การใช้ใบของต้นแก้ว (*Murraya paniculata*) เป็นดัชนีทางชีวภาพของมลพิษโลหะหนักในอากาศในพื้นที่กรุงเทพฯ

นาย ธีรเวช ทิตย์สีแสง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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LEAVES OF ORANGE JASMINE (*Murraya paniculata*) AS BIOINDICATORS OF AIRBORNE HEAVY METAL POLLUTION IN BANGKOK AREA

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ธีรเวช ทิตย์สีแสง : การใช้ใบของต้นแก้ว *(Murraya paniculata)* เป็นดัชนีทางชีวภาพของมลพิษโลหะหนักในอากาศในพื้นที่กรุงเทพฯ (LEAVES OF ORANGE JASMINE (*Murraya paniculata*) AS BIOINDICATORS OF AIRBORNE HEAVY METAL POLLUTION IN BANGKOK AREA) **อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. นพภาพร พานิช, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม ศ. ดร. ทิมโมธี เอส วูด,** 108 หน้า.

ทำการเก็บตัวอย่างใบของต้นแก้ว (*Murraya paniculata*) ดินชั้นบน และ อนุภาคฝุ่น PM10 จากพื้นที่สามแห่งในกรุงเทพฯ และปทุมธานี ซึ่งใช้เป็นพื้นที่อ้างอิง ได้แบ่งตัวอย่างใบไม้ออกเป็นสองประเภท คือใบที่ผ่านการล้าง และไม่ผ่านการล้าง นำตัวอย่างทั้งหมดมาย่อยและหาปริมาณของโลหะ Cu, Fe, Pb, Mn, Ni, Cr และ Zn ด้วยเครื่อง ICP-AES จากการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอน (SEM) พบว่าฝุ่นจากอากาศที่พบบริเวณผิวใบด้านบน (Adaxial) มีปริมาณมากกว่า ผิวใบด้านล่าง (Abaxial) โดยฝุ่นมีความแตกต่างกันทั้งด้านรูปร่างและขนาด การศึกษาลักษณะของปากใบ (Stomata) บริเวณผิวใบด้านล่างทั้งในใบแก่และใบอ่อน พบว่าไม่แตกต่างกันทั้ง รูปร่าง ขนาด และจำนวนของปากใบ การศึกษาปริมาณโลหะในฝุ่นในแต่ละพื้นที่พบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญของปริมาณโลหะ ในขณะที่ปริมาณโลหะที่พบในดินและในใบไม้ทั้งสองประเภทจากตัวอย่างที่เก็บในพื้นที่เมืองจะมีค่ามากกว่าในพื้นที่อ้างอิง การวัดระดับของความสัมพันธ์ต่างๆในการศึกษานี้ทำโดยใช้ค่าสัมประสิทธิ์สหสัมพัทธ์เพียร์สัน ซึ่งผลการศึกษาชี้ว่า ไม่พบความสัมพันธ์ของโลหะชนิดเดียวกัน ในใบไม้ที่ผ่านการล้างและดิน รวมถึง ใบไม้ที่ผ่านการล้างและฝุ่น ในการวิเคราะห์ Enrichment Factor (EF) ซึ่งช่วยในการแยกแหล่งกำเนิดของโลหะจากกิจกรรมของมนุษย์ ออกจากแหล่งกำเนิดจากพื้นผิวโลก ในใบไม้และฝุ่นโดยใช้ Fe เป็นธาตุอ้างอิง พบว่า รูปแบบของค่า EF ในใบไม้ และฝุ่น มีความเหมือนกัน ค่า EF ที่ได้ชี้ว่า โลหะ Cu, Ni, Pb และ Zn ไม่ได้มีแหล่งกำเนิดหลักจากธรรมชาติ ในขณะที่ Cr และ Mn มาจากธรรมชาติ ได้ทำการทดสอบอิทธิพลของการล้างใบ อายุใบ และ ฤดู(แล้ง และ ฝน) ที่มีผลต่อการสะสมของโลหะในใบไม้ โดยใช้ค่าทางสถิติ t-test ซึ่งพบว่าการล้างใบไม้จะลดปริมาณโลหะลงได้อย่างมีนัยสำคัญ โดย Fe เป็นธาตุที่ถูกขจัดออกไปได้มากที่สุด และพบว่าระดับความเข้มข้นของ Cu และ Fe ในใบไม้จะเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเวลาผ่านไป ในขณะที่ Mn และ Zn จะมีค่าคงที่ การศึกษายังพบว่าปริมาณโลหะที่พบในในฤดูฝนและฤดูแล้งในใบไม้ทั้งสองประเภทไม่แตกต่างกันอย่างมีนัยสำคัญ การศึกษาแหล่งกำเนิดของมลพิษโลหะในอากาศโดยใช้ใบไม้พบว่า ในพื้นที่เก็บตัวอย่างทั้งสามแห่งมีการปนเปื้อนด้วยโลหะมากกว่าในพื้นที่อ้างอิง โลหะที่พบในปริมาณมากที่สุดคือ Fe ในขณะที่พบ Pb น้อยที่สุด จากการวิเคราะห์ความสัมพันธ์ระหว่างโลหะ แสดงให้เห็นว่า มีความสัมพันธ์อย่างมากระหว่าง Cu-Mn, Cu-Zn, Cu-Pb และ Mn-Zn โดยที่ Fe ไม่สัมพันธ์กับโลหะอื่น การทดสอบโดยใช้การวิเคราะห์ความแปรปรวน (ANOVA) พบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญของ Pb ในแต่ละพื้นที่ โดยความแตกต่างของโลหะอื่นที่พบในแต่ละพื้นที่น่าจะเป็นผลจากกิจกรรมของมนุษย์ที่ต่างกัน การวิเคราะห์องค์ประกอบหลัก (PCA) แสดงให้เห็นว่าแหล่งกำเนิดของฝุ่นเกิดจาก 2 ปัจจัยหลัก โดยปัจจัยแรก ได้แก่แหล่งกำเนิดจากกิจกรรมของมนุษย์ (การจราจร) และปัจจัยที่สอง เกิดจาก ธรรมชาติ (ดิน) จากการศึกษาทั้งหมดอาจสรุปได้ว่าฝุ่นในอากาศในกรุงเทพฯ เป็นผลมาจากกิจกรรมของมนุษย์ โดยเฉพาะอย่างยิ่งจากการจราจร นอกจากนี้ยังแสดงให้เห็นว่า ใบของต้นแก้ว (*Murraya paniculata*) สามารถนำมาใช้เป็นดัชนีทางชีวภาพสำหรับมลพิษโลหะในอากาศได้

สาขาวิชาการจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต ....

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ....

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KEY WORD: BIOINDICATOR/ AIR POLLUTION / ORANGE JASMINE / METAL

TEERAWET TITSEESANG: LEAVES OF ORANGE JASMINE *(MURRAYA PANICULATA)* AS BIOINDICATORS OF AIRBORNE HEAVY METAL POLLUTION IN BANGKOK AREA. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. NOPPAPORN PANICH, D.Eng, THESIS CO-ADVISOR: PROF. TIMOTHY S WOOD, Ph.D, 108 pp.

Orange jasmine *(Murraya paniculata)* leaves, topsoil and particulate matter (PM10) were collected from three different sites in the area of Bangkok and in Pathumthani as reference site. The leaf samples were differently treated and divided into two groups: washed and unwashed leaves. All types of samples were digested and the concentrations of Cu, Fe, Pb, Mn, Ni, Cr and Zn were then quantified by using ICP-AES. The SEM study reveals that airborne particles are higher in density on the adaxial (upper surface) than abaxial (lower surface). The particles are heterogeneously distributed in forms and diameter lengths. The characteristics of stomata appeared on abaxial of old and young leaf in forms, sizes, and numbers are not different. M tal concentrations in PM10 are found insignificantly different between sites while the soil results indicate all metals are higher in urban areas relative to the reference site. All metals found in both types of leaves are higher in the sampling urban sites than the reference site. Pearson's correlation coefficient is used to determine the degree of relationship in the study. There is not significant correlation of the same metal concentrations between washed leaves and soils, and washed leaves and PM10. The enrichment factor (EF) using Fe as a reference element is employed to metals found in leaf and air particle samples to separate the anthropogenic source from the crustal source. The patterns of EF values of all sample types are similar. The EF values of Cu, Ni, Pb and Zn indicate that the dominant sources of these elements are not natural. On the contrary, the Cr and Mn are found their origin from natural source. The statistical t-test is used to test the effects of washing leaves, leaf ages and seasons (wet and dry) on metal accumulation in leaves. Washing the leaves reduces the concentrations of metals significantly. Fe is most removed by washing. The levels of Cu and Fe concentrations in leaf increase significantly in the course of time while the Mn and Zn are remained constant. Metal contents accumulated in both types of leaves are insignificantly different between wet and dry seasons. The study on source identification of air metal pollution using leaves show all three sampling sites are polluted with metals compared with the reference site. The highest mean concentration of the studied metals is Fe while Pb is the lowest. The correlation analysis shows that there is a high correlation coefficient between Cu-Mn, Cu-Zn, Cu-Pb, and Mn-Zn. However, Fe is not correlated to other metals. The ANOVA testing reveals that there is not a significant difference in Pb contents among sites. The significant difference in metals could be attributed to different anthropogenic activities among sites. The principal component analysis (PCA) shows two main factors according to the sources of metals found. Factor 1 and 2 are anthropogenic (traffic) and natural (soil) sources, respectively. Based on the overall study, it can be concluded that the airborne particles found in Bangkok are strongly impacted by anthropogenic sources especially traffic. The results also show that Orange jasmine *(Murraya paniculata)* leaves can be used as bioindicators for metal air pollution.

Field of study Environmental Management Student's signature T. Tit
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CHAPTER I

INTRODUCTION

1.1 General background

Increasing industrialization and human activities intensify the emission of various pollutants into the environment and introduce various harmful substances into the atmosphere. Interest in the effects of atmospheric particles especially in the inhalable fraction has increased during the last 10 years. Particulate matter with its diameter less than 10 µm (PM10) is considered harmful for human health because it can cause respiratory and cardiovascular diseases (Fang *et al*., 2006). Links between asthma attacks and coughs with particulates have also been reported (Freer-Smith *et al*., 1997). In large urban areas, the considerable amount of particulate matter in the air is a crucial problem. The primary source of airborne particulate matter comes from agricultural practices, vehicle traffic, industrial processes and fossil fuel stations (Beckett *et al*., 1998). Time series analyses, conducted in many industrial countries, have consistently shown that short-term changes in air pollution levels