L[1].	Rough, porous, thick	-	-	2.00 – 10.0	-	-	10.0 - 270	34.0 - 104	
Oyo Town, Nigeria	*Azadiractha indica*[2]	Rough, hard	-	ND - 11.5	3.00 - 29.2	82.0 - 320	-	1.10 - 4.30	5.75 - 44.5	Majolagbe *et al.*, 2010
	Ficus vopelli[2]	Smooth, tough	-	ND - 9.50	2.50 - 9.50	69.5 -241	-	0.95 - 6.20	4.08 – 17.5	
Buenos Aires, Argentina	*Fraxinus pennsylvanica*	Smooth, thick, fissure	469 – 1,130	1.81 - 12.1	31.9 - 99.3	454– 1,230	0.94 - 2.84	24.1 - 50.1	110 - 212	Fujiwara *et al.*, 2011
Thessaloniki, Greece	*Platanus orientalis* L.	Smooth, exfoliates	-	0.89-1.61	18.6-28	261-445	-	11.6-16.1	-	Sawidis *et al.*, 2011
Banja Luka, Bosnia and Herzegovina	*Tilia* (Linden)	Smooth, fissure	-	2.98–13.8	9.88 - 33.5	184–1,648	12.4–17.1	14.7–125	17.6–168	Skrbi´c *et al.*, 2012
Genova, Italy	*Quercus ilex* L.	Rough, thick	-	-	11.4-164.2	160-6,730	1.01-31.4	4.7-345	18.4-466	Drava *et al.*, 2016
Chiang Mai, Thailand	*Cassia fistula*	Smooth, thin	32.0 - 1,259	ND - 5.22	1.50 - 9.53	9.92 - 584.9	ND - 5.19	ND - 23.94	3.93 -104	This study
Phrao, Thailand	*Cassia fistula*	Smooth, thin	14.8 -59.6	ND	1.11 - 3.49	ND - 21.4	ND	ND	19.3 - 34.1	This study

Note: *[1,2] Sample collected from the same area; [ND] Non detected; - no data*

In order to estimate the leaching effects by the rain on metal accumulation on tree bark, metal concentrations from the same trees collected in dry and wet seasons were analyzed and compared (Table 3.12). It was found that the concentrations of Cr, Cu, Pb and Zn were not significantly different ($p > 0.05$) between seasons base on the result of statistic pair t-test, except for Al and Fe. The result was well agreed with the study of Cruz *et al*. (2012) in Protugal, who indicated that accumulation of metals (Cr, Fe and Zn) on the bark of *Cryptomeria japonica* was no significant difference between exposed periods in the summer and winter seasons. The reason might be due to the fact that tree bark exposed to metals from both atmosphere and steam flow. Even though, metals accumulated on tree bark were removed by the rain but they are also accumulated from wet deposition by leaching metals from atmosphere to attach on the bark.

According to the study of Catinon *et al*. (2012) in France, it was indicated that the concentration of metals in rain leaching from tree trunks was not different for each rain event. They also found that the total weight of dry matter leached (particles + water solution centrifuged and evaporated to dryness) from the 15 rain samples (104 L of total leachate volume) was 2 times higher than that from annual deposit content on the stem, meaning that free deposit formed on the tree bark had a quick renewal rate and could be fully recovered in a few months (2–3 months). In case of Al and Fe, their concentrations found in this study were higher in the wet season than those in the dry season due to contribution from soil particles. In wet season, soil on the road is mixed with rain water and can be flushed off, splashed and deposited on tree trunks located near the road. Moreover, soil or clay attached on the wheels of vehicle was other source of distribution of soil particle around the road side during the vehicle moving.

Table 3.12 Comparison of metal concentrations on tree bark between sampling seasons

Metals	Metal concentration (ng/cm^2)			
	Dry season* (n=40)		Wet season*(n=40)	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max
Al	892 ± 728^{aA}	180 - 4,044	1,252 ± 1,109^{bA}	30 - 5,408
Cr	1.38 ± 1.90^{aB}	ND - 6.90	0.90 ± 1.87^{aB}	ND - 7.40
Cu	19.6 ± 10.0^{aB}	7.8 - 49.4	23.7 ± 15.4^{aB}	2.5 - 65.6
Fe	534 ± 440^{aC}	64 - 2,024	705 ± 524^{bC}	78 - 2,214
Ni	0.58 ± 2.07^{aB}	ND - 8.57	0.21 ± 1.35^{aB}	ND - 8.78
Pb	3.08 ± 5.85^{aB}	ND - 16.2	4.45 ± 9.09^{aB}	ND - 34.6
Zn	151 ± 105^{aB}	31 - 644	171 ± 101^{aB}	ND - 475

Note: **The same trees; ND = Not detect (<LOD); $^{a,b\ and\ A,B,C}$ significant different ($p < 0.05$) between sampling seasons(pair t-test) and metal species (ANOVA test), respectively*

3.5.2 Metal concentrations in soil

In order to estimate the anthropogenic impacts on the bark of *Cassia fistula*, concentration of each metal accumulated on the bark was compared with its concentration found in local soil (enrichment factor). Therefore, 40 soil samples collected from road sides in the city of Chiang Mai were analyzed in this study. Meanwhile, 3 soil samples from Phrao district were also analyzed as background values. Metal concentrations in the soil samples are shown in Table 3.13. Mean concentration (mg/kg) of metals in soil samples from Chiang Mai City in descending order were Fe (17,742±6,214) > Al (14,174±5,428) >> Zn (150±70) > Cr (41.8±17.9) > Pb (34.6±22.8) > Cu (23.7±13.9) and Ni (22.0±10.7). The result showed that Fe and Al content in soil samples was about 2%. Their concentration were 100 times higher than Zn and about 500 times higher than a group of minimal content metals (Cr, Pb, Cu and Ni). Concentrations of most of the metals found in the city of Chiang Mai were about 1.5 times higher than those in background site. However, no significant difference between sites was observed. Unlikely the result from *Cassia fistula* bark, metal concentrations in urban area was significantly higher than in background area. It revealed that metals accumulated on tree bark could mainly come from atmospheric deposition and less influence from direct uptake from soil.

Metal concentrations found in roadside soils of Chiang Mai were compared with those in other cities around the world and the soil background values of the world were used as the reference values (Table 3.14). Concentrations of most of the metals (except for Zn) found in the soil of Chiang Mai were almost the same as those in Beijing, China and Galway, Ireland. However, they were lower than Banja Luka, Bosnia and Herzegovina. Additionally, concentration of Cr, Cu, Ni and Pb in soil samples of Chiang Mai were not over the world background values. Normally, Zn concentration of soil samples collected in Chiang Mai was 1.5 - 2 times higher than the background values (the world background and Phrao, Chiang Mai). The result indicated that soil in the city of Chiang Mai was probably polluted from vehicle pollutants emitted from vehicle tires (Apeagyei *et al.*, 2011).

3.5.3 Correlations of metals accumulated on *Cassia fistula* bark

Metal concentrations on the *Cassia fistula* barks in the city of Chiang Mai were used to calculate for the correlation among of metal species. Table 3.15 presents correlations of metals on bark samples from the city of Chiang Mai. Result of Pearson's correlation indicated that significant correlation ($p < 0.05$) between metals were observed for almost all of metal species. Most of them were classified as weak to moderate correlations ($r = 0.1 – 0.6$). Only Fe and Al presented strong correlation ($r > 0.9$). The metals accumulated on *Cassia fistula* bark were probably generated from the same sources. However, weak correlation between metals might be because of regional variation of natural background metal content on tree bark. Therefore, using the ratio of metal concentration found in study area and background area could be useful to reduce the variation between samples (Bergamaschi *et al.*, 2002; Zhang *et al.*, 2014).

Table 3.13 Metal concentrations in soil samples

Sampling sites (n)		Metal concentration (mg/kg) Al	Cr	Cu	Fe	Ni	Pb	Zn
Chiang Mai City site								
Urban area	Mean	13,879^{aA}	36.1^{aB}	25.0^{aB}	15,793^{abC}	18.3^{abB}	39.6^{aB}	156^{aB}
(26)	SD	5,118	13.1	16.6	4416	7.3	25.9	72
	Min	7,491	14.0	6.1	5512	3.7	9.1	33
	Max	23,389	79.4	82.0	24058	37.9	125.9	353
Sub-urban area	Mean	14,721^{aA}	52.4^{aB}	21.2^{aB}	21,361^{aC}	29.0^{aB}	25.5^{aB}	138^{aB}
(14)	SD	6,126	21.0	6.7	7,527	12.7	11.0	66
	Min	1,244	30.2	10.8	1,038	14.3	13.0	56
	Max	25,816	99.5	31.8	31,024	57.1	51.6	286
Background site								
Phrao	Mean	12,791^{aA}	34.4^{aB}	14.4^{aB}	13,573^{bC}	16.7^{bB}	22.5^{aB}	102^{aB}
(3)	SD	1,479	1.4	0.8	357	3.1	6.9	12
	Min	11,544	33.1	13.7	13,167	13.5	17.2	93
	Max	14,426	35.9	15.3	13,838	19.7	30.3	116

Note: *$^{a,b\ and\ A,B,C}$ significant different ($p < 0.05$) among sampling sites and metals species, respectively (ANOVA test)*

Table 3.14 Comparison of mean concentration of metals in roadside soil in the city of Chiang Mai and other cities

Locations	Mean concentrations (mg/kg)							References
	Al	Cr	Cu	Fe	Ni	Pb	Zn	
Osogbo, Nigeria	-	-	21.2	-	8.38	68.7	42.5	Fakayode and Olu-Owolabi, 2003
Beijing, China	-	61.9	29.7	-	26.7	35.4	92.1	Chen *et al*., 2010
Galway, Ireland	-	-	16.6	-	22.1	40.8	81.8	Dao *et al*., 2010
Banja Luka, Bosnia and Herzegovina,	-	4.39	32.2	11,801	45.6	74.7	139	Skrbi´c *et al*., 2012
Background values of the world	-	70.0	30.0	-	50.0	35.0	90.0	Chen *et al*., 2010
Chiang Mai City, Thailand	14,174	41.8	23.7	17,742	22.0	34.6	150	This study
Phrao, Thailand (Background site)	12,791	34.4	14.4	13,573	16.7	22.5	102	This study

Note:- *No data available*

Table 3.15 Correlation coefficients of metals accumulated on *Cassia fistula* bark

	Metals					
	Al	Cr	Cu	Fe	Ni	Pb
Cr	0.576**					
Cu	0.467**	0.346**				
Fe	**0.915****	0.639**	0.449**			
Ni	0.236*	0.341**	0.242**	0.267**		
Pb	0.482**	0.593**	0.330**	0.482**	0.233*	
Zn	0.224*	0.194*	0.215*	0.296**	0.161	0.158

Note: **,** significant correlation between metals at $p < 0.05$ and $p < 0.01$ levels, respectively; Bold letter: strong correlation ($r > 0.7$) between metal species*

3.5.4 Enrichment factors of metals

Enrichment factor values of each metal species was calculated for individual bark sample in order to reduce the variation between samples. Two types of enrichment factor were used in this study. Enrichment factor of metal in bark (EF_B) is calculated by using ratio between metal concentration found in the tree bark collected in Chiang Mai City and concentration of the same metal from the background area (Phrao district). Meanwhile, the ratio between the metal concentration found in tree bark in the city and its concentration found in local soil near the sampling trees was defined as enrichment factor in soil (EF_{TS}). Before calculation, the metal concentrations were normalized with the concentration of metal which rarely enters to environment from anthropogenic activity. Metals such as Sc, Ti, Zr and Al were widely used as the normalizing metals for calculating enrichment factors because of their low variability of occurrence, and fractionation effects of weathering (Zhang *et al.*, 2014). In calculation of EF_B and EF_{TS}, concentrations of metals were presented in Appendix D. In this study, the concentration of metals were assumed to be equal to their LOD values when their concentrations were lower than limit of detection value (< LOD value).

1) Enrichment factor in bark (EF_B) of metals accumulated on tree bark

Enrichment factor by comparing with the bark from background area (EF_B) was calculated in order to estimate accumulation rate of metal on tree bark. The EF_B values of metals in the city of Chiang Mai were Fe (20.2) > Al (9.07) > Zn (1.62) > Cu (1.44) > Cr (1.37) > Pb (1.13) > Ni (0.97). Comparison between urban and sub-urban area, it was found that the EF_B values of most of metals in urban area were 1.5 times higher than those in sub-urban area (Table 3.16). In order to present accumulation rates of metals on tree bark, the mean EE_B values were classified into 5 classes as proposed by Frati *et al*., (2005) (Table 3.17). It was found that the EF_B values of Fe and Al (EF_B > 1.75) were high meaning severe accumulation rate for both urban and sub-urban areas. A severe accumulation was also found from the EF_B value of Zn in urban area. Meanwhile, that EF_B value (0.75 – 1.25) found in sub-urban area was classified as normal rate accumulation. The EF_B values of Cr, Cu and Pb had fallen into a class of accumulation rate (1.25 – 1.75) in urban area while, those values found in sub-urban area were classified in normal rate (0.75 – 1.25). The comparing result between areas indicated that the accumulation rate of metals in urban area was higher than that in sub-urban area.

Table 3.16 Enrichment factor in bark (EF_B) of metals on *Cassia fistula* bark

Areas		EF_B						
(n)		Al	Cr	Cu	Fe	Ni	Pb	Zn
Urban	Mean	10.3^{aA}	1.56^{aB}	1.61^{aB}	22.7^{aC}	1.05^{aB}	1.28^{aB}	1.84^{aB}
(n = 79)	Min	0.070	0.017	0.038	0.092	0.017	0.024	0.025
	Max	63.3	11.8	8.841	163	11.3	11.3	12.0
Sub-urban	Mean	6.23^{bA}	0.958^{bB}	1.08^{bB}	14.4^{bC}	0.777^{aB}	0.796^{bB}	1.14^{bB}
(n = 36)	Min	0.465	0.091	0.162	2.68	0.091	0.091	0.123
	Max	23.5	5.17	2.62	60.8	2.85	2.85	3.02

Note: $^{a,b \text{ and } A,B,C}$ *significant different (p < 0.05) between sampling areas (t-test) and metals species (ANOVA test), respectively*

Table 3.17 Accumulation rate classifications for enrichment factor in bark (EF_B) of metals on *Cassia fistula* bark (Frati *et al*., 2005)

EF_B ranges	Accumulation rates	Mean EF_B values						
		Al	Cr	Cu	Fe	Ni	Pb	Zn
Urban area (n = 79)								
0 – 0.25	Severe loss							
0.25 – 0.75	Loss							
0.75 – 1.25	Normal					1.05		
1.25 – 1.75	Accumulation		1.56	1.61			1.28	
> 1.75	Severe accumulation	10.3			22.7			1.84
Sub-urban area (n = 36)								
0 – 0.25	Severe loss							
0.25 – 0.75	Loss							
0.75 – 1.25	Normal		0.96	1.08		0.78	0.80	1.14
1.25 – 1.75	Accumulation							
> 1.75	Severe accumulation	6.23			14.4			

2) Enrichment factor in soil (EF_{TS}) of metals accumulated on tree bark

In order to indicate natural or anthropogenic sources of metals in Chiang Mai City, the enrichment factor by comparing with local soil (EF_{TS}) values were applied in this study. It was found that the EF_{TS} values in descending order were Zn (3.37), Cu (2.56), Pb (1.46), Ni (1.14), Cr (0.26), Al (0.25) and Fe (0.11) as shown in Table 3.18. Based on the EF value of metals, a value higher than 1 can be considered from long-distance transport phenomena, from other natural and/or anthropogenic sources (Bergamaschi *et al*., 2002), while a value greater than 10 is indicated anthropogenic influence (Ferreira *et al*., 2012). Therefore, the possible sources of Zn (EF_{TS} = 3.37), Cu (EF_{TS} = 2.56), Pb (EF_{TS} =1.46) and Ni (EF_{TS} = 1.14) are from long-range transport of either natural or/and anthropogenic sources. The mean EF_{TS} values of Al, Cr and Fe (0.25, 0.26 and 0.11 respectively) were less than 1, meaning that they are originated from the surrounding crustal (Bergamaschi *et al*., 2002). For area comparison, the EF_{TS} values of metals from urban area were higher than that from sub-

urban area. Nevertheless, source of metals on the bark from both areas might come from the same source according to EF_{TS} value classification.

Table 3.18 Enrichment factor by soil (EF_{TS}) of metals on *Cassia fistula* bark

Areas (n)		EF_{TS}						
		Al	Cr	Cu	Fe	Ni	Pb	Zn
Urban	Mean	0.282^{aA}	0.298^{aA}	2.86^{aB}	0.121^{aA}	1.24^{aC}	1.65^{aC}	3.83^{aD}
(79)	Min	0.002	0.003	0.068	0.000	0.020	0.032	0.052
	Max	1.72	2.26	15.7	0.870	13.3	14.7	24.9
Sub-urban	Mean	0.170^{bA}	0.183^{aA}	1.92^{bB}	0.076^{bA}	0.914^{aC}	1.03^{bC}	2.36^{bD}
(36)	Min	0.013	0.017	0.288	0.014	0.106	0.117	0.256
	Max	0.645	0.987	4.65	0.323	3.35	3.68	6.27

Note: $^{a,b \text{ and } A,B,C,D}$ *significantly different ($p < 0.05$) between sampling area (t-test) and metals species (ANOVA test), respectively*

In comparison of EF_B and EF_{TS} values, it was found that EF_{TS} of Cu, Ni, Pb and Zn were higher than EF_B. The exception was observed only for Al and Fe. It might be because the composite of Al and Fe content in soil (~ 2%) was relatively high comparing to other metals (~ 0.002 – 0.02%). Therefore, the ratio of metals on bark sample and soil sample (EF_{TS} values) was low.

The result of EF_{TS} values indicated that the city of Chiang Mai was polluted with metals generated from both natural and anthropogenic sources. However, using EF_{TS} values to estimate source is inappropriate because it can indicate only natural or anthropogenic source. Therefore, principal component analysis (PCA) and cluster analysis (CA) were applied for source estimation.

2) Correlations of enrichment factors values of metal accumulated on tree barks

Both EF_B and EF_{TS} values of metals were calculated for correlations between metal species. Table 3.19 present correlation result of enrichment factors value of metals on bark sample from the city of Chiang Mai. The results showed strong correlation (r = 0.65 – 0.97 with p <0.01) for all metals. The correlation coefficients among metal enrichment factors (EF_B and EF_{TS}) presented stronger than those among metal concentrations, meaning that correlations. It might be because the variation of metal between samples (different of background metal content on barks) was reduced by from normalizing with natural source metal (Mn).

Table 3.19 Correlation coefficients of enrichment factors values of metals on tree bark

Enrichment factors		Pearson correlation coefficients (r)					
		Al	Cr	Cu	Fe	Ni	Pb
EF_B	Cr	**0.770****					
	Cu	**0.801****	**0.876****				
	Fe	**0.953****	**0.762****	**0.782****			
	Ni	0.688**	**0.876****	**0.893****	0.651**		
	Pb	**0.728****	**0.948****	**0.887****	0.699**	**0.917****	
	Zn	0.660**	**0.813****	**0.835****	0.665**	**0.877****	**0.836****
EF_{TS}	Cr	**0.790****					
	Cu	**0.771****	**0.870****				
	Fe	**0.965****	**0.759****	**0.748****			
	Ni	0.695**	**0.926****	**0.858****	0.654**		
	Pb	**0.751****	**0.960****	**0.878****	**0.702****	**0.954****	
	Zn	0.662**	**0.824****	**0.787****	0.654**	**0.851****	**0.853****

Note:** *significant correlation between metal at p < 0.01 level; bold letter : strong correlation (r>0.7) between metal species*

3) Estimation the origin of metals accumulated on tree bark

Metals are emitted from many activities including transportation i.e. direct vehicle emission (fuel combustion and vehicle part erosion) and direct emission such as dispersion of particle attached on vehicle wheels and road surface. Therefore, the origin of metals was identified by using principal component analysis (PCA) and cluster analysis (CA).

PCA for enrichment factors were calculated by using the varimax rotation of principal component patterns in order to improve the differentiation between sources. Ayers and Yeung (1996) mentioned that only factor loading higher than 0.5 was indicated to be statistically significant. Table 3.20 shows PCA results of both EF_B and EF_{TS}. Two principal components (PCs) were classified with a cumulative explained variance of about 93% for both EF_B and EF_{TS}. Moreover, their loading patterns were also similar. The first principal component was characterized by high coefficient values of Cr, Cu, Ni, Pb and Zn (> 0.7) and accounts for 56% of the total variance. Cu and Zn are always used as marker of traffic because there were major metal component of break ware and tyre ware (Apeagyei *et al*., 2011; Pant and Harrison, 2013). Cr was an important constituent of metal alloys (e.g., Ni–Cr–Mo, Cr–V, Cr–W), while Ni was observed from exhaust-emission (Pant and Harrison, 2013; Zannoni *et al*., 2016). Moreover, Pulles *et al.*, (2012) mentioned that Cr, Cu, Pb, and Zn were the dominant metal species emitted from lubricant oil combustion. Therefore, it was suggested that the metals found on tree bark are from representative source of vehicle emission.

The second loading exhibited significant coefficients for Al and Fe (> 0.8) and accounts for about 37% of the total variance. Those metals were reported in literature that released from crust material (Yongjie *et al.*, 2009; Enamorado-Báez *et al*, 2015). However, Fe was also found as composition in brake pads and tires of vehicles (Thorpe and Harrison, 2008; Apeagyei *et al*., 2011). Therefore, it could not be cleared for the source of metals existed in this PC. Consequently, CA was applied to estimate the source metals from presenting of a similar pattern of EF_{TS} value.

Table 3.20 Principal component analysis of enrichment factors

Components	EF_B		EF_{TS}	
	1	2	1	2
Al	0.412	**0.896**	0.412	**0.896**
Cr	**0.810**	0.502	**0.810**	0.501
Cu	**0.783**	0.542	**0.783**	0.542
Fe	0.383	**0.910**	0.383	**0.910**
Ni	**0.911**	0.335	**0.911**	0.335
Pb	**0.876**	0.408	**0.876**	0.408
Zn	**0.860**	0.342	**0.860**	0.342
% of Variance	56.04	36.76	56.04	36.75
Cumulative (%)	56.04	92.79	56.04	92.79

Note: *The bold letter: the greater value in classified component of metal*

Cluster analysis was performed on enrichment factors (EF_B and EF_{TS}) values of the seven metals on roadside bark samples. Prior to calculation, the individual EF_B and EF_{TS} values of metals were transformed by dividing the values with its maximum value (maximum magnitude of 1 method) in order to avoid the overpowering of the higher concentration on classification the metal into groups. Then the average linkage (between-groups linkage) and Squared Euclidean distance were calculated for cluster classification. The cluster results were similar on metal classification pattern for both EF_B and EF_{TS} values. There were three distinct clusters preformed at a low distance criterion less than 10 (Figure 3.11). The first cluster composed of Ni, Pb and Cr, while Cu and Zn were classified to the second cluster. The third cluster contained Al and Fe. However, the distance between cluster I and II was relative short and likely separated from cluster III. Moreover, Cr, Cu, Ni, Pb and Zn were reported in literatures as tracer of vehicle source (Péreza *et al*., 2010; Amato *et al*., 2011; Song and Gao, 2011). In addition, the metals clustered were well agreed with PCA result. Consequently, Cr, Cu, Ni, Pb and Zn were grouped and indicated as vehicle pollutants in this study.

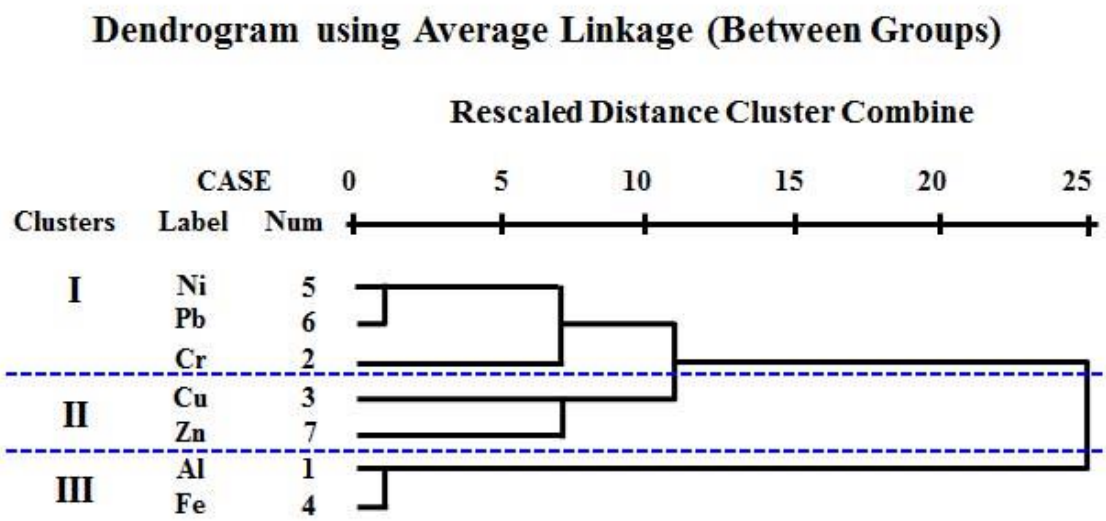


Figure 3.11 Dendrogram for enrichment factors value of 7 metals

In case of cluster III, very close distance (< 5) was observed between Al and Fe, meaning very close relationship of both metals. Al is always used as crustal tracer because it is an abundant metal composite in the Earth's crust, which is comprising 7% of its mass (Dong *et al.,* 2002). It was reported that Fe can be generated from both vehicle and soil particle (Apeagyei *et al.,* 2011; Enamorado-Báez *et al.*, 2015) but close relationship between Fe and Al including very strong correlation (r = 0.915) between their concentration, CA result as well as PCA result. Therefore, Al and Fe should be generated from soil particle attached on wheel or road surface, which are distributed from mobile source. Consequently, Al and Fe were group in soil pollutant in this study.

3.6 Pollution indices for metal pollution assessment

The pollution indices including contamination factor (CF), geoaccumulation index (I_{geo}) and potential load index (PLI) were applied in this study in order to present relative contaminants in Chiang Mai City and background area. The indices were presents in terms of individual metal and group of metals by origin including vehicle pollutants (Cr, Cu, Ni, Pb and Zn), soil pollutants (Al, Fe) and mix pollutants (the 7 observed metals). Indices calculation, the concentration of metals were

assumed to be equal their LOD values when the concentrations were lower than limit of detection value (< LOD value).

3.6.1 Contamination factor and pollution load index of metals

The contamination factor (CF) is a relative of contaminant in study area with background area. The CF value was used for metal contamination level expression in sediment (Salah *et al*., 2012). In this study, this values was applied in order to present contamination status on the bark of *Cassia fistula* in Chiang Mai City comparing to the background site (Phrao district). The CF value of metals on tree barks in the city was calculated (Appendix D) and the result was showed in Table 3.21. The result found that the CF value of metals in a group of soil pollutants (Al and Fe) were 5 – 25 times higher than those in the group of vehicle pollutants (Cr, Cu, Ni, Pb and Zn). The CF values of most of the metals in urban area were higher than those that in sub-urban area. However there were no significant difference between the areas. Contamination levels of metals in the city of Chiang Mai were classified into four levels base on the CF values (Salah *et al*., 2012) as shown in Table 3.22. Mean CF values of Cr, Ni and Pb were about 1.03 - 1.58 and classified as moderate contamination level ($1 \leq CF < 3$), accounting for 94 - 100% of sampling areas (Figure 3.12). It means that this study area was moderately contaminated with Cr, Ni and Pb. The CF values of Cu and Zn ranged from 0.38 – 7.85. Their mean values were 2.33 (Cu) and 1.97 (Zn). Their CF values were categorized as moderate contamination level and accounting for 68 -78% of total sampling points. Moreover, while 19% of the samples were classified as considerable contamination level ($3 \leq CF < 6$). Considering soil pollutants, mean CF values of Al were 12.54 (urban) and 10.28 (sub-urban) and those of Fe were 12.54 (urban) and 10.28 (sub-urban). About 64 – 89% of total area of the city were classified as very high contamination level ($CF \geq 6$). The result indicated that the city of Chiang Mai is very high contaminated with Al and Fe.

Table 3.21 The contamination factor (CF) and pollution load index (PLI) values of metals on *Cassia fistula* bark in the city of Chiang Mai

Sampling areas		CF							PLI		
(n)		Al	Cr	Cu	Fe	Ni	Pb	Zn	Vehicle	Soil	Mix
Urban area	Mean	12.544^{aA}	1.584^{aB}	2.468^{aB}	26.486^{aC}	1.033^{aB}	1.335^{aB}	2.108^{aB}	1.523^{aA}	17.966^{aB}	2.952^{aA}
(79)	SD	11.900	1.647	1.312	21.171	0.144	1.300	1.161	0.628	15.230	1.491
	Min	1.725	1.000	0.558	2.299	1.000	1.000	0.483	0.915	1.992	1.379
	Max	66.676	12.763	7.159	98.308	2.100	11.577	7.422	5.636	80.962	12.068
Sub-urban	Mean	10.277^{aA}	1.352^{aB}	2.030^{aB}	23.423^{aC}	1.040^{aB}	1.054^{aB}	1.673^{aB}	1.309^{aA}	15.405^{aB}	2.540^{aA}
area	SD	8.064	0.782	0.976	18.524	0.151	0.199	1.220	0.273	12.040	0.840
(36)	Min	1.815	1.000	0.704	2.291	1.000	1.000	0.381	0.855	2.039	1.239
	Max	38.655	4.761	5.443	79.685	1.680	2.029	7.845	1.970	53.368	4.696

Note: $^{a,b \text{ and } A,B,C}$ *significantly different ($p < 0.05$) between sampling areas (t-test)and metals species(ANOVA test), respectively*

Table 3.22 Classification of contamination levels for contamination factor (CF) in the city of Chiang Mai (Salah *et al*., 2012)

Areas	Metals	Contamination levels			
		Low (CF < 1)	Moderate (1 ≤ CF < 3)	Considerable (3 ≤ CF < 6)	Very high (CF > 6)
Urban area (n =79)	Al				12.544
	Cr		1.584		
	Cu		2.468		
	Fe				26.486
	Ni		1.033		
	Pb		1.335		
	Zn		2.108		
Sub-urban area (n =36)	Al				10.277
	Cr		1.352		
	Cu		2.030		
	Fe				23.423
	Ni		1.040		
	Pb		1.054		
	Zn		1.673		

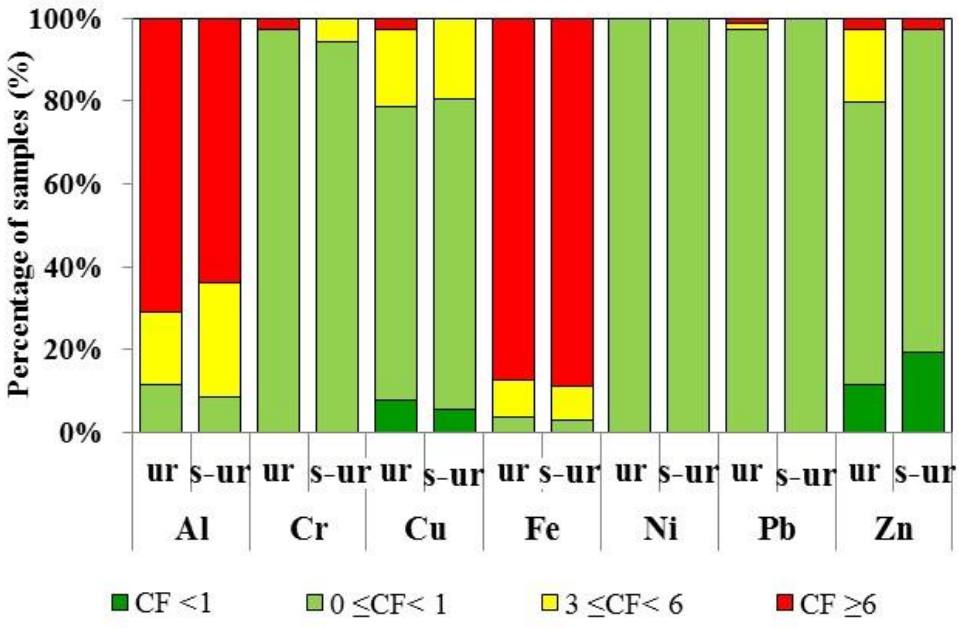


Figure 3.12 Contamination factor (CF) showing characteristics of metals in Chiang Mai City

The pollution load index (PLI) was proposed by Tomlinsonet *et al*. in 1980 which is calculated from multiplication of CF values for all of interest metal species. This study, the PLI was used to qualify the pollution effect of various elements grouped by sources including vehicle pollutants (Cr, Cu, Ni, Pb and Zn), soil pollutants (Al and Fe) and mixed pollutants (all 7 metals). The PLI values of pollutants on the tree bark were calculated as the detail in Appendix D. The result (Table 3.21) shows that PLI values of soil pollutants were 12 times higher than those of vehicle pollutants for both urban and sub-urban areas. The mean PLI values in both urban and sub-urban area were classified into seven class of pollutant degrees as shown in Table 3.23. It was found that mean PLI values of vehicle pollutants in urban and sub-urban areas were classified into unpolluted to moderately polluted ($1 < PLI \leq 2$), while those of soil pollutants, 17.966 (urban) and 15.405 (sub-urban), were in a class of very highly polluted degree ($PLI > 5$). The mean PLI values of mix pollutants were classified in moderate polluted degree ($2 < PLI \leq 3$). However, the individual PLI value of soil pollutants varied from 2.00 to 80.96. The variation of PLI value is shown in Figure 3.13. It was found that the area with PLI value of soil pollutants were greater than five ($PLI > 5$), was about 82-83% of total area indicating that this study area was very highly polluted due to soil pollutants. Meanwhile, 91- 92% of samples presented the PLI values of vehicle pollutants in a range of 1 - 2, therefore the city was classified as unpolluted to moderately polluted with vehicle pollutants. In case of mix pollutants, its mean PLI value illustrated a moderately polluted degree ($2 < PLI \leq 3$). However, 64 – 73% of the samples present PLI value greater than 2 ($PLI > 2$) which indicating that the city of Chiang Mai was moderately to highly polluted.

Table 3.23 Classification of pollution degrees for pollution load index (PLI) in the city of Chiang Mai (Guéguen *et al*., 2012; Chen *et al*., 2015)

PLI ranges	Pollution degrees	Mean PLI values					
		Urban area			Sub-urban area		
		Vehicle	Soil	Mix	Vehicle	Soil	Mix
PLI= 0	Background concentration						
0 < PLI ≤ 1	Unpolluted						
1 < PLI ≤ 2	Unpolluted to moderately polluted	1.523			1.309		
2 < PLI ≤ 3	Moderately polluted		2.952				2.540
3 < PLI ≤ 4	Moderately to highly polluted						
4 < PLI ≤ 5	Highly polluted						
PLI > 5	Very highly polluted		17.966			15.405	

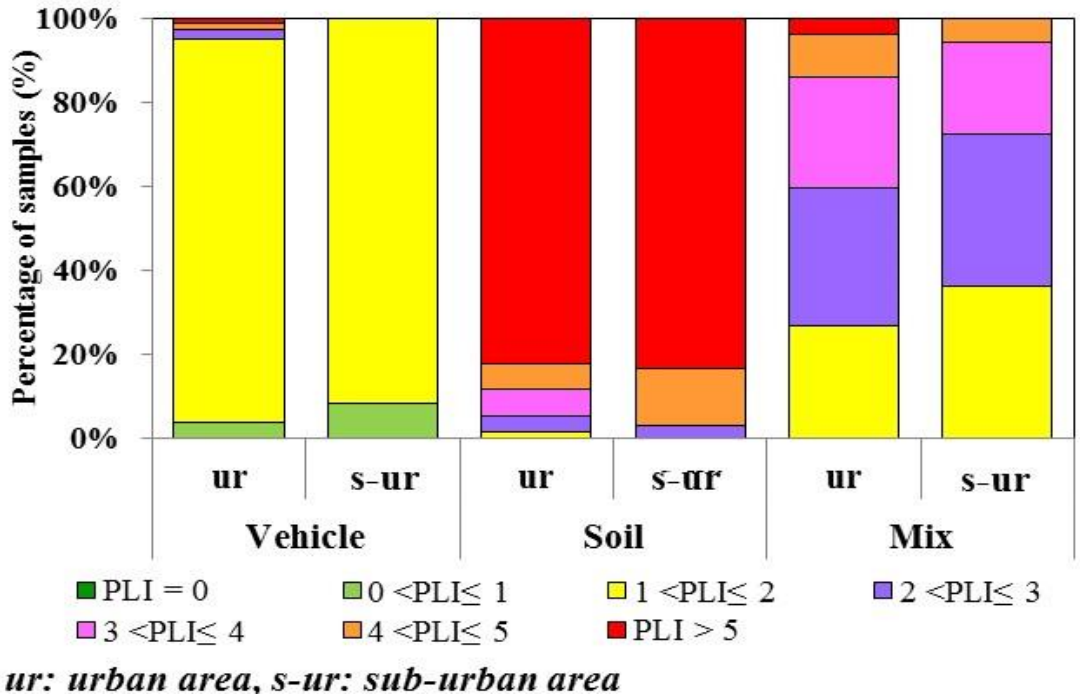


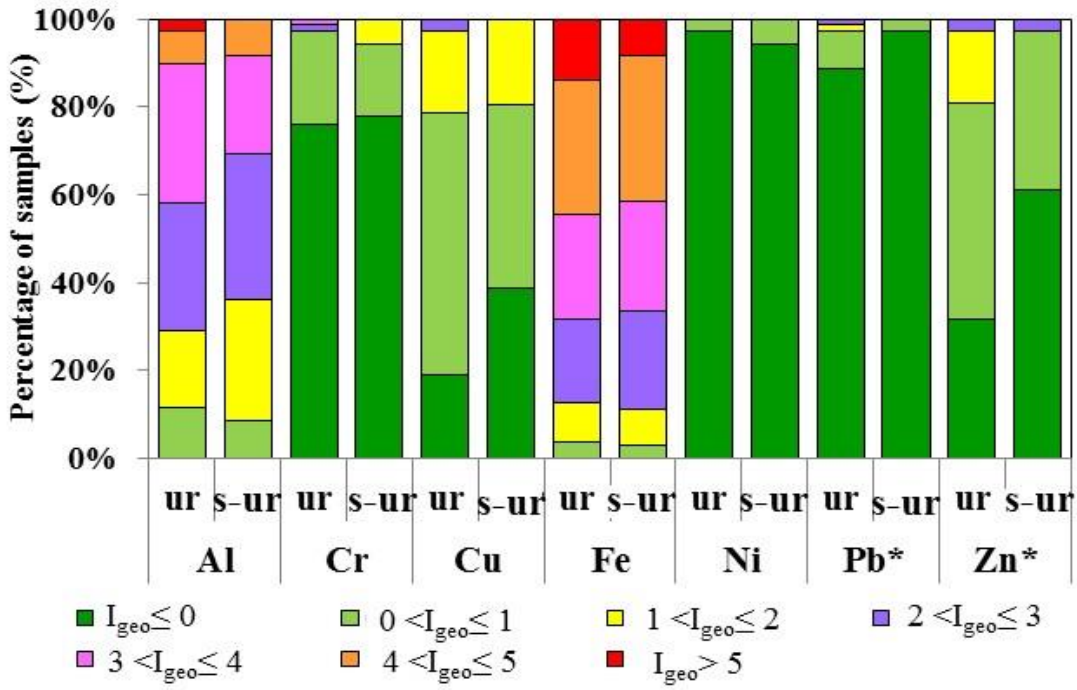
Figure 3.13 Pollution load index (PLI) characteristics in Chiang Mai City

3.6.2 Geoaccumulation index of metals

The geoaccumulation index (I_{geo}), was proposed by Müller in 1969, is calculation of metal concentration in study area above background or baseline concentration (Abrahim and Parker, 2008). This index is commonly applied to assess the pollution status in soil, sediment and tree bark in many area (Abrahim and Parker, 2008; Guéguen *et al.*, 2012; Chen *et al.*, 2015). On the other hand, total geoaccumulation index ($I_{GEO\text{-}tot}$), a new index which is integration of I_{geo} and PLI for n metals, was proposed by Guéguen *et al.* in 2012. This new index is useful to indicate pollution level in each study area. In this study, individual geoaccumulation index (I_{geo}) was calculated the individual metal concentration found on the tree bark in Chiang Mai City relative to its concentration in Phrao District (background area), while the geoaccumulation index ($I_{GEO\text{-}tot}$) was applied for group of pollutants including vehicle pollutants, soil pollutant as well as mix pollutants (Appendix D). The result showed that The I_{geo} values were varied greatly among metal species (Table 3.24). The highest I_{geo} value was observed for Fe (0.611 - 6.034), while Ni showed the lowest I_{geo} value (-0.620 - 0.486). Moreover, the I_{geo} values of soil pollutant group (Al and Fe) were higher than those of vehicle pollutant group (Cr, Cu, Ni, Pb and Zn), which were similar to the result of the CF values. In additional, the I_{geo} value of almost metals found in urban area were higher than those in sub-urban area but no significant difference were observed. Exception was observed for Pb and Zn, which had significant difference between areas.

The mean I_{geo} values of Ni were -0.55 (urban) and - 0.54 (sub-urban), while those of Pb were -0.35 (urban) and - 0.53 (sub-urban). Those values were negative value ($I_{geo} \leq 0$), which were classified into class 0 meaning non to weakly polluted (Table 3.25). However, the I_{geo} values of Ni and Pb varied from -0.62 to 2.95. The negative I_{geo} value ($I_{geo} \leq 0$) were accounted for 94 - 98% (Ni) and 89 - 97 % (Pb) of the total study area (Figure 3.14). It means that the city of Chiang Mai was very low contaminated with Ni and Pb. The mean I_{geo} values of Cr were -0.17 and -0.28 for urban and sub-urban areas, respectively. Their values were classified as non to weak polluted ($I_{geo} \leq 0$). Nevertheless, more than 22% of the bark samples were classified as being polluted with Cr. The I_{geo} value of Cu and Zn ranged from -1.98 to 2.39 and more than 60% of the

areas were fallen in to weakly to moderately polluted level. However, only 39% of samples from sub-urban area were polluted with Zn. In case of I_{geo} of Al and Fe, their mean I_{geo} values were also classified into 2 classes. Al (I_{geo} = 2.58 (urban) and 2.41 (sub-urban)) was fallen as moderately to strongly polluted class ($2 < I_{geo} \leq 3$), while Fe (I_{geo} = 3.62 (urban) and 3.52 (sub-urban)) was classified as strongly polluted class ($3 < I_{geo} \leq 4$). Moreover, more than 64% of sampling areas were categorized as moderately to excessively polluted (I_{geo} >2). Consequently, the result illustrated that the city was highly polluted with Al and Fe.



ur: urban area, s-ur: sub-urban area

Note: ** significantly different ($p < 0.05$) between sampling areas*

Figure 3.14 Geoaccumulation index (I_{geo}) characteristics of metals in Chiang Mai City

Table 3.24 Individual geoaccumulation index (I_{geo}) and total geoaccumulation index ($I_{GEO-tot}$) values of metals on *Cassia fistula* barks in the city of Chiang Mai.

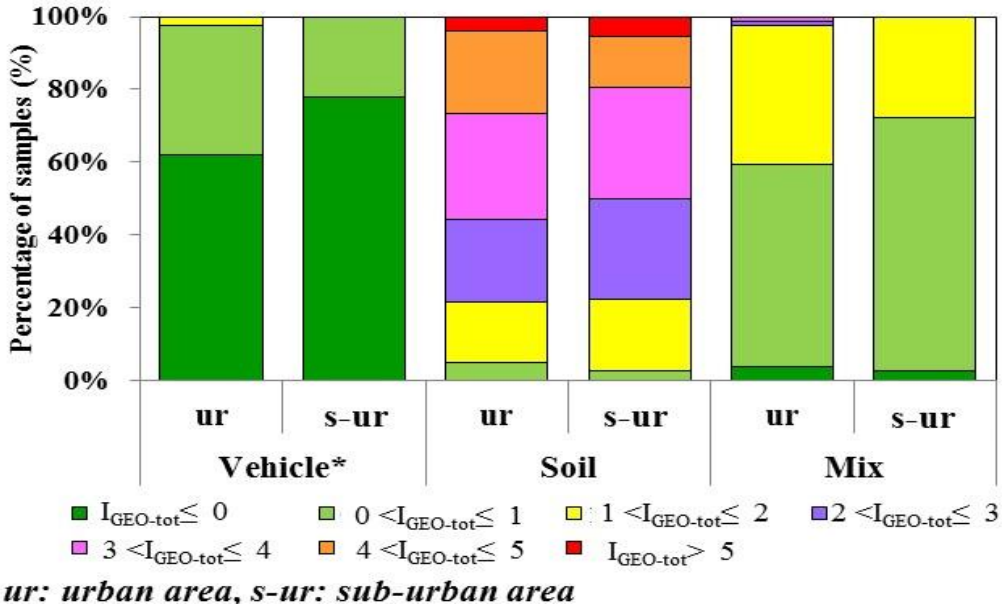
Sampling areas (n)		I_{geo}							$I_{GEO-tot}$		
		Al	Cr	Cu	Fe	Ni	Pb	Zn	Vehicle	Soil	Mix
Urban area (79)	Mean	2.577^{aA}	-0.174^{aB}	0.540^{aC}	3.622^{aD}	-0.548^{aE}	-0.345^{aBE}	0.303^{aC}	-0.045aA	3.099aB	0.854aC
	SD	1.190	0.681	0.723	1.333	0.150	0.533	0.745	0.394	1.237	0.567
	Min	0.202	-0.585	-1.427	0.616	-0.585	-0.585	-1.635	-0.713	0.409	-0.122
	Max	5.474	3.089	2.255	6.034	0.486	2.948	2.307	1.910	5.754	3.008
Sub-urban area (36)	Mean	2.405^{aA}	-0.281^{aBE}	0.285^{aC}	3.518^{aD}	-0.539^{aE}	-0.526^{bE}	-0.067^{bB}	-0.226bA	2.962aB	0.685aC
	SD	1.048	0.545	0.672	1.207	0.170	0.209	0.774	0.292	1.110	0.469
	Min	0.275	-0.585	-1.091	0.611	-0.620	-0.585	-1.977	-0.811	0.443	-0.275
	Max	4.688	1.666	1.860	5.731	0.164	0.436	2.387	0.393	5.153	1.646

Note: $^{a,b \text{ and } A-E}$ *significant different ($p < 0.05$) between sampling areas (t-test)and metals species(ANOVA test), respectively*

Table 3.25 The pollution degree of geoaccumulation index (I_{geo}) and total geoaccumulation index ($I_{GEO-tot}$) values of metals on *Cassia fistula* barks in the city of Chiang Mai (Guéguen *et al*., 2012; Chen *et al*., 2015)

I_{geo} ranges	Classes	Pollution degrees	I_{geo}							$I_{GEO-tot}$		
			Al	Cr	Cu	Fe	Ni	Pb	Zn	Vehicle	Soil	Mix
Urban area (n =79)												
$I_{geo} \leq 0$	0	Not to weakly polluted		-0.174			-0.548	-0.345		-0.045		
$0 < I_{geo} \leq 1$	1	Weakly to moderately polluted			0.540				0.303			0.854
$1 < I_{geo} \leq 2$	2	Moderately polluted										
$2 < I_{geo} \leq 3$	3	Moderately to strongly polluted	2.577									
$3 < I_{geo} \leq 4$	4	Strongly polluted				3.622					3.099	
$4 < I_{geo} \leq 5$	5	Strongly to excessively polluted										
$I_{geo} > 5$	6	Excessively polluted										
Sub-urban area (n=36)												
$I_{geo} < 0$	0	Not to weakly polluted		-0.281			-0.539	-0.526	-0.067	-0.226		
$0 < I_{geo} < 1$	1	Weakly to moderately polluted			0.285							0.685
$1 < I_{geo} < 2$	2	Moderately polluted										
$2 < I_{geo} < 3$	3	Moderately to strongly polluted	2.405								2.962	
$3 < I_{geo} < 4$	4	Strongly polluted				3.518						
$4 < I_{geo} < 5$	5	Strongly to excessively polluted										
$I_{geo} > 5$	6	Excessively polluted										

$I_{GEO\text{-}tot}$ value was used to present a polluted degree of group of metals from the emitted source including vehicle, soil and mixed pollutants. The result found that the value of soil pollutants were higher than that of vehicle and mix pollutants (Table 3.24). The $I_{GEO\text{-}tot}$ values of vehicle pollutants varied from -0.81 to 1.91. The mean values were -0.05 and -0.23 for urban and sur-urban areas, respectively. Event though, those $I_{GEO\text{-}tot}$ values were classified into non to weakly polluted (Table 3.25) but there were significantly different ($p < 0.05$) between urban and sub-urban areas. From the characteristic of $I_{GEO\text{-}tot}$ in the city of Chiang Mai (Figure 3.15), it was found that the areas with $I_{GEO\text{-}tot}$ greater than 0 ($I_{GEO\text{-}tot} > 0$) were 38% for urban area and only 22% for sub-urban area. Therefore, it was assumed that urban area was more polluted with vehicle pollutants than sub-urban area. Meanwhile, the mean $I_{GEO\text{-}tot}$ values of soil pollutants: urban area ($I_{GEO\text{-}tot} = 3.10$) and sub-urban area ($I_{GEO\text{-}tot} = 2.96$), were fallen into strongly polluted class and moderately to strongly polluted class, respectively. Moreover, 95 – 97% of areas in the city had $I_{GEO\text{-}tot} > 1$, meaning that the city was moderately polluted to excessively polluted with soil pollutants. On the other hand, the value of mix pollutants were classified as weakly to moderately polluted ($0 < I_{GEO\text{-}tot} \leq 2$) 94 – 97% of bark samples. It was indicated that the city of Chiang Mai were slightly contaminated with pollutants generated from traffic.



Note: ** significantly different ($p < 0.05$) between sampling areas*

Figure 3.15 Total geoaccumulation index ($I_{GEO\text{-}tot}$) characteristics in Chiang Mai City

Comparing of polluted level classification between urban and sub-urban area, it was found that no significant difference ($p > 0.05$) between areas was observed from CF and PLI value calculation. It was indicated that those indices were unable to distinguish contamination levels between areas. On the other hand, significant different between areas ($p < 0.05$) were illustrated from the values of I_{geo} (Pb and Zn) and $I_{GEO\text{-}tot}$ (vehicle pollutants), meaning that those indices was able to distinguish the difference of polluted levels between areas. Therefore, I_{geo} and $I_{GEO\text{-}tot}$ indices should be better than CF and PLI indices in terms of pollution level classification based on the result of statistic analysis between areas.

It was found that PLI and $I_{GEO\text{-}tot}$ values of soil pollutants were 12 times higher than those of vehicle pollutants. Moreover, those values of soil pollutants were classified as strongly polluted level. It is illustrated that the city of Chiang Mai is polluted with soil particles (attached on vehicle wheels or road surface) due to mobile on road source.

3.7 Air quality mapping in the city of Chiang Mai

Regarding level of metals accumulated on tree barks, the pollution levels in the city of Chiang Mai varied from unpollute to excessively pollute with vehicle pollutants. Therefore, a GIS software (QGIS) was used to produce spatial air quality maps including enrichment factors and the pollution indices of Chiang Mai City based on metal levels. The QGIS software is free software which was available online at http://www.qgis.org/en/site/forusers/download.html.

In order to produce air quality mapping, important information was obtained from various sources.

Traffic volume: the average daily vehicle volume on main roads in Chiang Mai city (period 2009 – 2012) were from the report (Sopajaree *et al*., 2011) and the accumulated 24 hrs of vehicle volume from Chiang Mai Traffic Control and Training Center of Chiang Mai Municipality, the office of Chiang Mai rural roads and the office of Chiang Mai highway.

Building area: the main road network in Chiang Mai city in which indicated traffic volume was created by using GIS software from digitization road line from web mapping service of Google.com. The GIS database of building area in Chiang Mai city in 2004 was performed by digitization the building shape from high resolution of aerial photograph. The database was provided by Department of Civil Engineering, Faculty of Engineering, Chiang Mai University and Geo-Information and Space Technology Centre (Northern Region).

3.7.1 Traffic volume and building density

In order to estimate the effects of road traffic, a 500 m x 500 m (0.25 km^2) grid cells was overlain on the Chiang Mai City covering the main road network and the infrastructure, i.e. buildings. The total 1,088 grid cells with 0.25 km^2 area were overlaid over the 272 km^2 area of the city. The features of main roads and buildings were identified within the grid to produce datasets of short line lengths of the road and buildings contained within each grid. Total traffic volume in each grid was calculated from a summation of multiplication for traffic volume per day on main roads with the ratio between length of the main road in each grid and original length of the main road. The total building area was the summation of the area of all buildings existing in the grid. The calculation of total traffic volume and building area in grid cell were explain in Appendix E. It was assumed that the building area was direct variated with building density in the area, which used to estimate air ventilation capacity.

The total building area per grid (m^2/grid) and the traffic volume on main roads (vehicles/day) were employed for a map construction in order to indicate characteristic of building density and traffic volume in the city (Figure 3.16). Daily vehicle volume on main roads of the city of Chiang Mai varied from 3,000 – 80,000 vehicles. The highest vehicle volume was found on Suthep road which Chiang Mai University, market and hospital are located on this road, while Yothathikarn Chiang Mai-Lumphun road had the lowest traffic volume on road (along with railway). In urban area (inner part of the white circle), the mean volume of vehicle in the inner part of Chiang Mai moat was 18,000 vehicles per day and it was lower than that in the outer part of the moat (26,000 vehicles per day). The volume in sub-urban area was high (29,000 vehicles per day). In the city, characteristic of roads are short and narrow,

therefore traffic volume was lower than in sub-urban area, where roads are wider and longer. For total building area in each grid (0.25 km^2), the highest building area was found at the area of Waroros Market to Tha Phae gate (0.11 – 0.12 km^2/grid). Comparing between areas, the building area in urban (~ 0.04 km^2/grid) was about 4 times higher than that in sub-urban (~ 0.01 km^2/grid). Building area was found to be related to population density. It was 3 times higher in urban area, (~ 3,000 people/ km^2) than in sub-urban (~ 1,000 people/ km^2). Moreover, it was strongly correlated ($p < 0.01$) with traffic volume with Spearman's rank correlation coefficient (r_s) equal to 0.712.

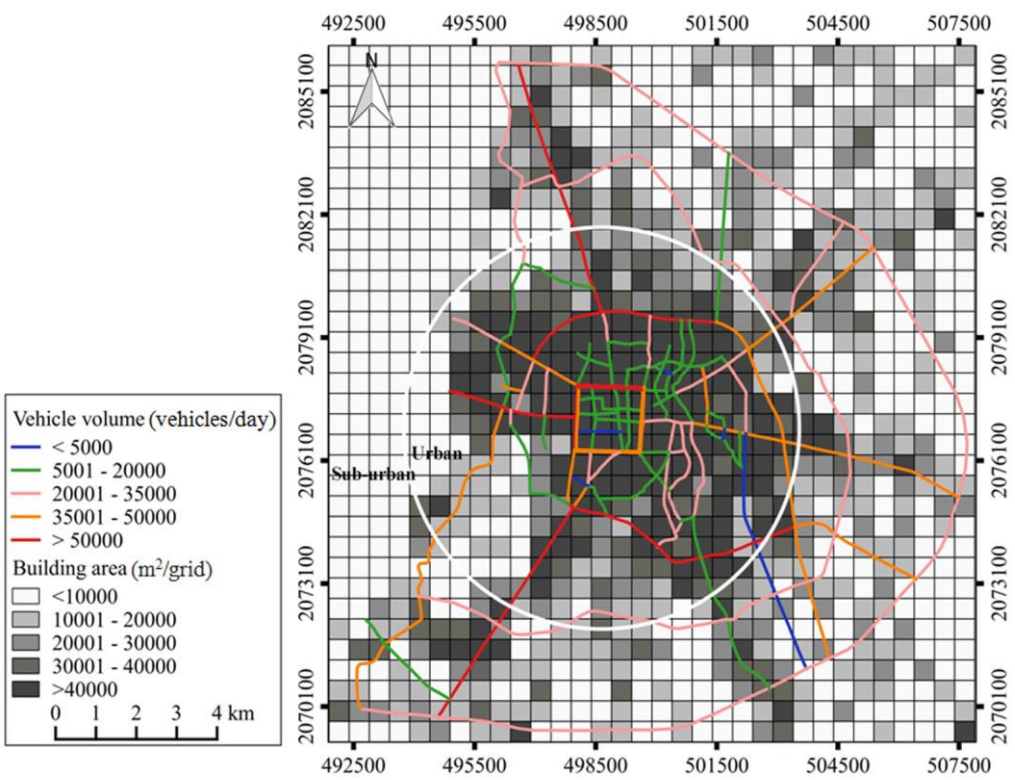


Figure 3.16 Characteristic of building area and vehicle volume on main road in the city of Chiang Mai

3.7.2 Spatial distribution of metals concentration

According to a various level for metals content on the *Cassia fistula* bark, therefore classification of metal concentrations for mapping were calculated based on the minimum to maximum value of their concentration. Six class of metal concentrations were classified as showed in Table 3.26.

Table 3.26 Classification of metal concentrations in Chiang Mai City

class	Range of concentration (ng/cm^2)						
	Soil pollutants		Vehicle pollutants				
	Al	Fe	Cr	Cu	Ni	Pb	Zn
1	≤ 500	≤ 300	≤ 1.50	≤ 8.00	≤ 2.00	≤ 5.00	≤ 50
2	501 -1,000	301 - 600	1.51 - 3.00	8.01 - 16.00	2.01 - 4.00	5.01 - 10.00	50.1 - 100
3	1,001 - 1,500	601 - 900	3.01 -4.50	16.01 - 24.00	4.01 - 6.00	10.01 - 15.00	101- 150
4	1,501 - 2,000	901 - 1,200	4.51 - 6.00	24.01 - 32.00	6.01 - 8.00	15.01 - 20.00	151 - 200
5	2,001 -2,500	1,201 - 1,500	6.01 - 7.50	32.01 - 40.00	8.01 - 10.00	20.01 - 25.00	201-250
6	> 2,500	> 1,500	> 7.50	> 40.00	> 10.00	> 25.00	> 250

Spatial distribution of metals, including Al, Cr, Cu, Fe, Ni, Pb and Zn in the city of Chiang Mai are presents in Figure 3.17 – 3.18. The result showed that high values (red color) of all metal species were mainly observed in urban area. All metals showed the high values at the intersection of Kaewnawarat road and Ratanakosin road (the pink circle in the map), which is a big intersection located at ~ 3 km far from the city center in the northeast direction. This area always have heavy traffic congestion because it is surrounded by schools, hospitals and a bus terminal. Moreover, high values of metals were also found at the north-west corner (Hua Rin corner) of Chiang Mai moat (the orange circle in the map). This area is a busy intersection and closed to the hospitals and a high schools.

Moreover, the distribution maps showed that the concentrations of Pb and Zn were significantly correlated ($p < 0.05$) with building area and traffic volume in the city of Chiang Mai. Cu and Fe were correlated with building area, while Cr was correlated with only traffic volume (Table 3.27). The correlation result indicated that metals in group of vehicle pollutants (Cr, Cu, Pb and Zn) were related with factors of vehicle volume and building area (air ventilation efficiency) in grid, there means that

those factors were influence on vehicle pollutant metals accumulated in the area. On the other than, while Al and Fe (soil pollutants group) were not significantly correlated with both building area and vehicle volume, exception was observed for Fe concentration and building area. It might be because Al and Fe can be generated from both traffic and other activities i.e. construction.

Table 3.27 Spearman's correlations of metal concentrations with building area and traffic volume in Chiang Mai City

Concentration	Spearman's rank correlation coefficient (r_s)	
	building area	vehicle volume
Al	0.099	0.049
Cr	0.155	0.270**
Cu	0.253**	0.146
Fe	0.190*	0.143
Ni	0.055	0.053
Pb	0.327**	0.430**
Zn	0.364**	0.208*

Note: *** Significant level at 0.01 (2-tailed), * Significant level at 0.05 (2-tailed)*

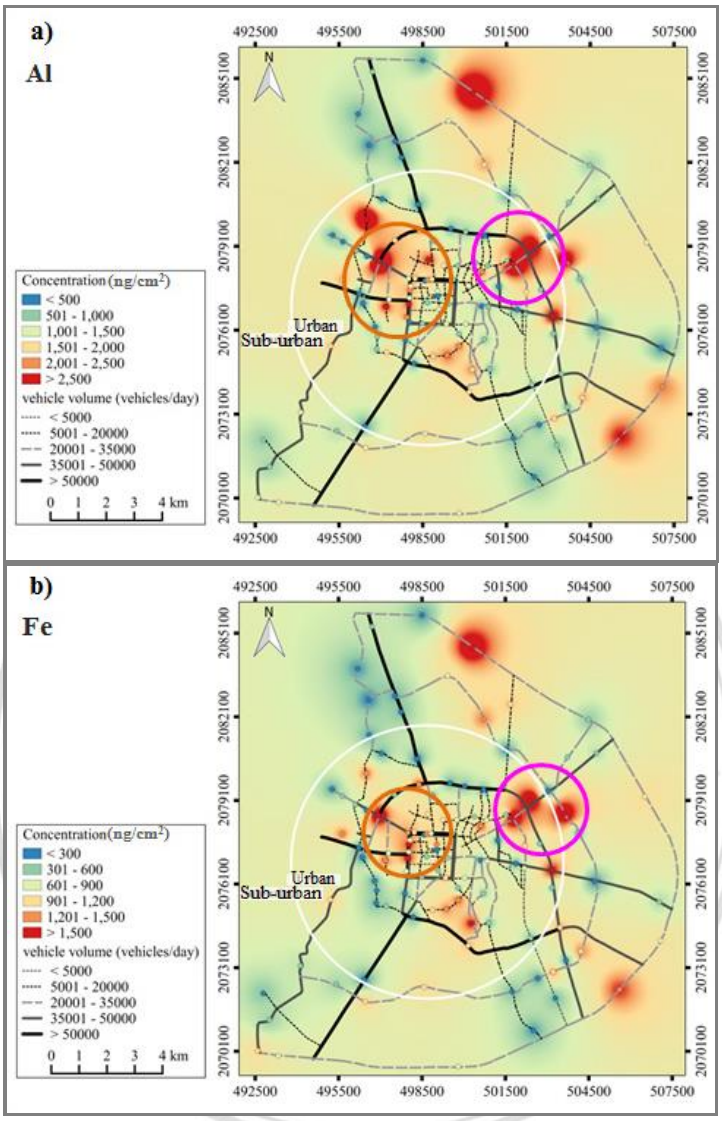


Figure 3.17 Spatial distribution of metals concentration (soil pollutants) on *Cassia fistula* bark in the city of Chiang Mai a) Al, b) Fe

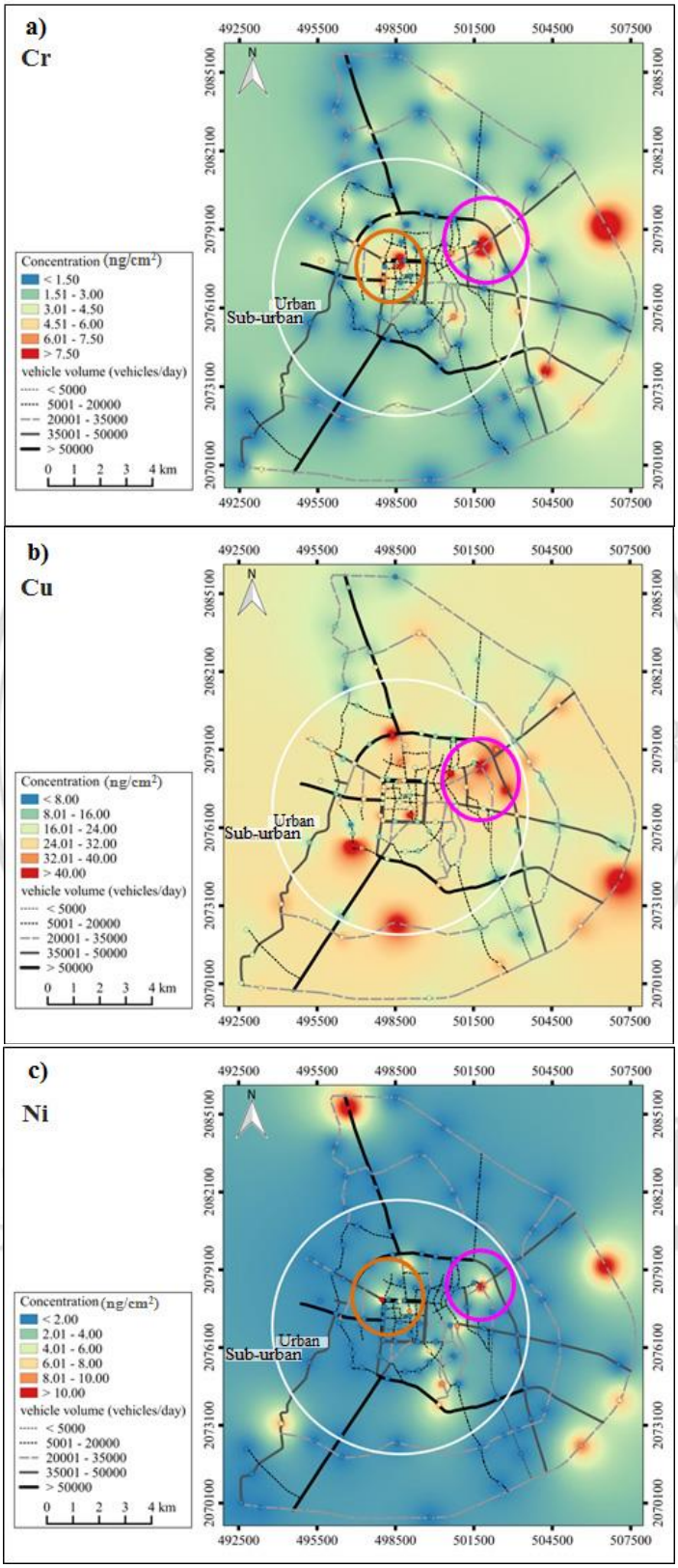


Figure 3.18 Spatial distribution of metals concentration (vehicle pollutants) on *Cassia fistula* bark in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn

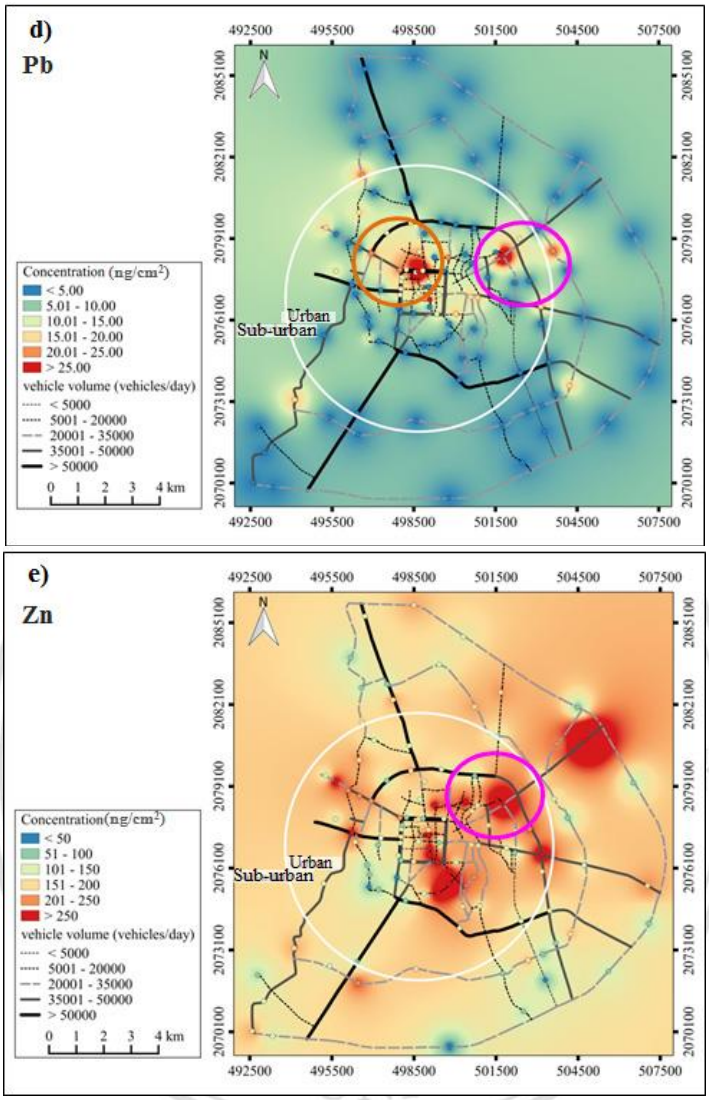


Figure 3.18 Spatial distribution of metals concentration (vehicle pollutants) on *Cassia fistula* bark in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn (Cont.)

3.7.3 Spatial distribution of metal enrichment factors

In order to indicate the area with high accumulation of metals in the city of Chiang Mai, the metal enrichment from background level (EF_B) were used to generate spatial distribution maps. The EF_B values in the map were classified into 5 levels based on accumulation rate classification (Frati *et al*., 2005). The EF_B distribution maps of vehicle pollutants are shown in Figure 3.19. It was found that vehicle pollutants (Cr, Cu, Pb, Ni and Zn) indicated severe accumulation at two areas. The first one is at the Hua Rin corner of Chiang Mai moat, which is around 1 km far from the city center in north-west direction. The other severe point us about 4-5 km in north-east direction of the city center where heavy traffic always observed. This area is a business area with high building density including hospitals. Moreover, the maps were clearly illustrated that accumulation rate of vehicle pollutants on tree bark in urban area was higher than that in sub-urban area. Moreover, soil pollutants (Al and Fe) and mix pollutants presented severe accumulation ($EF_B > 1.75$) for all areas in the city (Figure 3.20-3.21).

According to spatial distribution maps of EF_B values of the bark in the city of Chiang Mai, they were found that 5 intervals of the EF_B values classified based on 5 classes of accumulation rate could not indicate the variation of metal accumulation rate on the tree bark in the city. Therefore, the EF_B values were re-classified into 7 intervals base on the minimum to maximum range of their values (Table 3.28) and spatial distribution maps of re-classification of EF_B values were shown in Figure 3.22-3.24. The maps of all metals (both vehicle and soil pollutants) indicated that the area at Hua Rin coner was poor air quality (the orange circle in the maps). Moreover, the vehicle pollutants also indicated the relative high polluted area at about 5 km distance in the northeast from the city center. This area is around 2 intersections on the Supperhighway Chiang Mai – Lampang road (the pick circle in the maps). This area had high traffic volume because hospital, market and shopping mall were located on this area.

Table 3.28 Classification of enrichment factors (EF_B) of the bark in Chiang Mai City

class	Range of EF_B									
	Al	Fe	soil pollutants	Cr	Cu	Ni	Pb	Zn	vehicle pollutants	Mix pollutants
Accumulation rate classification (Frati *et al*., 2005)										
1	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
2	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75	0.26 – 0.75
3	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25	0.76 – 1.25
4	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75	1.26 – 1.75
5	>1.75	>1.75	>1.75	>1.75	>1.75	>1.75	>1.75	>1.75	>1.75	>1.75
Min – Max range classification (*Re-classification*)										
1	≤4.00	≤ 8.00	≤ 5.00	≤ 0.30	≤ 0.30	≤ 0.20	≤ 0.30	≤ 0.50	≤ 0.30	≤ 0.70
2	4.01-8.00	8.01-16.00	5.01-10.00	0.31-0.60	0.31-0.60	0.21-0.40	0.31-0.60	0.51-1.00	0.31-0.60	0.71-1.40
3	8.01-12.00	16.01-24.00	10.01-15.00	0.61-0.90	0.61-0.90	0.41-0.60	0.61-0.90	1.01-1.50	0.61-0.90	1.41-2.10
4	12.01-16.00	24.01-32.00	15.01-20.00	0.91-1.20	0.91-1.20	0.61-0.80	0.91-1.20	1.51-2.00	0.91-1.20	2.11-2.80
5	16.01-20.00	32.01-40.00	20.01-25.00	1.21-1.50	1.21-1.50	0.81-1.00	1.21-1.50	2.01-2.50	1.21-1.50	2.81-3.50
6	20.01-24.00	40.01-48.00	25.01-30.00	1.51-1.80	1.51-1.80	1.01-1.20	1.51-1.80	2.51-3.00	1.51-1.80	3.51-4.20
7	>24.00	>48.00	>30.00	>1.80	>1.80	>1.20	>1.80	>3.00	>1.80	>4.20

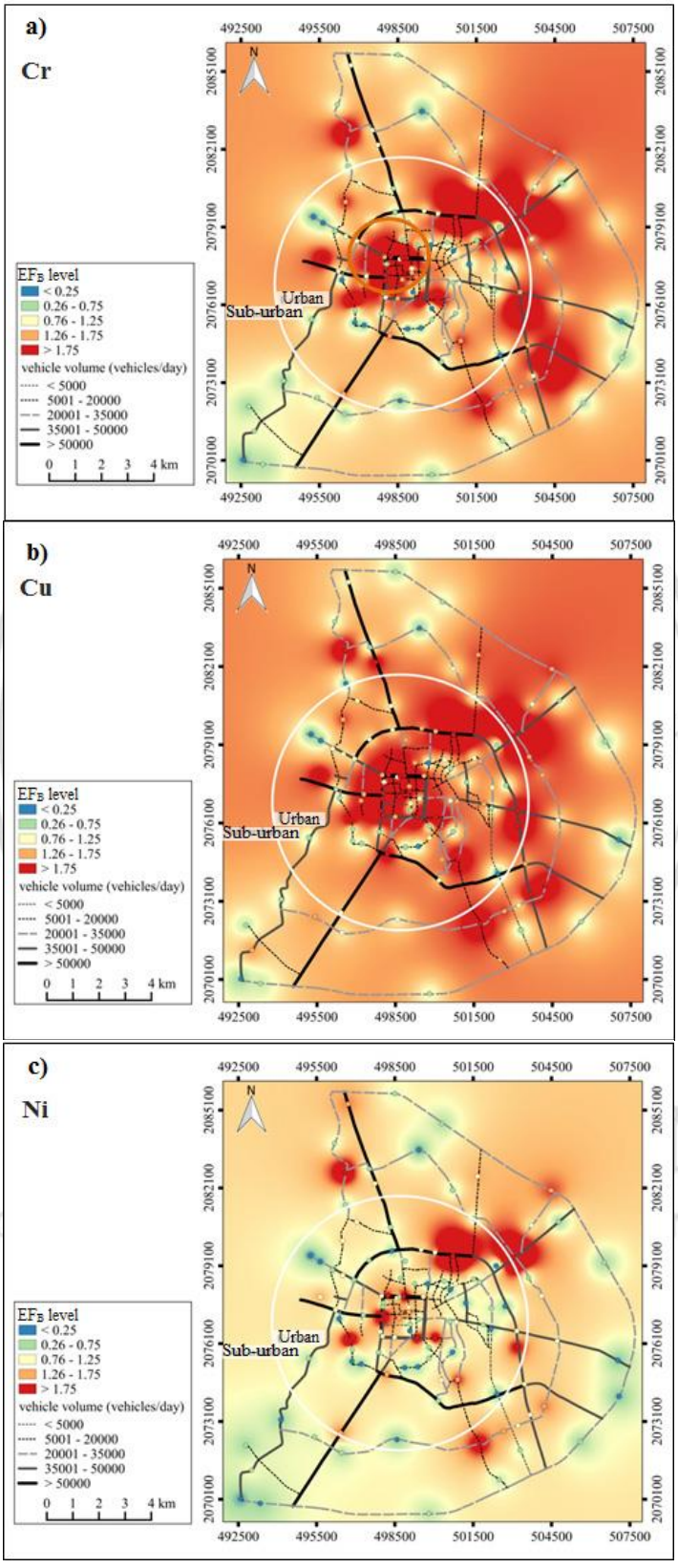


Figure 3.19 Spatial distribution of vehicle pollutants based on metal enrichment factor (EF_B) in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn, f) vehicle pollutants

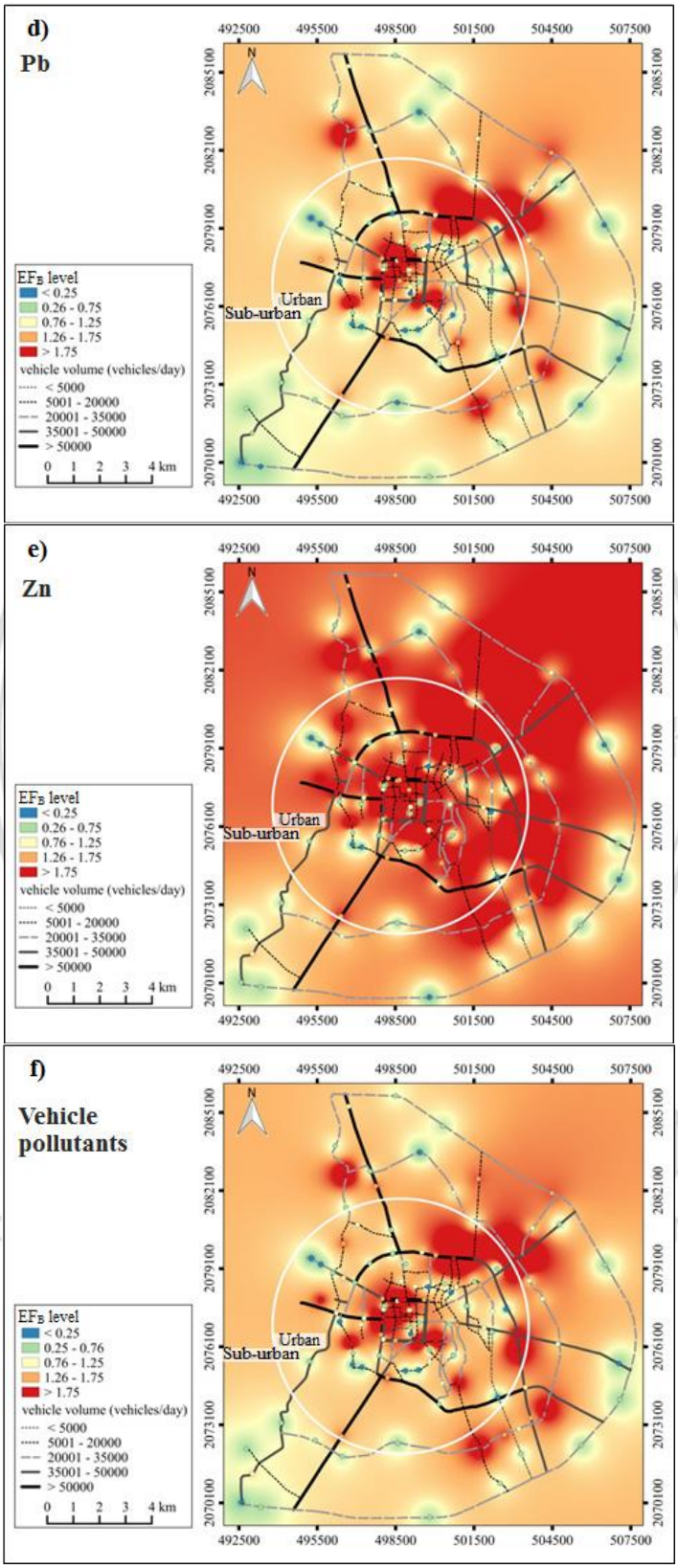


Figure 3.19 Spatial distribution of vehicle pollutants based on metal enrichment factor (EF_B) in the city of Chiang Mai a) Cr, b)Cu, c)Ni, d) Pb, e)Zn, f) vehicle pollutants (Cont.)

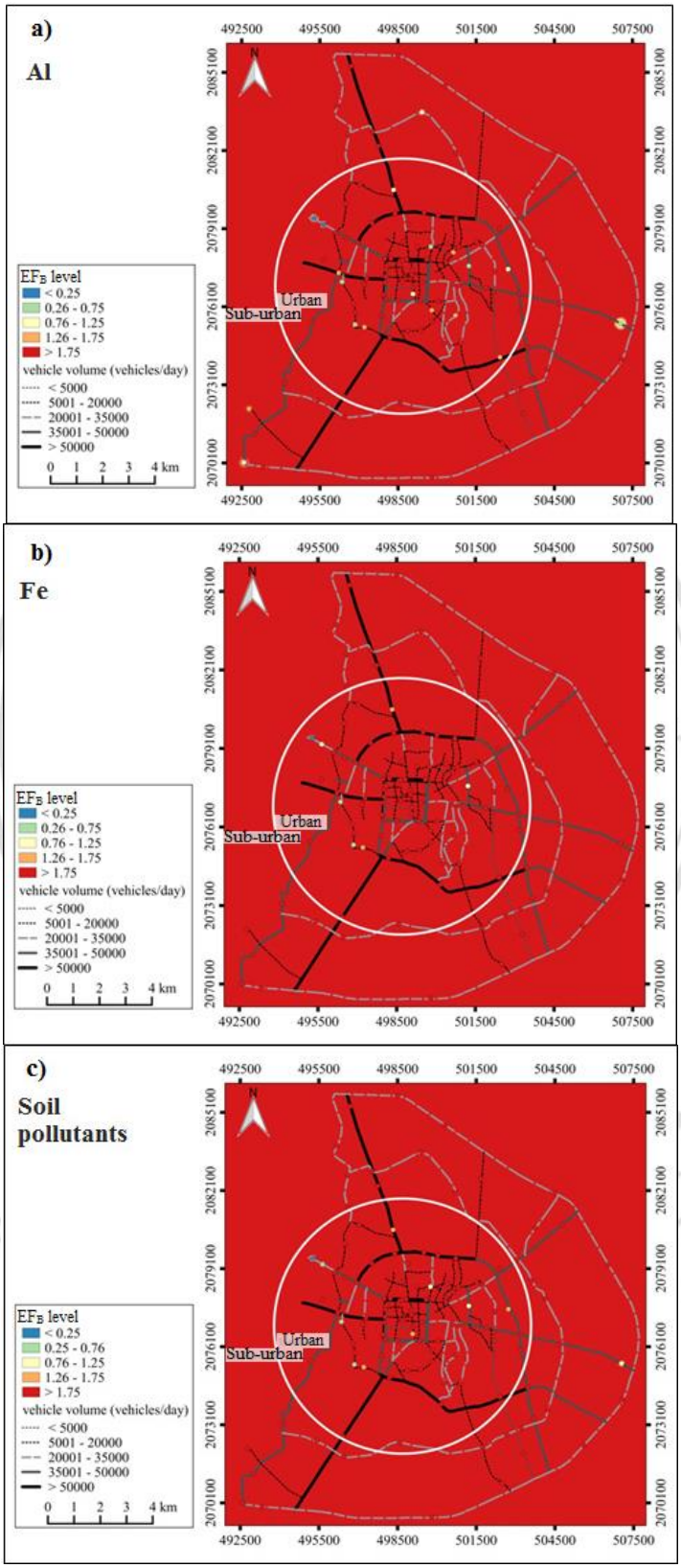


Figure 3.20 Spatial distribution of soil pollutants based on metal enrichment factor (EF_B) in the city of Chiang Mai a) Al, b) Fe, c) soil pollutants

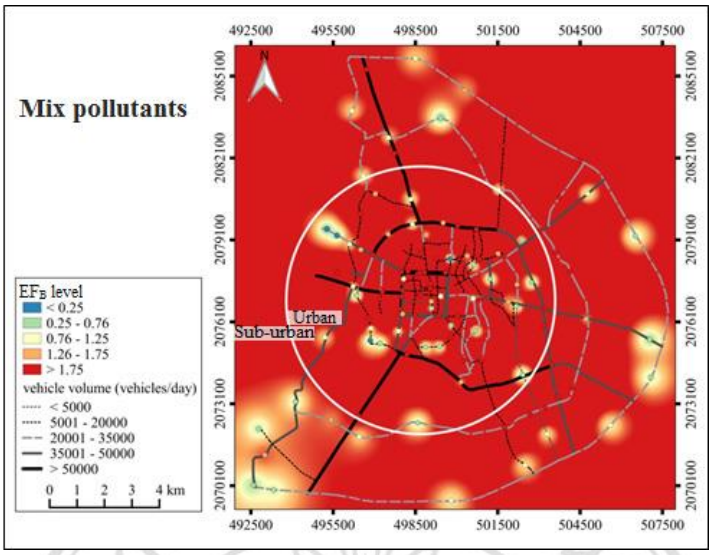


Figure 3.21 Spatial distribution of mix pollutants based on metal enrichment factor (EF_B) in the city of Chiang Mai

The EF_B values of all metals were significantly correlated with vehicle volume (Table 3.29), those correlations were confirmed with the strongly correlation among of metal enrichment factors values. This leads to the conclusion that all metal species must be generated from many traffic activities.

Table 3.29 Spearman's correlations of EF_B values with building area and traffic volume in Chiang Mai City

Metal pollutants	Spearman's rank correlation coefficient (r_s)	
	building area	vehicle volume
Al	0.170	0.236*
Cr	0.169	0.319**
Cu	0.305**	0.372**
Fe	0.227*	0.275**
Ni	0.120	0.247**
Pb	0.178	0.323**
Zn	0.285**	0.306**
vehicle pollutants	0.233*	0.335**
soil pollutants	0.197*	0.257**
mix pollutants	0.236*	0.331**

Note: *** Significant level at 0.01 (2-tailed), * Significant level at 0.05 (2-tailed)*

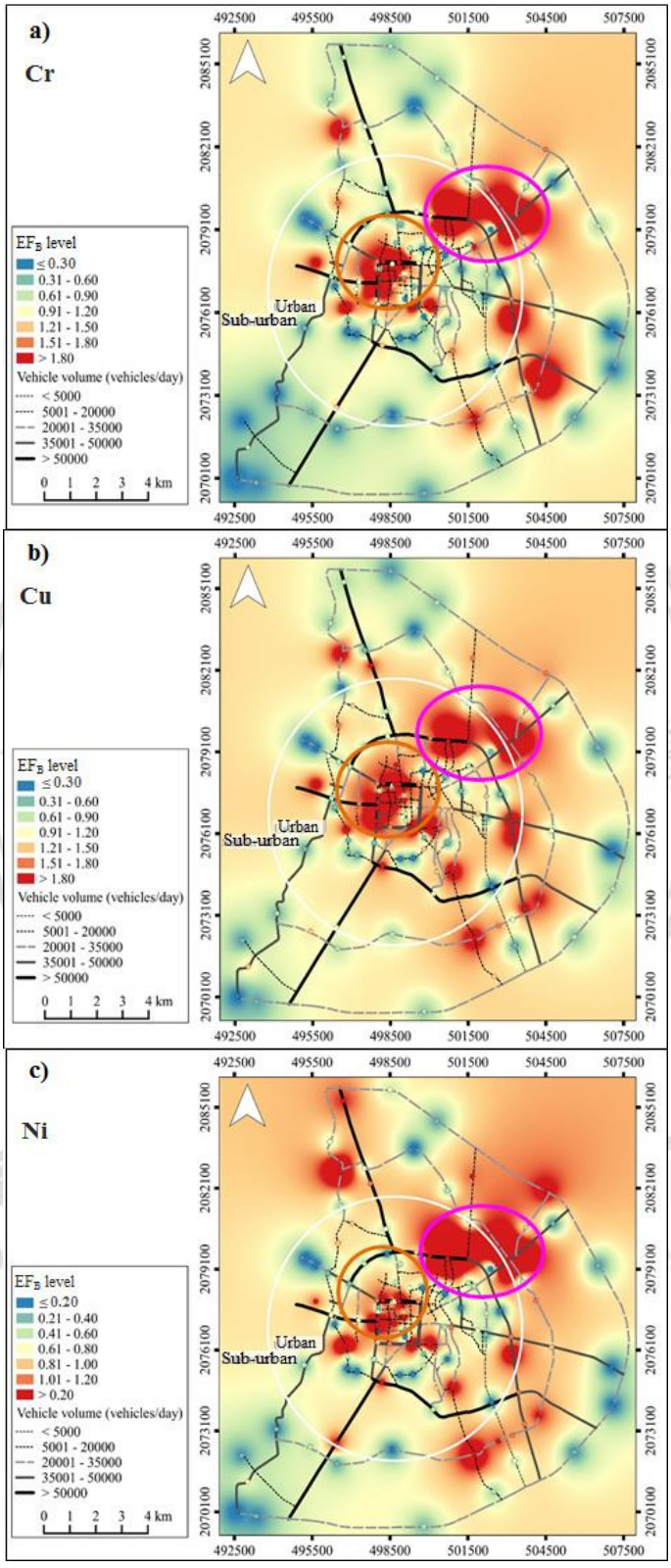


Figure 3.22 Spatial distribution of vehicle pollutants based on metal enrichment factor (EF_B) (new classification) in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn, f) vehicle pollutants

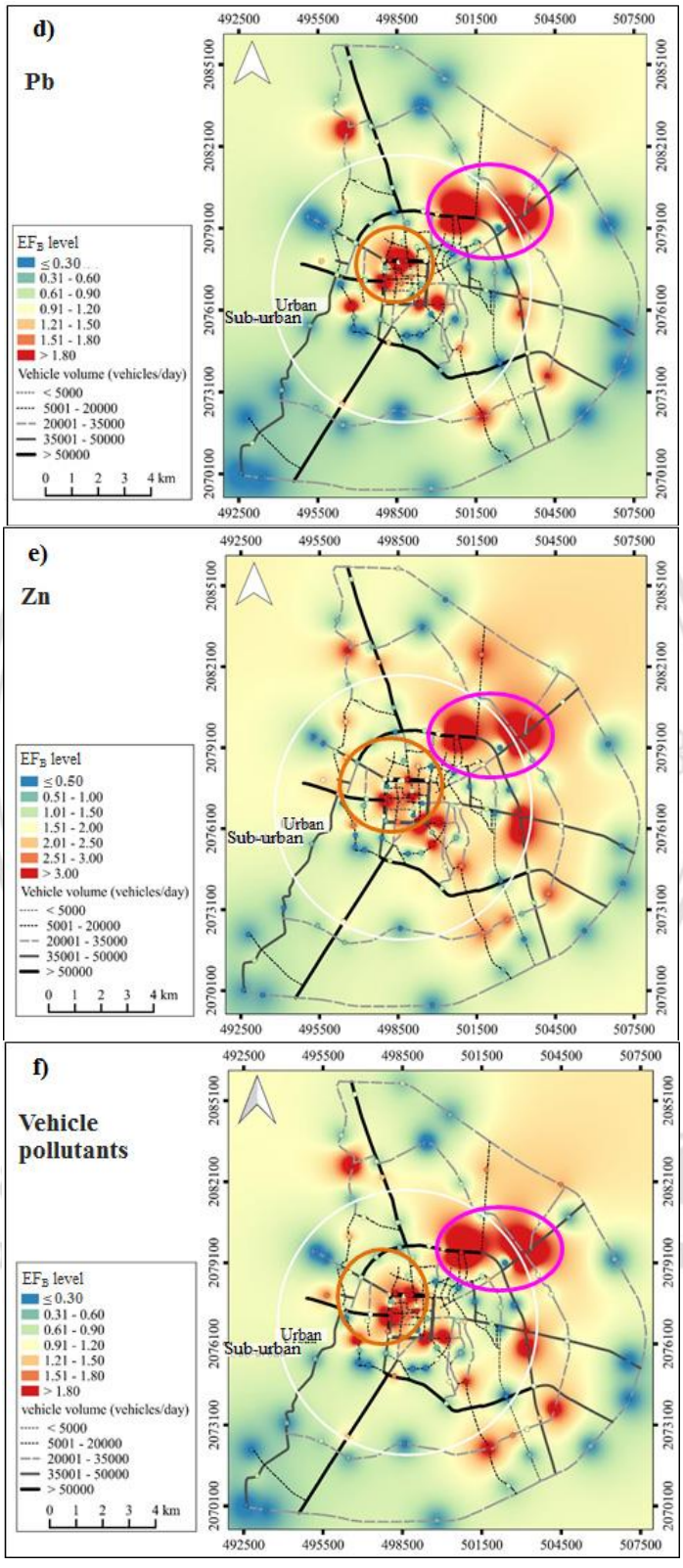


Figure 3.22 Spatial distribution of vehicle pollutants based on metal enrichment factor (EF_B) (new classification) in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn, f) vehicle pollutants (Cont.)

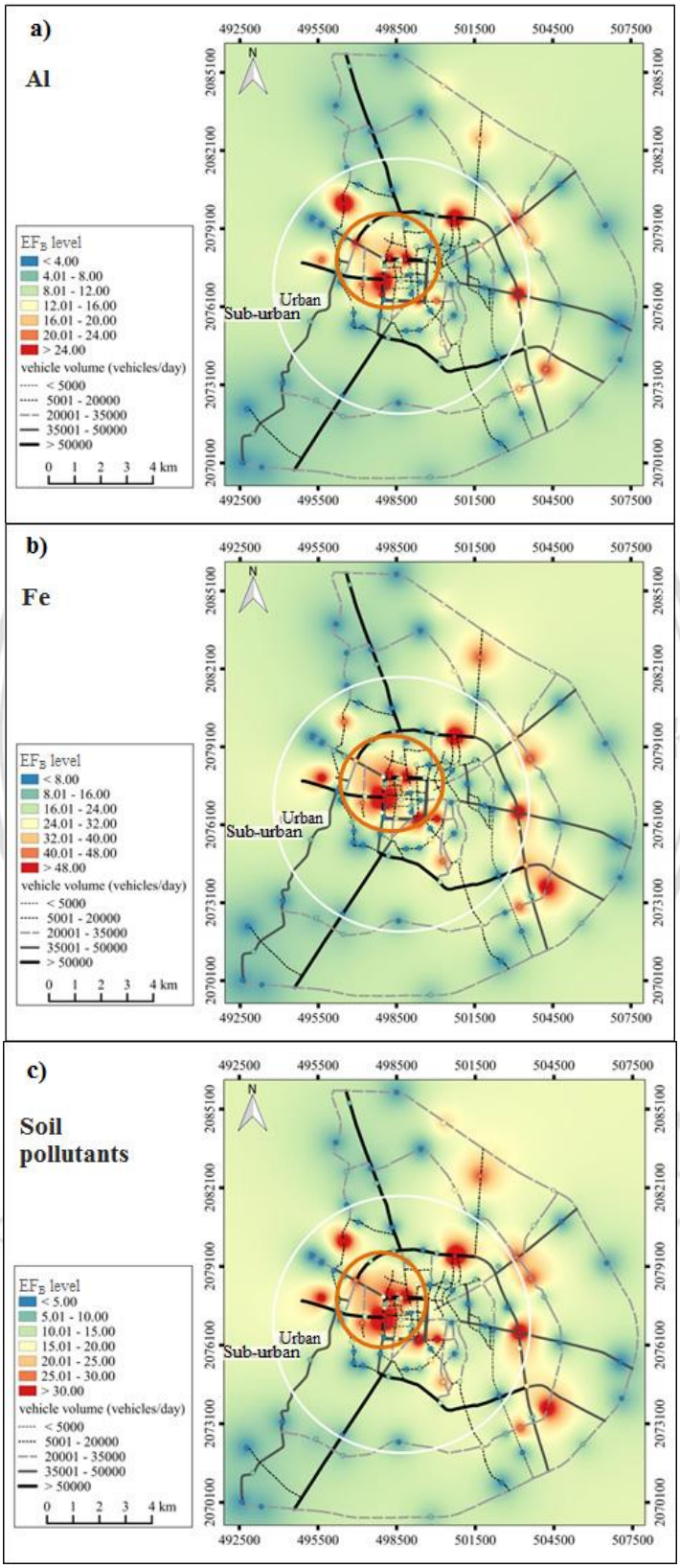


Figure 3.23 Spatial distribution of soil pollutants EF_B (re-classified) in the city of Chiang Mai a) Al, b) Fe, c) soil pollutants

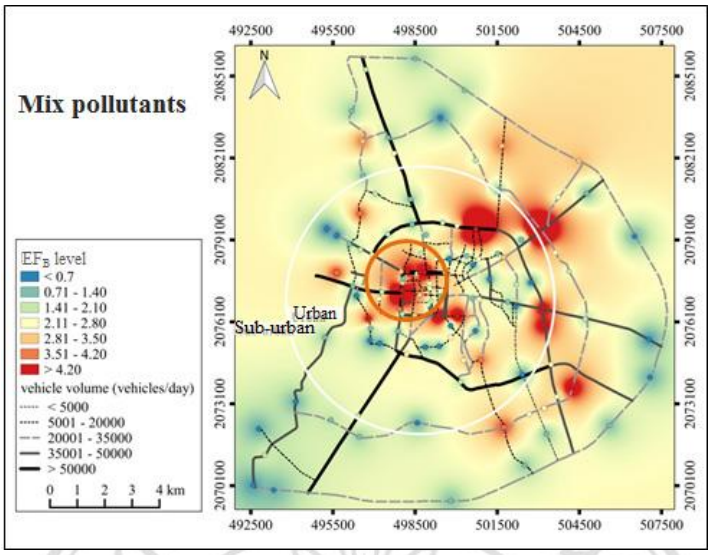


Figure 3.24 Spatial distribution of mix pollutants EF_B (re-classified) in the city of Chiang Mai

3.7.4 Spatial distribution of contamination factor and pollution load index

In order to indicate contamination level in each area in the city of Chiang Mai, spatial distribution of metals contamination factor (CF) values were generated. Figure 3.25 presents CF mapping of vehicle pollutants, which were classified into 4 levels based on contamination levels classification (Salah *et al*., 2 0 1 2). At some particular area (the intersection of Kaewnawarat road and Ratanakosin road (pink circle in maps)), most of the vehicle pollutants including Cr, Cu, Pb and Zn were fallen into high contamination level (CF > 3). The result was well agreed with the distribution map of metal concentration (Figure 3.18).

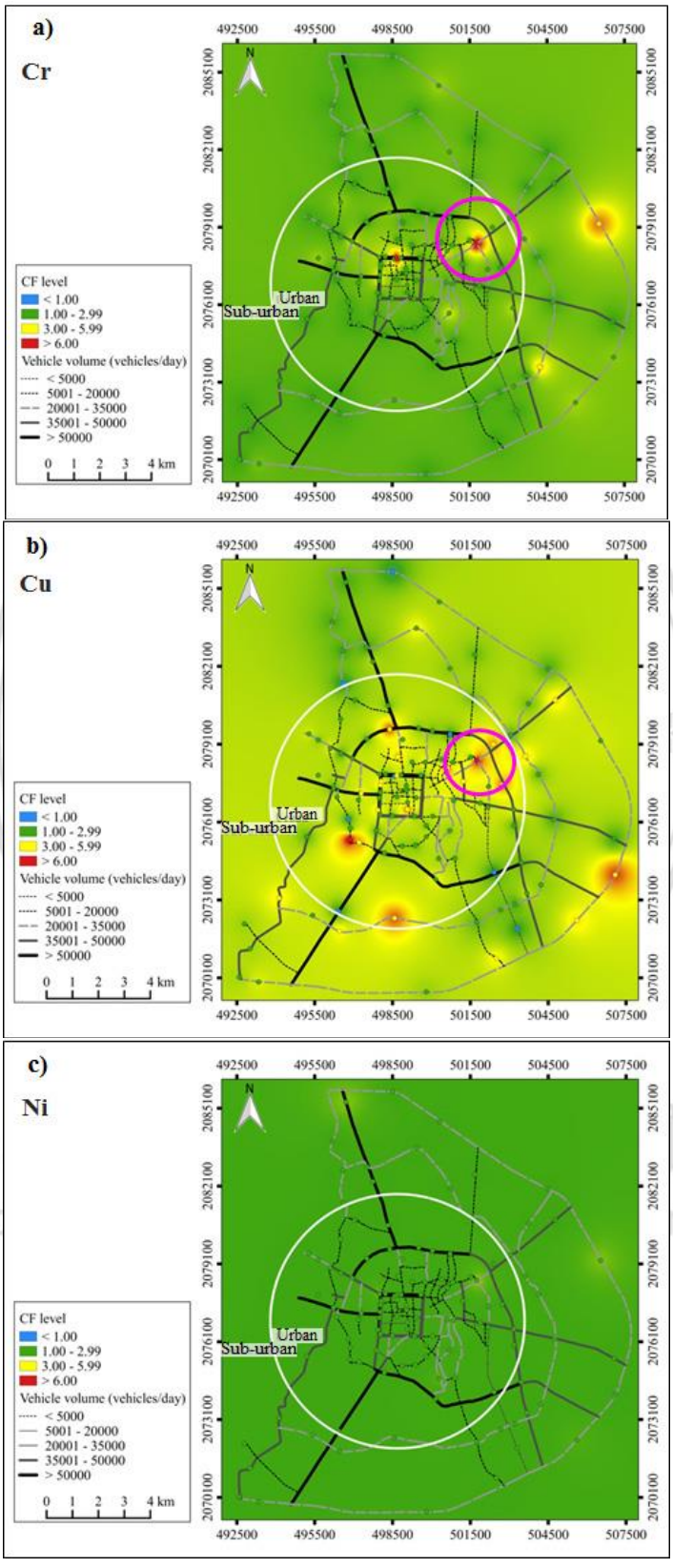


Figure 3.25 Spatial distribution of vehicle pollutants based on metal contamination factor (CF) in the city of Chiang Mai a) Cr, b) Cu, c) Ni, d) Pb, e) Zn

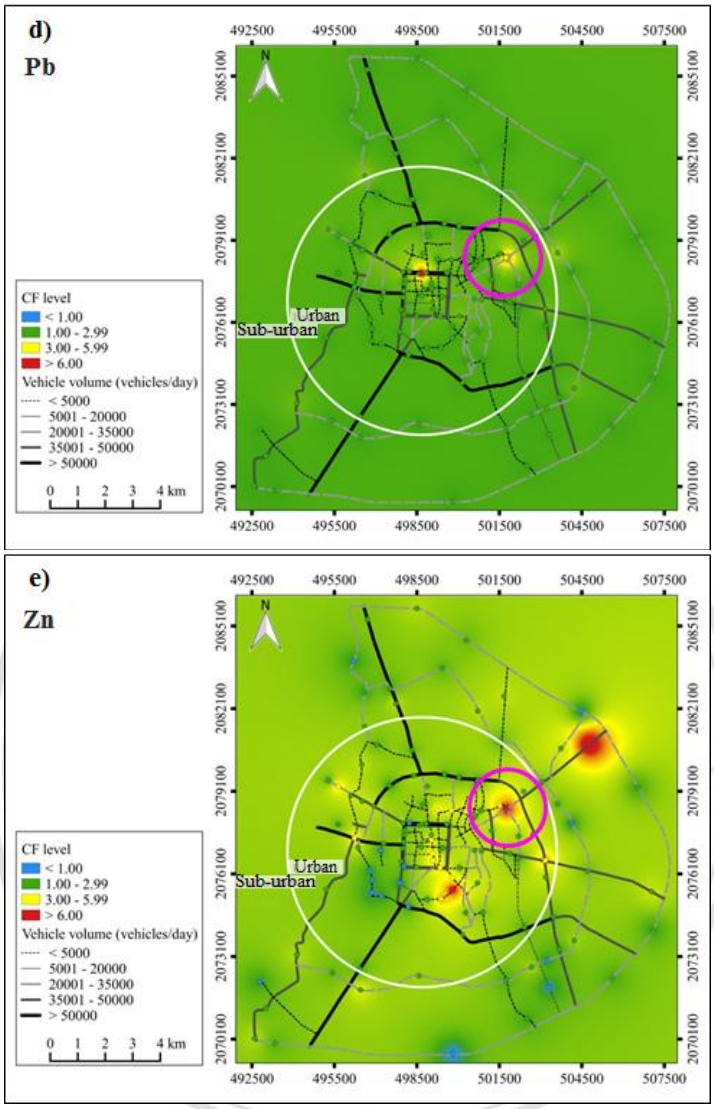


Figure 3.25 Spatial distribution of vehicle pollutants based on metal contamination factor (CF) in the city of Chiang Mai: a) Cr, b) Cu, c) Ni, d) Pb, e) Zn (Cont.)

In case of soil pollutants including Al and Fe, the distribution maps illustrated that there were very high contamination level (CF > 6) for all areas in the city (Figure 3.26). It was found that most of the values were in the same level and the map was not able to distinguish between areas. Therefore, new classification of CF values for Al and Fe were performed and presented in Table 3.30 and Figure 3.27. Based on the new classification, high CF values were found at the area around intersection of Kaewnawarat road and Ratanakosin road (pink circle in maps) to supper high way (Chiang Mai- Lampang road) and at the area around 1 – 5 km far from the city center in

north-west direction (orange circle in maps). The result was well agreed with the map of vehicle pollutants.

Table 3.30 Classification of soil pollutants contamination factor (CF) of the bark in Chiang Mai City

class	Range of CF values			
	Contamination level classification*		Min-Max value classification (re-classification)	
	Al	Fe	Al	Fe
1	< 1.00	≤0.25	≤ 4.00	≤ 8.00
2	1.00 – 2.99	0.26 – 0.75	4.01-8.00	8.01-16.00
3	3.00 – 5.99	0.76 – 1.25	8.01-12.00	16.01-24.00
4	≥ 6.00	1.26 – 1.75	12.01-16.00	24.01-32.00
5	-	-	16.01-20.00	32.01-40.00
6	-	-	20.01-24.00	40.01-48.00
7	-	-	>24.00	>48.00

Note: ** reference (Salah et al., 2012).*

In case of pollution load index (PLI), it was integrated of CF value of metals in the same group of pollutants including vehicle pollutants (Cr, Cu, Ni, Pb and Zn), soil pollutants (Al and Fe) and mix pollutants (all of 7 metals). Spatial distribution maps of PLI values of *Cassia fistula* bark in were generated in order to indicate degree of pollution in the city of Chiang Mai from various source of pollutants. The maps of PLI values, which were classified into 7 levels by contamination degree classification (Chen *et al*., 2015), are presented in Figure 3.28. It was found that most of the area in the city was classified as unpolluted to moderately polluted for vehicle pollutants, while soil pollutants had fallen into very highly polluted degree. However, these maps were unable to distinguish between low and high polluted areas. Therefore, new PLI classification were classified by considering only the minimum to maximum range of PLI values (Table 3.31 and Figure 3.29). The new maps were clearly illustrated that area around the intersection of Kaewnawarat road and Ratanakosin road was the highest polluted zone (pink circle in maps). Moreover, the area around Hua Rin corner also high polluted area in the city (orange circle in maps).

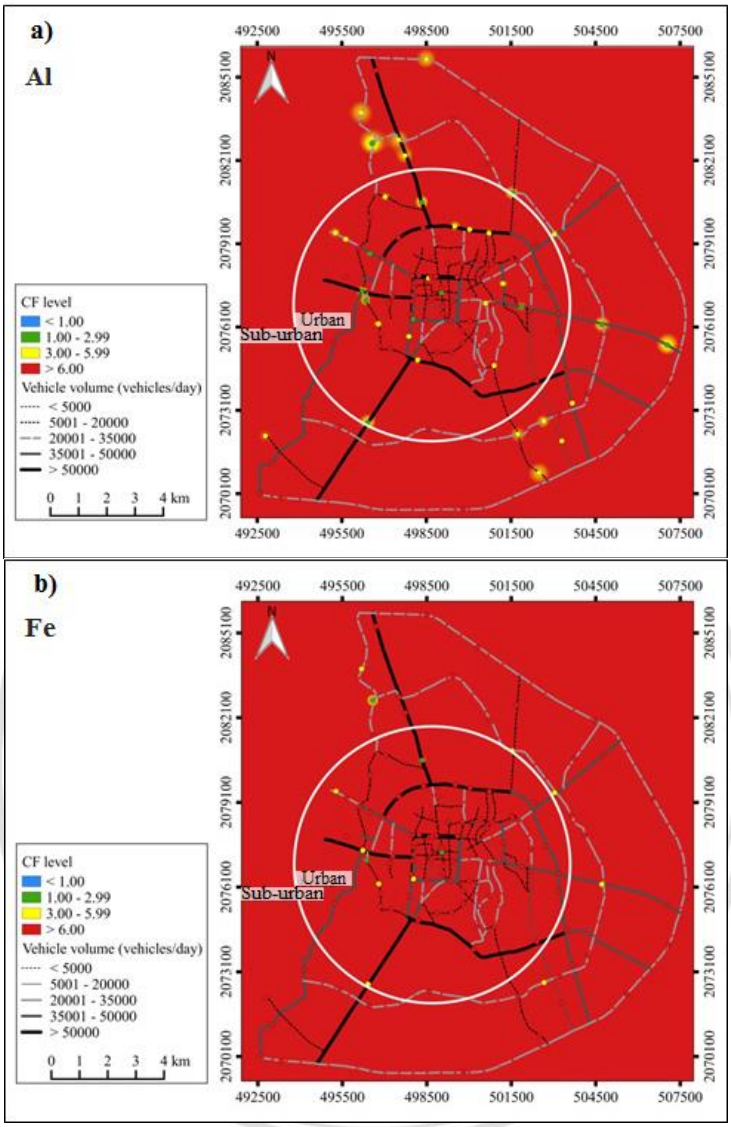


Figure 3.26 Spatial distribution of soil pollutants contamination factor (CF) in the city of Chiang Mai a) Al, b) Fe

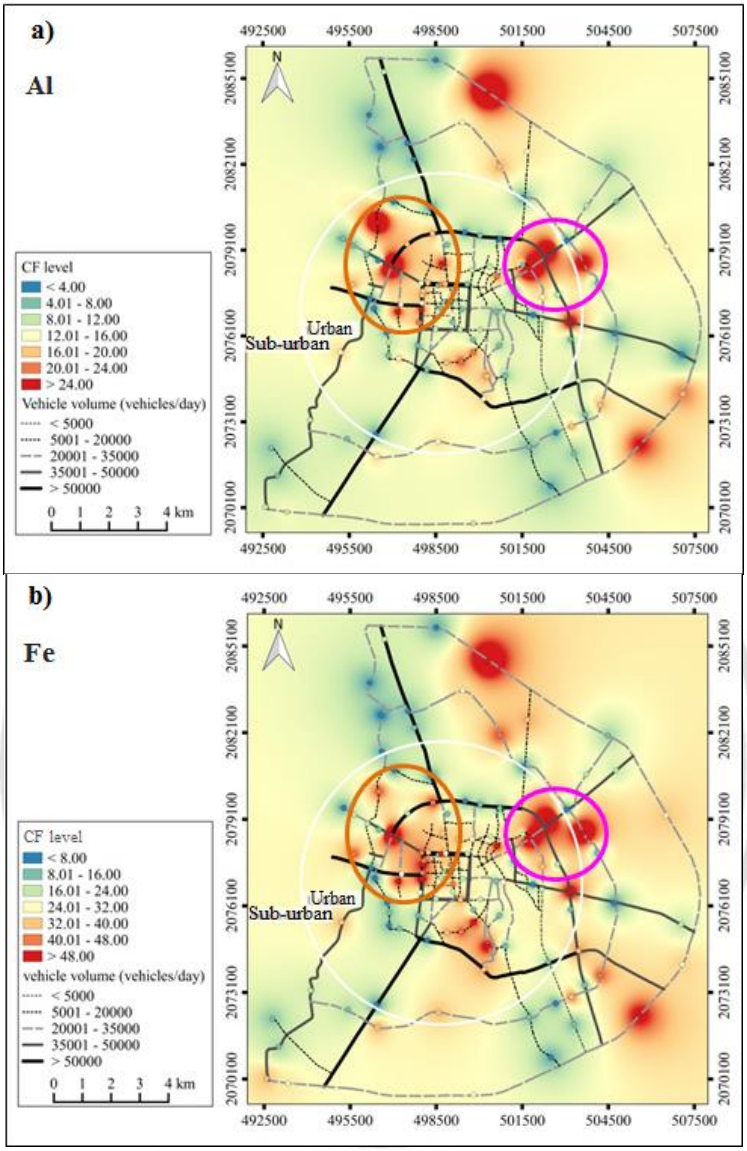


Figure 3.27 Spatial distributions of soil pollutants CF (new classified) in the city of Chiang Mai a) Al, b) Fe

Table 3.31 Classification of Pollution load index (PLI) of the bark in Chiang Mai City

Class	Range of PLI value					
	Pollutant degree classification*			Min-Max value classification (re-classification)		
	Vehicle pollutants	Soil pollutants	Mix pollutants	Vehicle pollutants	Soil pollutants	Mix pollutants
1	≤ 0	≤ 0	≤ 0	≤ 1.00	≤ 7.00	≤ 2.00
2	0.01-1.00	0.01-1.00	0.01-1.00	1.01-1.20	7.01-14.00	2.01-2.50
3	1.01-2.00	1.01-2.00	1.01-2.00	1.21-1.40	14.01-21.00	2.51-3.00
4	2.01-3.00	2.01-3.00	2.01-3.00	1.41-1.60	21.01-28.00	3.01-3.50
5	3.01-4.00	3.01-4.00	3.01-4.00	1.61-1.80	28.01-35.00	3.51-4.00
6	4.01-5.00	4.01-5.00	4.01-5.00	1.81-2.00	35.01-42.00	4.01-4.50
7	>5.00	>5.00	>5.00	>2.00	>42.00	>4.50

Note: ** reference (Chen et al., 2015).*

Table 3.32 shows correlation results of CF and PLI values with building area and traffic volume in the city of Chiang Mai. The result showed that the CF values of metals in vehicle pollutants group (Cr, Cu, Pb and Zn) were significantly correlated with building area and traffic volume in grid. It was similar to the correlation result of PLI values of vehicle pollutants. However, both building area and traffic volume were no significant correlation with PLI value of soil pollutants. In addition, the PLI value of all pollutants was related with building area only. The correlation results distinguished that degree of contamination of vehicle pollutants was influenced by both volume of source and air ventilation efficiency in the area.

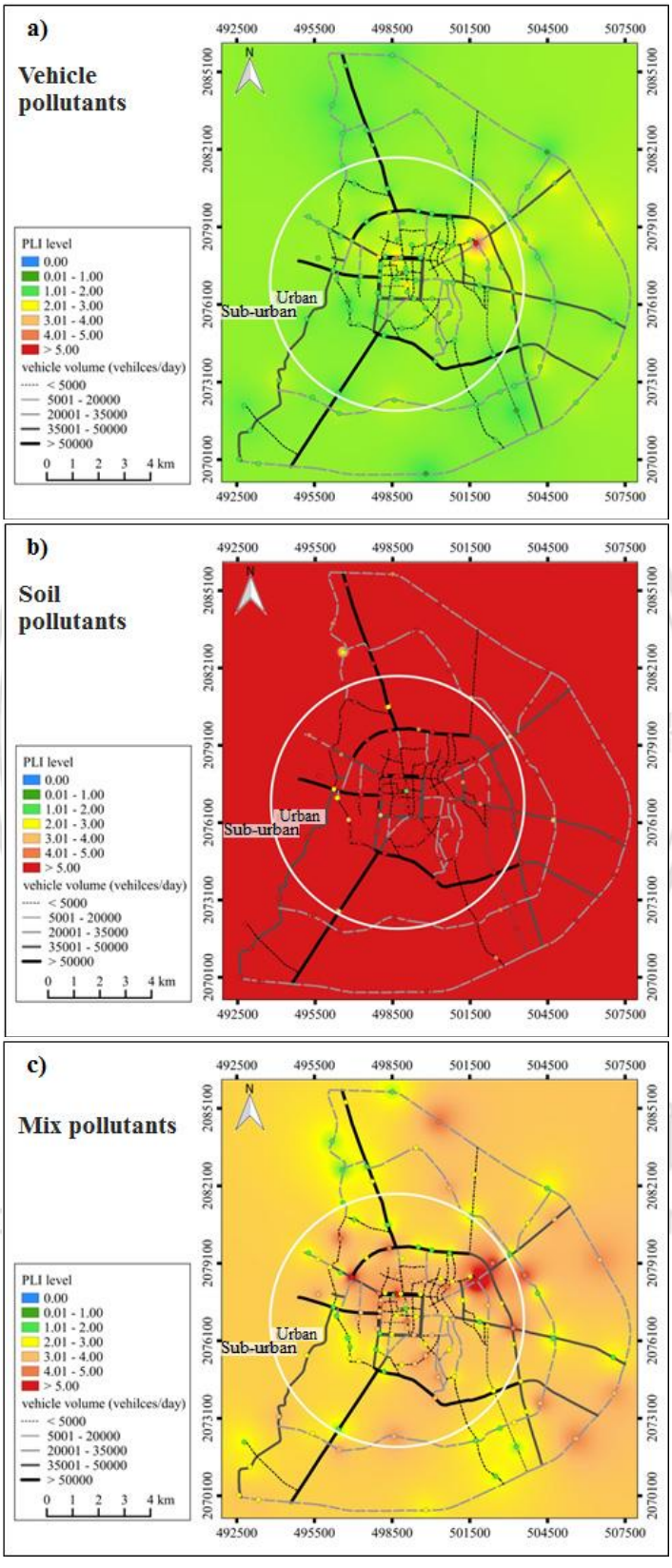


Figure 3.28 Spatial distribution of pollution load index (PLI) in the city of Chiang Mai a) vehicle pollutants, b) soil pollutants, c) mix pollutants

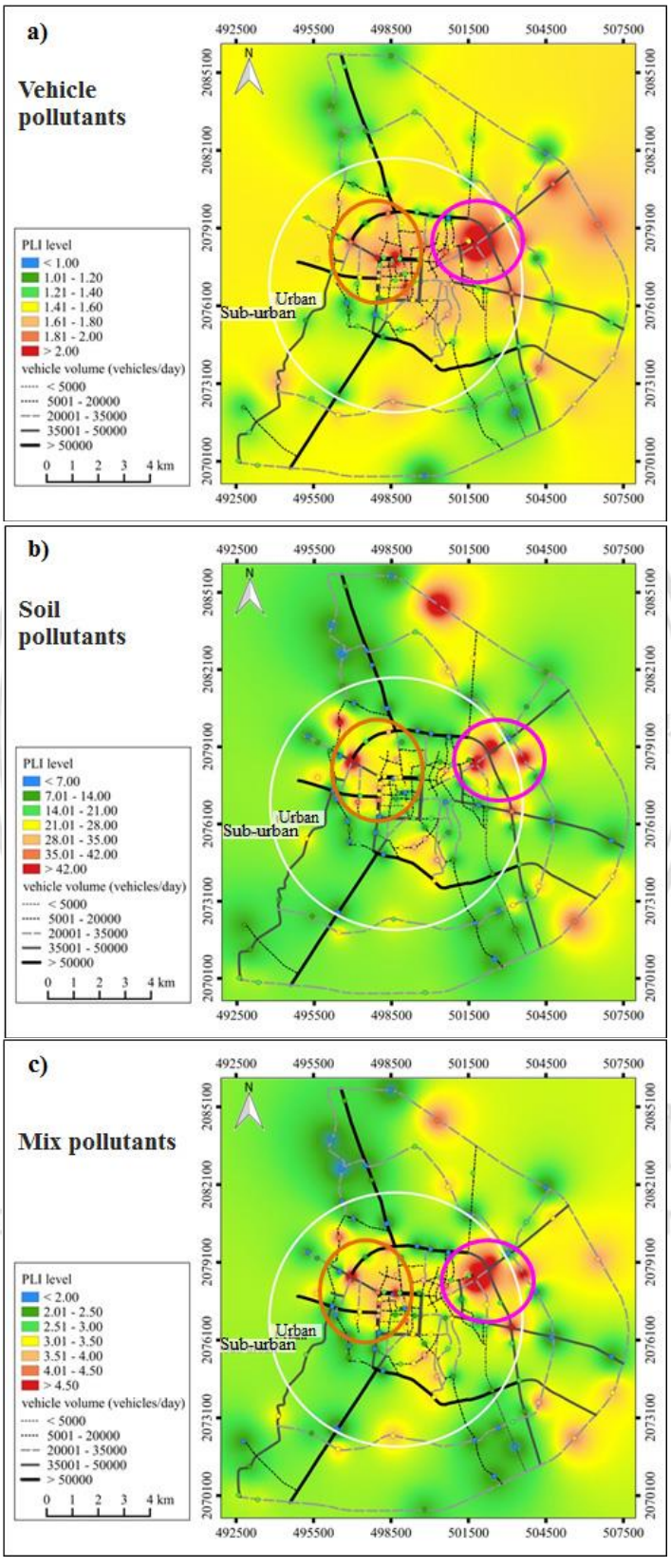


Figure 3.29 Spatial distribution of pollution load index (PLI) (new classified) in the city of Chiang Mai a) vehicle pollutants, b) soil pollutants, c) mix pollutants

Table 3.32 Spearman's correlations of CF and PLI values with building area and traffic volume in Chiang Mai City

Indices	Pollutants	Spearman's rank correlation coefficient (r_s)	
		building area	vehicle volume
CF	Al	0.099	0.049
	Cr	0.146	0.253**
	Cu	0.252**	0.142
	Fe	0.190*	0.142
	Ni	0.128	0.081
	Pb	0.327**	0.431**
	Zn	0.364**	0.207*
PLI	vehicle pollutants	0.376**	0.261**
	soil pollutants	0.148	0.100
	mix pollutants	0.243**	0.156

Note: *** Significant level at 0.01 (2-tailed),* * Significant level at 0.05 (2-tailed)*

3.7.5 Spatial distribution of geoaccumulation index and total geoaccumulation index

Spatial distribution maps of individual metal geoaccumulation index (I_{geo}) were generated in order to present degree of pollution from traffic activities. The I_{geo} values in the maps were classified into 7 classes based on pollution degree classification proposed by Müller (1969). Most of the area in the city presented various degrees of pollution from not polluted to moderately polluted (I_{geo} value ≤ 2) referring to metals in a group of vehicle pollutants (Figure 3.30). The I_{geo} values of Cu, Pb and Zn were higher in urban area than those in sub-urban area, which significant difference between areas were observed from Pb and Zn I_{geo} value based on statistic result ($p<0.05$).

In order to indicate the highest contaminated area, of I_{geo} values were re-classification and re-calculated as shown in Table 3.33 and Figure 3.31. All maps illustrated that the highest polluted area was observed at the area around intersection of Kaewnawarat road and Ratanakosin road (pink circle in maps). Moreover, the high polluted area was also found at the area around Hua Rin corner (orange circle in maps) for Cr, Ni and Pb.

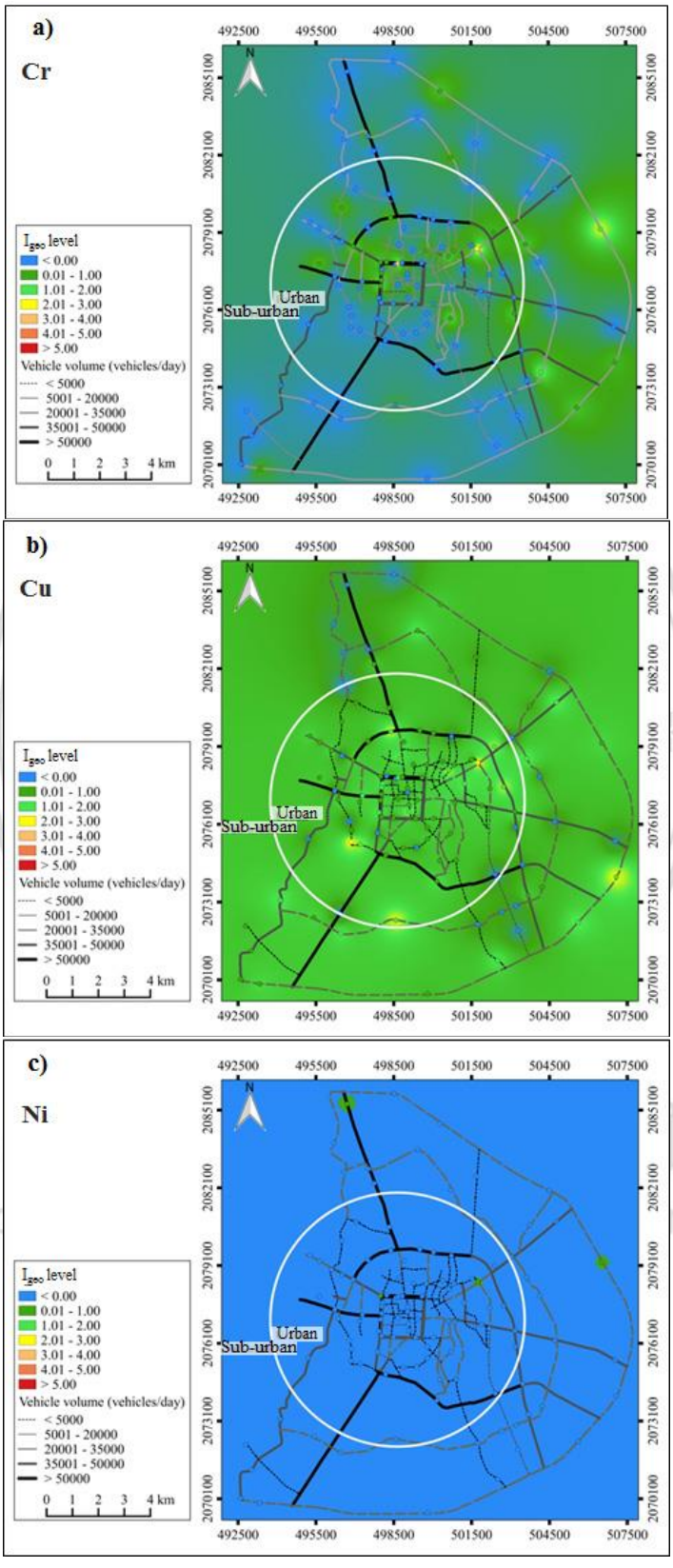


Figure 3.30 Spatial distribution of vehicle pollutants geoaccumulation index (I_{geo}) in Chiang Mai City a) Cr, b) Cu, c) Ni, d) Pb, e) Zn

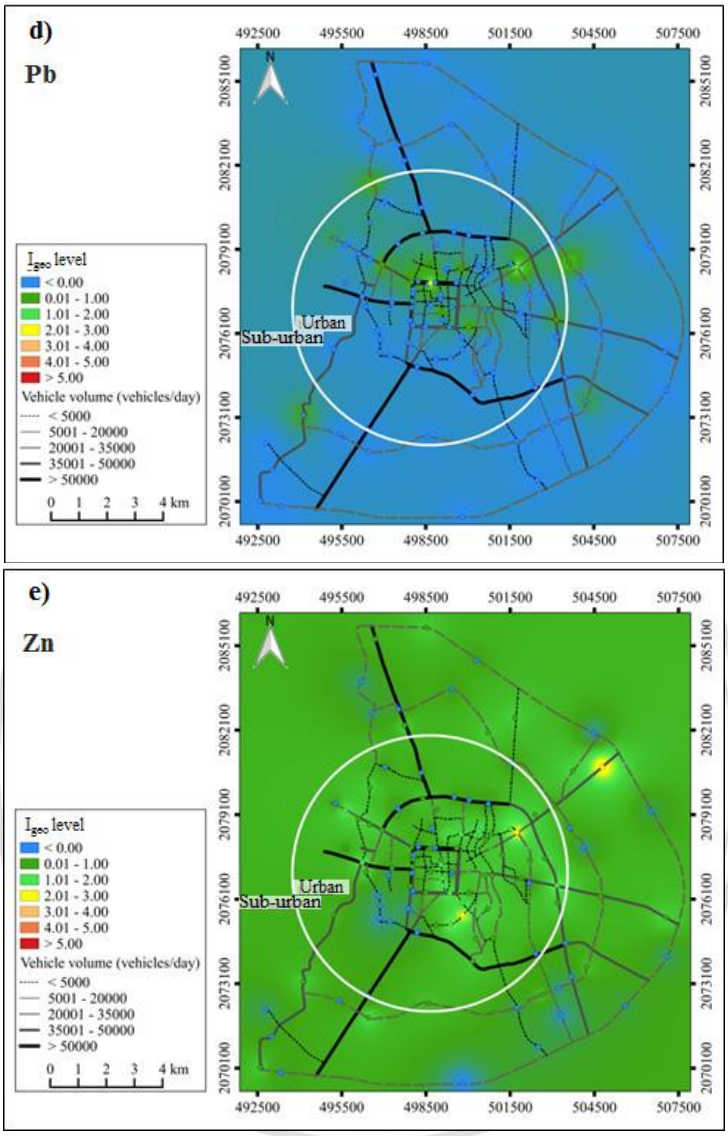


Figure 3.30 Spatial distribution of vehicle pollutants geoaccumulation index (I_{geo}) in Chiang Mai City a) Cr, b) Cu, c) Ni, d) Pb, e) Zn (Cont.)

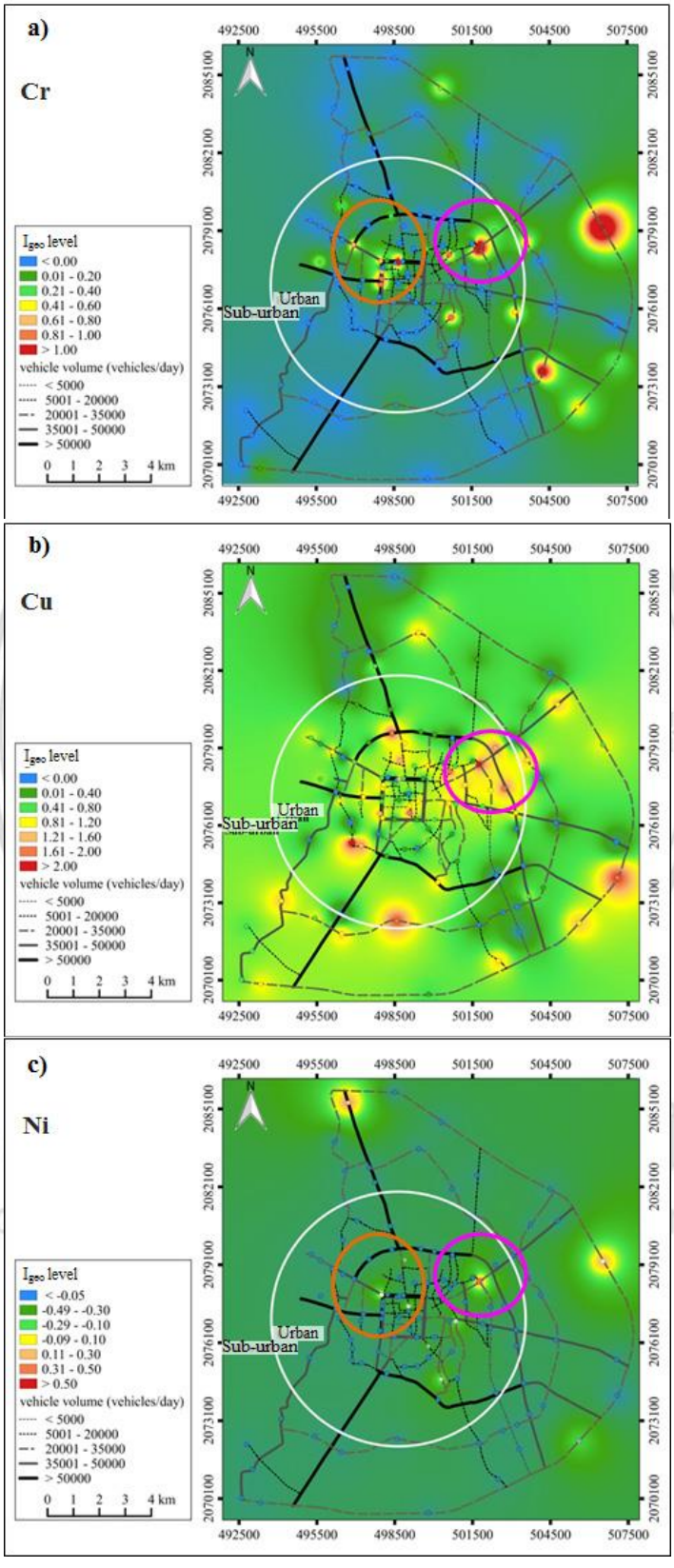


Figure 3.31 Spatial distribution of vehicle pollutants geoaccumulation index (I_{geo}) (new classification) in Chiang Mai City a) Cr, b) Cu, c) Ni, d) Pb, e) Zn

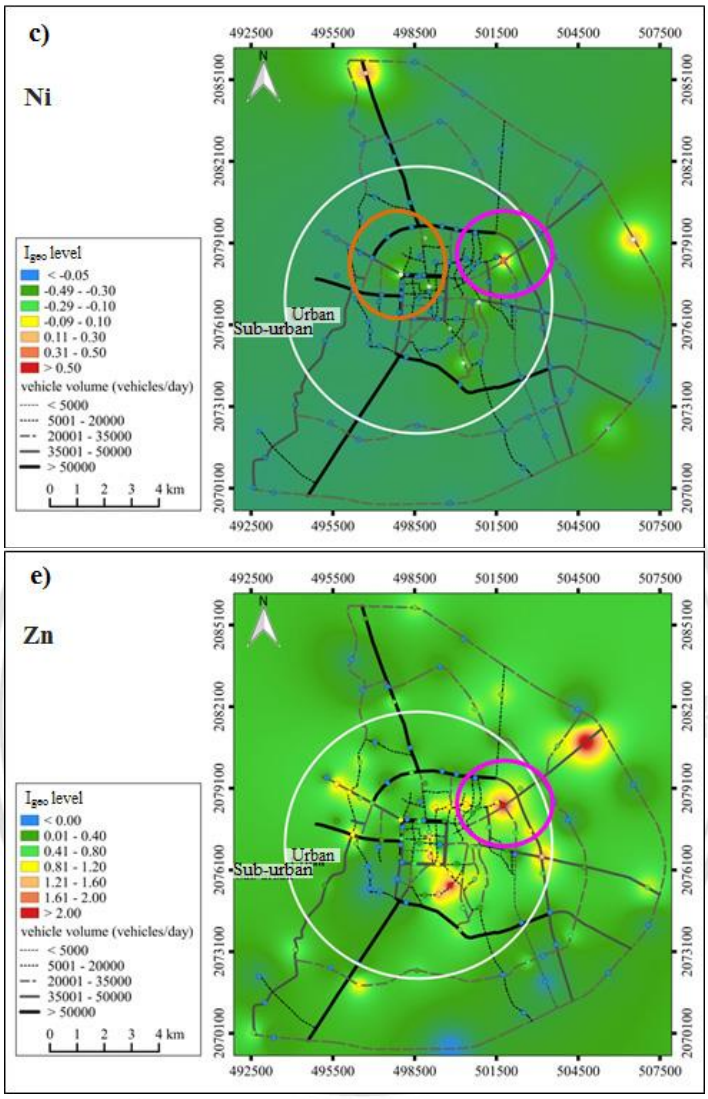


Figure 3.31 Spatial distribution of vehicle pollutants geoaccumulation index (I_{geo}) (new classification) in Chiang Mai City a) Cr, b) Cu, c) Ni, d) Pb, e) Zn (Cont.)

In case of Al and Fe (soil pollutants), the I_{geo} maps with the 7-classes of pollution degree are presented in Figure 3.32. Most of the areas in the city was classified as moderately polluted to excessively polluted (I_{geo} value $\geq$ 2) with those pollutants. However, new classification of the I_{geo} value (Table 3.33) was also useful to indicated polluted area of soil pollutants in the city of Chiang Mai (Figure 3.33). The result illustrated that the high polluted areas were observed at the same place with vehicle pollutants (pink and orange circles in Figure 3.31).

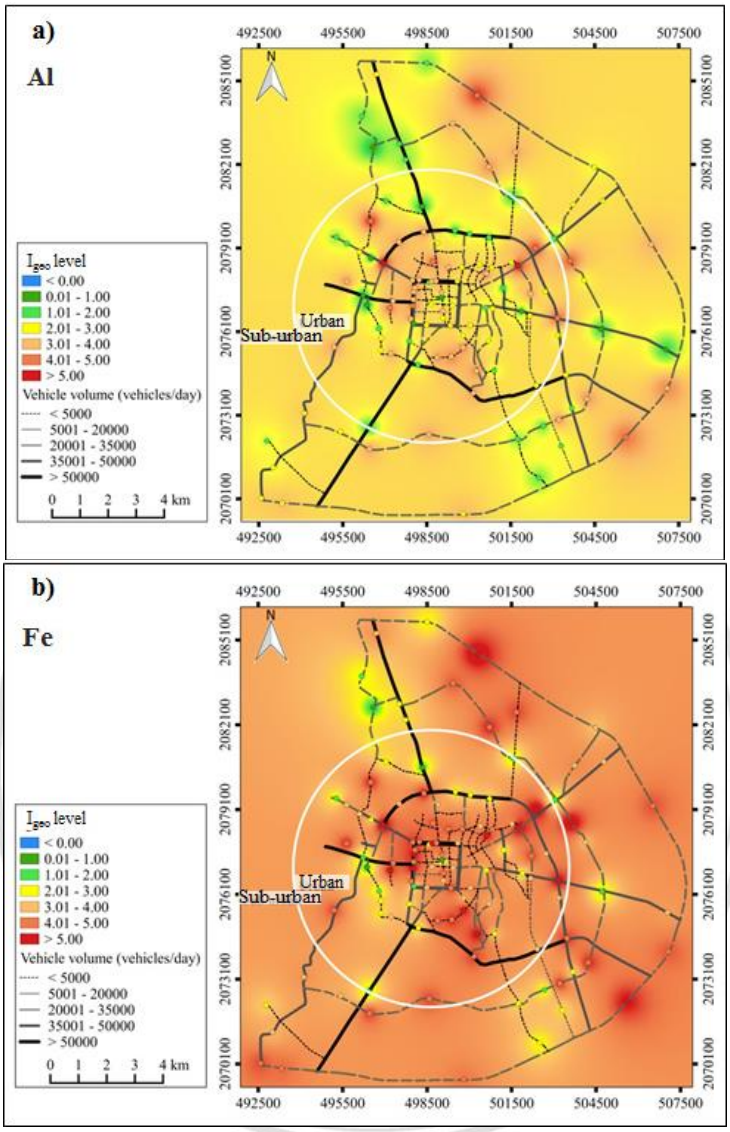


Figure 3.32 Spatial distribution of soil pollutants geoaccumulation index (I_{geo}) in Chiang Mai City a) Al, b) Fe

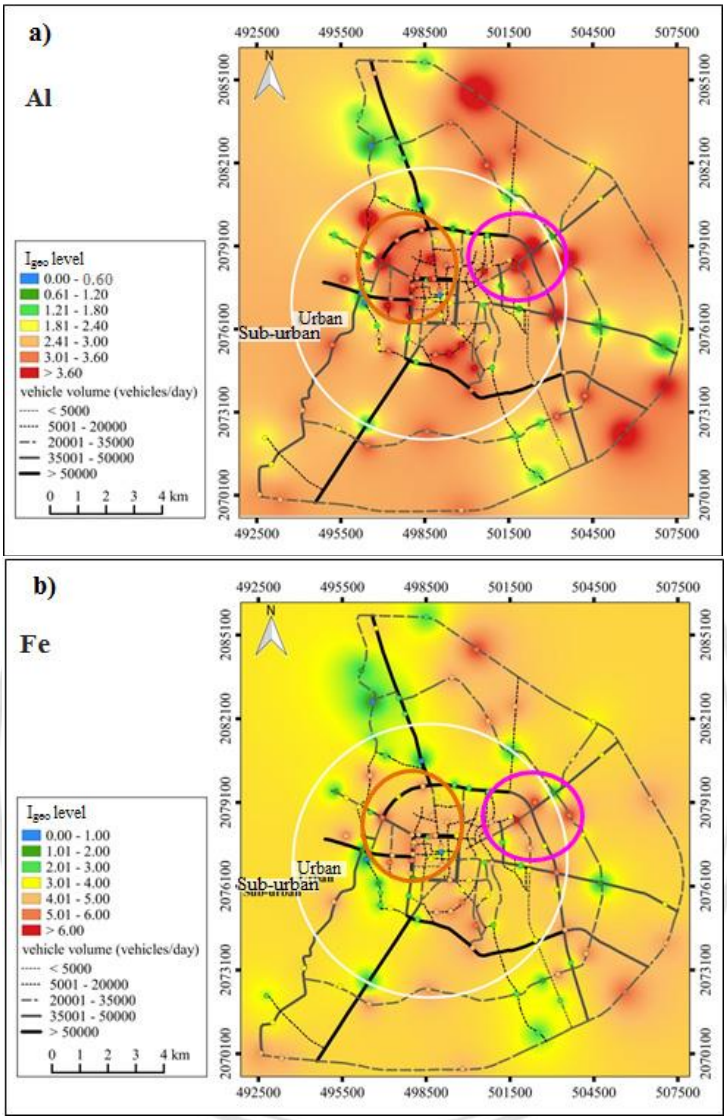


Figure 3.33 Spatial distribution of soil pollutants geoaccumulation index (I_{geo}) (new classification) in Chiang Mai City a) Al, b) Fe

Table 3.33 Classification of geoaccumulation index (I_{geo}) of the bark in the city of Chiang Mai

Class	Range of I_{geo} values						
	Vehicle pollutants					Soil pollutants	
	Cr	Cu	Ni	Pb	Zn	Al	Fe
Pollution degree classification (*Guéguen et al., 2012; Chen et al., 2015*)							
1	≤ 0	≤ 0	≤ 0	≤ 0	≤ 0	≤ 0	≤ 0
2	0.01-1.00	0.01-1.00	0.01-1.00	0.01-1.00	0.01-1.00	0.01-1.00	0.01-1.00
3	1.01-2.00	1.01-2.00	1.01-2.00	1.01-2.00	1.01-2.00	1.01-2.00	1.01-2.00
4	2.01-3.00	2.01-3.00	2.01-3.00	2.01-3.00	2.01-3.00	2.01-3.00	2.01-3.00
5	3.01-4.00	3.01-4.00	3.01-4.00	3.01-4.00	3.01-4.00	3.01-4.00	3.01-4.00
6	4.01-5.00	4.01-5.00	4.01-5.00	4.01-5.00	4.01-5.00	4.01-5.00	4.01-5.00
7	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
Min – Max range classification (*Re-classification*)							
1	≤ 0.00	≤ 0.00	≤ -0.50	≤ 0.00	≤ 0.00	0.00-0.60	0.00-1.00
2	0.01-0.20	0.01-0.40	-0.49- -0.30	0.01-0.10	0.01-0.40	0.61-1.20	1.01-2.00
3	0.21-0.40	0.41-0.80	-0.29- -0.10	0.11-0.20	0.41-0.80	1.21-1.80	2.01-3.00
4	0.41-0.60	0.81-1.20	-0.09-0.10	0.21-0.30	0.81-1.20	1.81-2.40	3.01-4.00
5	0.61-0.80	1.21-1.60	0.11-0.03	0.31-0.40	1.21-1.60	2.41-3.00	4.01-5.00
6	0.81-1.00	1.61-2.00	0.31-0.50	0.41-0.50	1.61-2.00	3.01 -3.60	5.01-6.00
7	>1.00	>2.00	>0.50	>0.50	>2.00	>3.60	>6.00

Total geoaccumulation index ($I_{GEO\text{-}tot}$) is integrated of metal I_{geo} values in group of metals including vehicle pollutants (Cr, Cu, Ni, Pb and Zn), soil pollutants (Al and Fe) and mix pollutants (all of 7 metals). Figure 3.34 presents $I_{GEO\text{-}tot}$ maps of vehicle pollutants, soil pollutants and mix pollutants in the city of Chiang Mai, in which the $I_{GEO\text{-}tot}$ value was classified in to 7 classes based on pollution degree classification. Vehicle pollutants illustrated non to moderately polluted ($I_{GEO\text{-}tot} < 1$) for all area in the city, while soil pollutants presented moderately to excessively polluted ($I_{GEO\text{-}tot} \geq 1$). For mix pollutants, moderately polluted ($1 \leq I_{GEO\text{-}tot} \leq 2$) was observed in the city. However, $I_{GEO\text{-}tot}$ maps of pollutants classified based on the pollution degree classification were indistinguish the difference of pollutant contamination between areas, therefore new classification was recalculated (Table 3.34). Spatial distribution maps of the re-classification of $I_{GEO\text{-}tot}$ values were showed in Figure 3.35. The result illustrated the same result in which the area at intersection of Kaewnawarat road and Ratanakosin road (pink circle in maps) and Hua Rin corner (orange circle in maps) distinguished the most polluted zone in the city of Chiang Mai.

Table 3.34 Classification of total geoaccumulation index ($I_{GEO-tot}$) of the bark in Chiang Mai City

Class	Range of $I_{GEO-tot}$ value					
	Pollutant degree classification*			Min-Max value classification (re-classification)		
	Vehicle pollutants	Soil pollutants	Mix pollutants	Vehicle pollutants	Soil pollutants	Mix pollutants
1	≤ 0	≤ 0	≤ 0	≤ 0.00	≤ 0.00	≤ 0.00
2	0.01-1.00	0.01-1.00	0.01-1.00	0.01-0.07	0.01-0.70	0.01-0.40
3	1.01-2.00	1.01-2.00	1.01-2.00	0.08-0.14	0.71-1.40	0.41-0.80
4	2.01-3.00	2.01-3.00	2.01-3.00	0.15-0.21	1.41-2.10	0.81-1.20
5	3.01-4.00	3.01-4.00	3.01-4.00	0.22-0.28	2.11-2.80	1.21-1.60
6	4.01-5.00	4.01-5.00	4.01-5.00	0.29-0.35	2.81-3.50	1.61-2.00
7	>5.00	>5.00	>5.00	>0.35	>3.5	>2.00

Note: ** reference (Guéguen et al., 2012; Chen et al., 2015).*

Correlation results of I_{geo} value with building area and traffic volume (Table 3.35) were similar to the result of PLI value in which significant correlation was observed from both parameters and vehicle pollutants (Cr, Cu, Pb and Zn).

Table 3.35 Spearman's correlations of geoaccumulation index (I_{geo}) and total geoaccumulation index ($I_{GEO-tot}$) with building area and traffic volume in Chiang Mai City

Indices		Spearman's rank correlation coefficient (r_s)	
		building area	vehicle volume
I_{geo}	Al	0.099	0.049
	Cr	0.146	0.253**
	Cu	0.252**	0.142
	Fe	0.190*	0.142
	Ni	0.128	0.081
	Pb	0.327**	0.431**
	Zn	0.364**	0.207*
$I_{GEO-tot}$	vehicle pollutants	0.376**	0.261**
	soil pollutants	0.148	0.100
	mix pollutants	0.243**	0.156

Note: *** Significant level at 0.01 (2-tailed), * Significant level at 0.05 (2-tailed)*

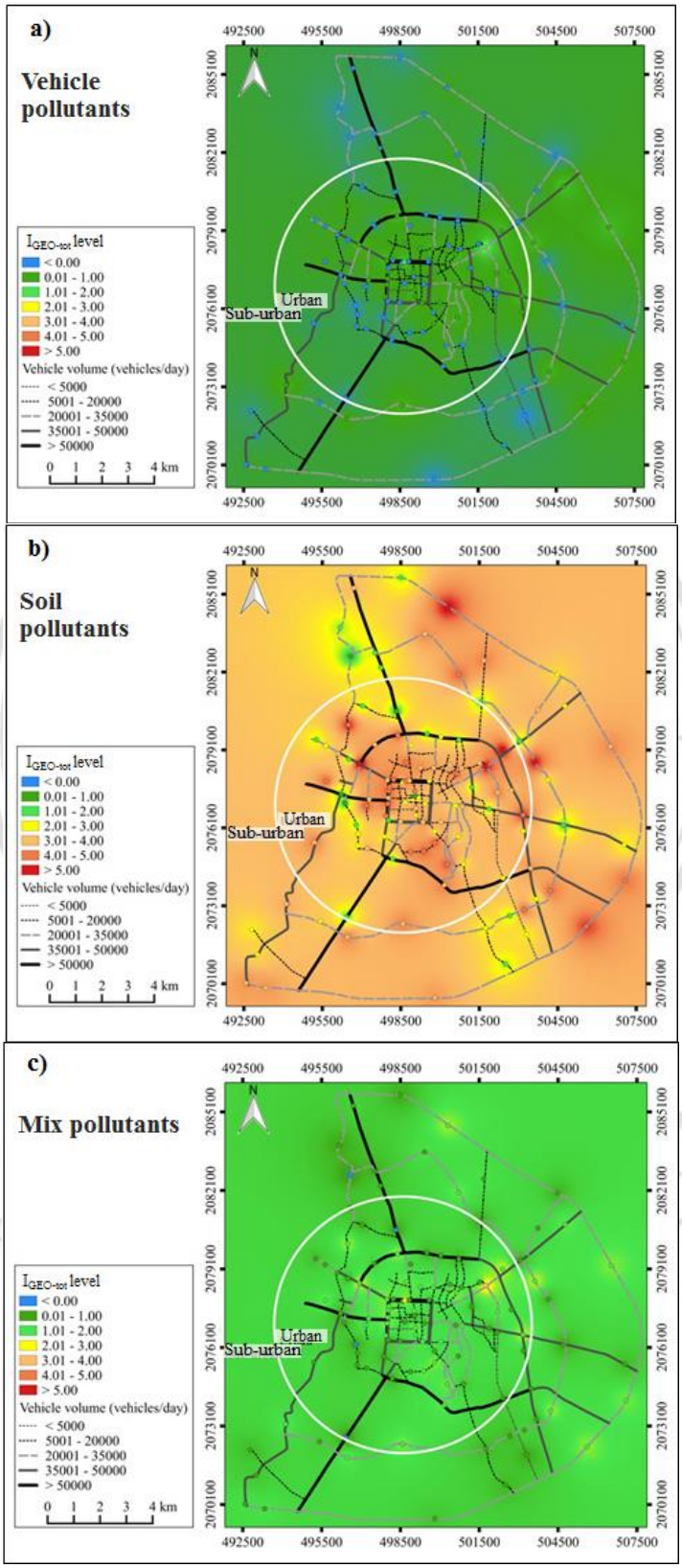


Figure 3.34 Spatial distribution maps of total geoaccumulation index ($I_{GEO-tot}$) in the city of Chiang Mai a) vehicle pollutants, b) soil pollutants, c) mix pollutants

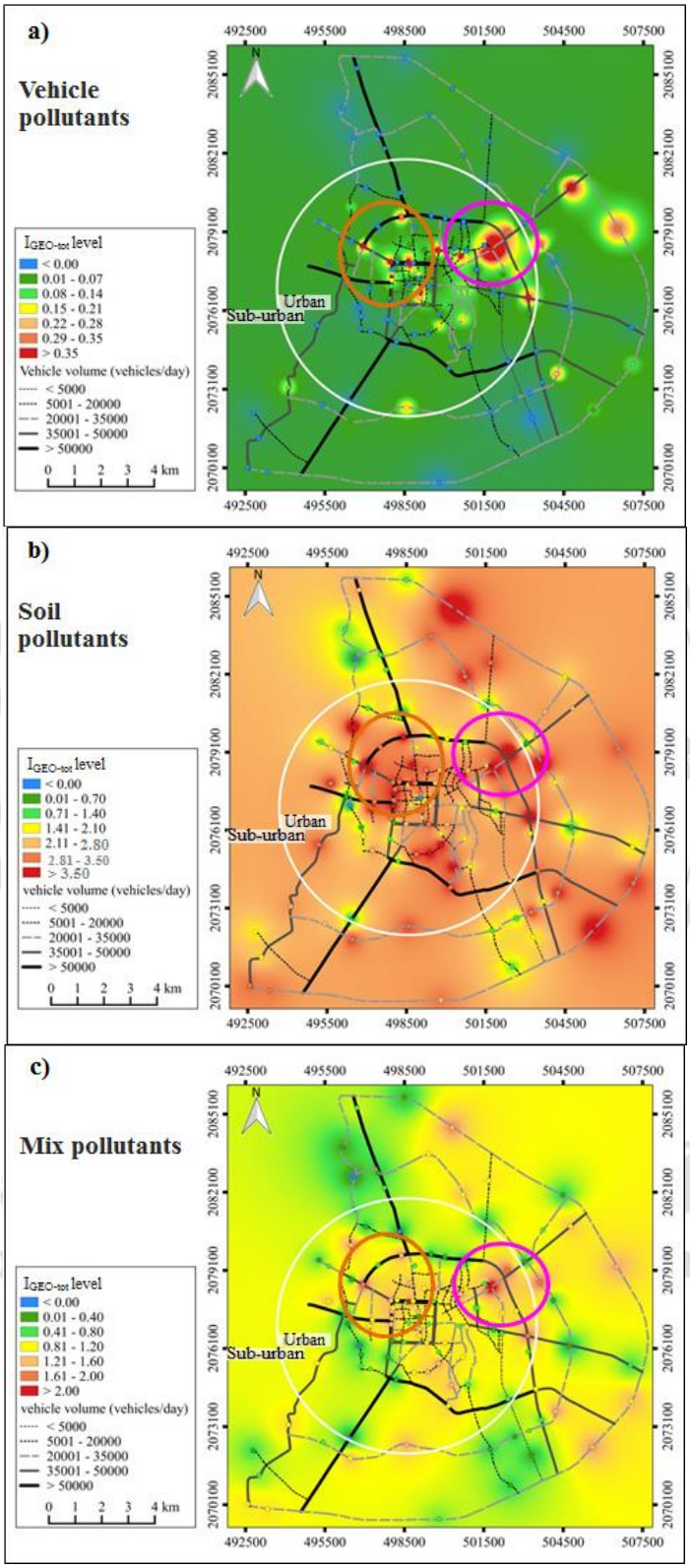


Figure 3.35 Spatial distribution maps of total geoaccumulation index ($I_{GEO-tot}$) (new classification) in Chiang Mai City a) vehicle pollutants, b) soil pollutants, c) mix pollutants

Spearman's correlations of metal concentration and pollution indices with building area and vehicle volume, significant correlation ($p< 0.05$) were mainly observed on vehicle pollutants including Cr, Cu, Pb and Zn, while no significant correlation between soil pollutants and vehicle volume was found. There were indicated that those metals in group of vehicle pollutants were emitted from vehicle and directly accumulated on *Cassia fistula* bark, while Al and Fe might be mainly generated from soil particle attached on road surface. They were distributed in the surrounding area due to wind or mobile vehicles.

All distribution maps illustrated that intersection of Kaewonawarat road and Ratanakosin road and Hua Rin corner of Chiang Mai moat are the area with high pollution area in the city of Chiang Mai. They are located at high density of building and traffic volume and closed to the hospitals and high schools. Moreover, there were distinguished that air quality at the north-west though north-east directions of city center seem to be poorer that that at other directions. It might be due to those direction were located at down wind direction because the wind in the city mainly came from South and South-west direction (Figure 3.36). The result of this study was similar to the study of Chantara (2000) who use mango tree as bioindicator for polycyclic aromatic hydrocarbon (PAHs) which are pollutants emitted from combustion of vehicle exhaust in the city of Chiang Mai in 1999. The result showed that high contamination of PAHs at area around intersection of Kaewonawarat road and Ratanakosin road. Moreover, the result also indicated the area at Northeast to East direction the Chiang Mai Moat was more polluted than the area in Western area of the moat. In addition, Nattakarn (2013) also indicated the same result that area around Hua Rin corner and the East part of Chiang Mai city had poor air quality from using lichen as bioindicator to determine air quality in the city. The result illustrated that *Cassia fistula* tree bark was the high efficient bioindicator for air quality from traffic area.

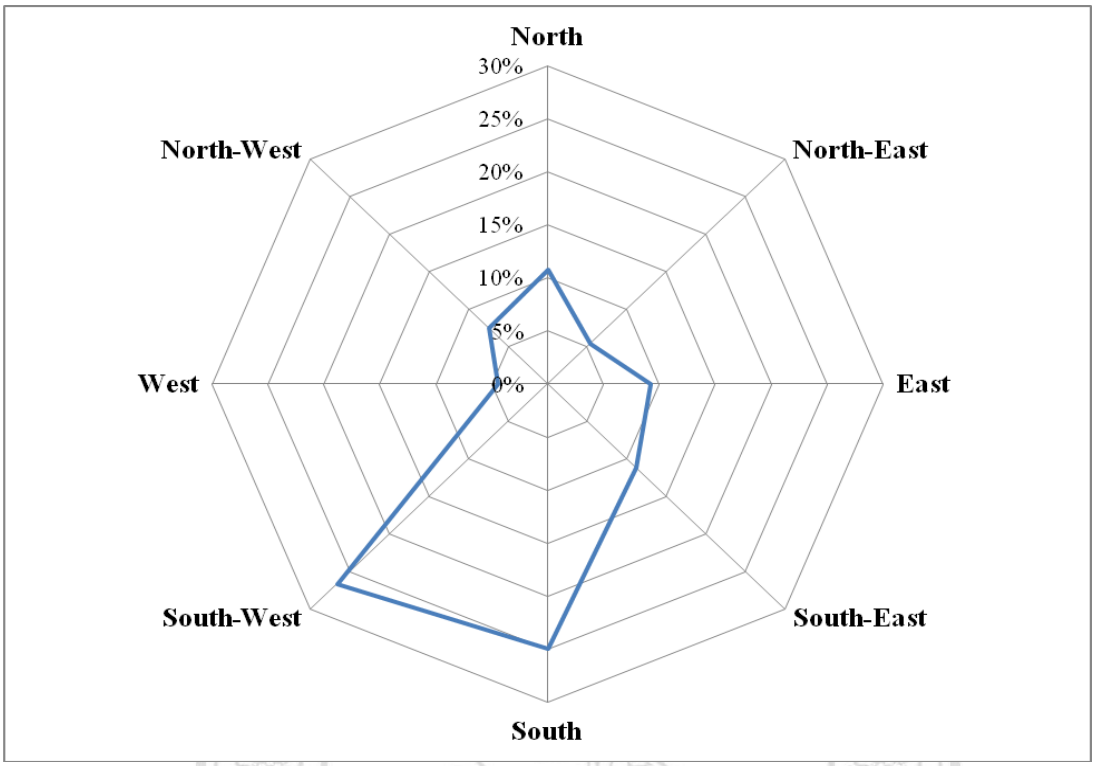


Figure 3.36 Percentage of origin direction of maximum wind speed in Chiang Mai City during 2005 - 2014

CHAPTER 4

CONCLUSION

Use of trees as pollutant bioindicator in South East Asia (SEA) is still limited. Only small number of researches were carried out in this region. Therefore, this study provides useful information of sampling criteria and sampling method for using bark of *Cassia fistula* (golden shower tree), which is a native tree species of SEA as bioindicator for metals emitted from transportation including Al, Cr, Cu, Fe, Ni, Pb and Zn. Moreover, its application for determination of atmospheric metals emitted from road traffic in the city of Chiang Mai was also presented in this study.

Metal accumulation on bark layer of *Cassia fistula* tree was found the highest amount in cork layer (outermost layer) which was mainly influenced by atmospheric pollution. The level was increased in the inner layers, chlorenchyma and phloem layers, respectively. Then the concentration increased again in the phloem&vascular cambium layers (innermost layer), which are connected to xylem layer, therefore the metal found here should be supplied from soil by root system. It can be concluded that use of *Cassia fistula* tree bark for atmospheric metals monitoring should be done by collecting cork layer. Moreover, this study indicated that the age of trees between 4 – 20 years or their DBH size in a range of 5 – 30 cm can be used. Moreover, exposed direction to the traffic has no effect on metal accumulation.

Comparing bark collecting between adhesive collecting methods including Japanese and Thai synthetic resin brands, and scratch collecting method was carried out in order to find the effective bark sampling method and cause less damage on tree. The result showed that scrape method was better than the adhesive method because it presented the high precision on metals analysis in term of low variation of metal concentrations detected within the study area. Moreover, this method can detected and distinguished concentration of metals content on *Cassia fistula* bark between low and high polluted areas, it means scrape method had high accuracy in metal analysis.

In addition, high variation of metal concentrations was observed from adhesive collecting method because metals were found as material composition of all brands of the synthesis resin. After that, the sample size of bark was tested and it was found that 4 x 5 cm^2 area size was the appropriate size for *Cassia fistula* bark sample with scrape collecting method. This size was able to distinguish the accumulation level for Cr and Pb on tree barks in relation to low and high polluted areas. For this group of metal, bigger sample size providing higher metal concentration and they can be detected by the instrument.

The selected collecting method and sampling criteria of *Cassia fistula* bark was applied to collect bark samples from main roads in the city of Chiang Mai. Sampling sites were divided into urban area (~ 5 km radius from city center) and sub-urban area (> 5 km radius from city center). Moreover, bark samples from the background site (Chiang Mai-Phrao road, Phrao District) were also collected. Amount of metals accumulated on the bark samples in descending order were Al > Fe > Zn > Cu > Pb> Cr > Ni.

Comparing between areas, the highest concentration was found in urban area and it was significantly different from the background site. Moreover, metal enrichment factors which is ratio of individual concentrations on *Cassia fistula* bark with the mean concentration (n=4) from the background site (EF_B), or local soil composition (EF_{TS}) were used to indicate accumulation rate of tree bark and distinguish between natural and anthropogenic sources of metals. Base on the EF_B values of Al and Fe, they were classified as severe accumulation rate, while those of Cr, Cu and Zn were in a class of accumulation rate. The EF_B of Pb and Ni were classified in normal rate.

In case of enrichment factor by comparing with the local soil (EF_{TS}) values, they illustrated that Zn, Cu, Pb and Ni possibly came from long-range transport of either natural or/and anthropogenic sources, whereas Al, Cr and Fe could be originated from the surrounding crustal. Nevertheless, principal component analysis (PCA) and cluster analysis (CA) of EF_B and EF_{TS} values could categorize metals into two groups based on their possible sources. Al and Fe were originated from soil particulates (soil pollutants), while Cr, Cu, Pb, Ni and Zn were emitted from vehicles (vehicle pollutants).

In order to present contamination status of the city of Chiang Mai from metals, pollution indices including CF, PLI, I_{geo} and $I_{GEO\text{-}tot}$ were calculated. The result indicated

that the city was classified as moderately to excessively pollute from soil pollutants, while it was categorized as unpolluted to moderately polluted from vehicle pollutants. Furthermore, it was found that I_{geo} and $I_{GEO\text{-}tot}$ were better than CF and PLI indices because they were able to distinguish polluted levels between urban and sub-urban areas. Additionally, pollution indices and geographic information of Chiang Mai city were applied to perform air quality map in the city.

The maps of all indices indicated that urban areas seem to be more polluted than sub-urban area. The area at Kaewnawarat road and Ratanakosin road intersection, and a north-west corner or Hua Rin corner of Chiang Mai moat were high polluted area of the city. The areas were have high traffic density due to location of schools, hospitals and a bus terminal nearby. Moreover, those areas have poor air quality because they were located at the downwind direction of the city.

The study found that degree of pollution originated from vehicles were related with traffic volume and building density in relation to air ventilation capacity. However, their correlations were relatively weak, it may be because some of uncontrolled parameters, such as vehicle moving rate, wind direction and velocity, building arrangements and shapes, aspect ratio (building height to street width), which influence on pollutants dispersion.

Even though, *Cassia fistula* tree is an effective bioindicator for monitoring of pollutants emitted from traffic in the city of Chiang Mai. However, use of one tree species can be limited due to its distribution in the city or in the area of interest. *Cassia fistula* tree was not found on some main roads. Consequently, study of pollutant accumulation on other tree species should be done in the future in order to fulfill information of air quality map in Chiang Mai city.

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Rungruang Janta, 2012. "Use of Synthetic Resin Adhesive for Sampling of Heavy Metals on Tree Bark". Graduate Seminar in Environmental Science, SCB 1-720, Science Complex Building I, Faculty of Science, Chiang Mai University.

Rungruang Janta, 2014. "Use of *Cassia fistula* Tree as Bioindicator for Heavy Metal Accumulation". Graduate Seminar in Environmental Science, SCB 1-720, Science Complex Building I, Faculty of Science, Chiang Mai University.

Oral presentation

Rungruang Janta, Somporn Chantara, Angkhana Inta, Munetsugu Kawashima, and Kenichi Satake, 2014. "Levels of Road Traffic Heavy Metals in Tree Bark Layers of *Cassia fistula* Tree." 2014, 4th International Conference on Environment and BioScience (ICEBS 2014), Jinju South Korea, 8-9 Otober 2014.

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APPENDIX

APPENDIX A

Calculation of metal concentrations

A-1 Metals content on the bark of *Cassia fistula* (µg/g)

1) Calculation of metal concentration on the bark sample (µg/g)

$$M_{Bi} = \frac{C_i \times V_B}{W_B}$$

M_{Bi} = interest metal content on the bark (µg/g)

C_i = concentration of interest metal in sample solution (µg/ml)

V_B = volume of sample solution for bark sample (10 ml)

W_B = weight of bark sample (g)

2) Calculation of total metal concentration on the bark (µg/g)

$$Total_i = \frac{((C_{1i} \times W_1) + (C_{2i} \times W_2) + (C_{3i} \times W_3) + (C_{4i} \times W_4)) \times V_B}{W_1 + W_2 + W_3 + W_4}$$

$Total_i$ = total of interest metal content on the bark (µg/g)

C_{1i}, C_{2i}, C_{3i}, C_{4i} = concentration of interest metal in sample solution (µg/ml) of cork, chlorenchyma, phloem, phloem&vascular cambium layer, respectively

W_1, W_2, W_3, W_4 = weight (g) of cork, chlorenchyma, phloem, phloem&vascular cambium layer, respectively

V_B = volume of sample solution (10 ml)

A-2 Metals content on the bark by surface area (µg/cm^2)

$$M_{Bi/a} = \frac{C_i \times V_B}{A_B}$$

$M_{Bi/a}$ = interest metal content on the bark (µg/cm^2)

C_i = concentration of interest metal in sample solution (µg/ml)

V_B = volume of sample solution of bark sample (10 ml)

A_B = surface area of bark (cm^2)

A-3 Metals content in soil (µg/g)

$$M_{Si} = \frac{C_i \times V_S}{W_S}$$

M_{Si} = interest metal content in soil (µg/g)

C_i = concentration of interest metal in sample solution (µg/ml)

V_S = volume of sample solution of soil sample (25 ml)

W_S = weight of soil sample (g)

APPENDIX B

Pollutant indices

B-1 Calculation of enrichment factors

1) Calculation of enrichment factor in bark

$$EF_B = \frac{[X_i]x[\overline{X}_{Mn,B}]}{[X_{Mn}]x[\overline{X}_{i,B}]}$$ (Abril *et al*., 2014)

$[X_i]$ = individual concentration of an interest metal in bark sample collected from study area

$[X_{Mn}]$ = individual concentration of Mn in bark sample collected from study area

$[\overline{X}_{i,B}]$ = average concentration of interest metal in bark sample collected from background area

$[\overline{X}_{Mn,B}]$ = average concentration of Mn in bark sample collected from background area

2) Calculation of enrichment factor in soil

$$EF_{TS} = \frac{[X_i]x[\overline{X}_{Mn,S}]}{[X_{Mn}]x[\overline{X}_{i,S}]}$$ (Abril *et al*., 2014)

$[X_i]$ = individual concentration of an interest metal in bark sample collected from study area

$[X_{Mn}]$ = individual concentration of Mn in bark sample collected from study area

$[\overline{X}_{i,S}]$ = average concentration of interest metal in soil sample collected from study area

$[\overline{X}_{Mn,S}]$ = average concentration of Mn in soil sample collected from study area

3) Calculation of enrichment factor by group of metals

Grouping of enrichment factor value by metal source was calculated by using the concept of PLI

$$EF\ A-group = (EF_{i1} x EF_{i2} x EF_{i3} x EF_{i4} x \ldots\ldots EF_{in})^{1/n}$$

EF = enrichment factor

i = specie of interest metal

n = the number of interest metal

B-2 Calculation of contaminate factor (CF) and pollution load index (PLI)

1) Calculation of contaminate factor (CF)

$$CF = \frac{[X_i]}{[\overline{X}_{i,B}]}$$ (Salah *et al*., 2012)

$[X_i]$ = concentration of the interest metal collected from study area

$[\overline{X}_{i,B}]$ = average concentration of the interest metal collected from background area

2) Calculation of pollution load index (PLI)

Potential Load Index (PLI) is calculated from integration of CF for n metal species. The calculation of PLI is shown as below (Guéguen *et al*., 2012).

$$PLI = \left(CF_{i1} x CF_{i2} x CF_{i3} x CF_{i4} x ..... CF_{in}\right)^{1/n}$$

CF = contamination factor

i = specie of interest metal

n = the number of interest metal

B-3 Calculation of geoaccumulation index (I_{geo}) and total geoaccumulation index ($I_{GEO\text{-}tot}$)

1) Calculation of geoaccumulation index (I_{geo})

$$I_{geo} = \log_2 \left[\frac{[X_i]}{1.5 x [\overline{X}_{i,B}]} \right]$$ (Abrahim and Parker, 2008)

$[X_i]$ = concentration of the interest metal collected from study area

$[\overline{X}_{i,B}]$ = average concentration of the interest metal collected from background area

The factor 1.5 was used in order to detect a small influence from anthropogenic (Chen *et al.*, 2015).

2) Calculation of total geoaccumulation index ($I_{GEO\text{-}tot}$)

Geoaccumulation index ($I_{GEO\text{-}tot}$), a new index which is integration of I_{geo} and PLI for n metals, was proposed by Guéguen *et al.* in 2012. This new index is useful for easy specification of pollution level in each study area.

$$I_{GEO-tot} = \left(\frac{\Sigma_i I_{geo,i}}{n} \right) = \log_2 \left[\frac{PLI}{1.5} \right]$$ (Guéguen *et al.*, 2012)

$I_{geo,i}$ = I_{geo} value of interest metal

n = number of total interest metal

PLI = PLI value of study area

APPENDIX C

Air quality mapping of Chiang Mai City

C-1 Calculation of total traffic volume

Total traffic volume in grid cell is defined as total of average daily vehicle volume existed in 0.25 km^2 grid area (Figure C-1). The total traffic volume in each grid is summation of multiplication of traffic volume of main road (R_n) with the ratio between length of main road in each grid and original length of main road ($L_{n.i}/L_n$).

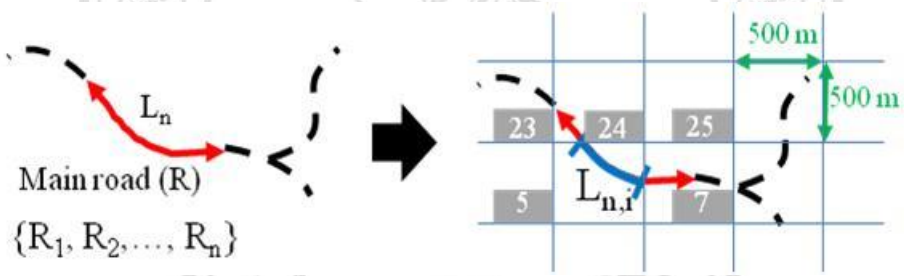

Figure E-1 Separation of traffic volume in each grid

$$T_i = \Sigma\left(R_n x \frac{L_{n,i}}{L_n} \right)$$

T_i = total traffic volume in grid i

R_n = traffic volume of main road n

$L_{n,i}$ = length of main road n in grid i

L_n = length of main road n

C-2 Calculation of building area

Building area in grid cell is defined as summation of building area existed in 0.25 km^2 grid area (Figure C-1).

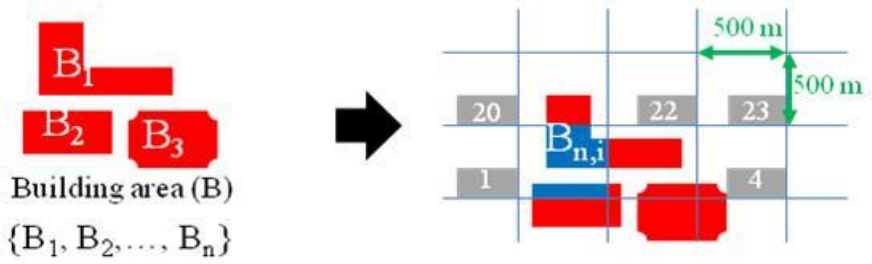

Figure E-2 Separation of building area in the grid

$$A_i = \Sigma B_{n,i}$$

A_i = total building area in grid i

$B_{n,i}$ = building area of building n in grid i

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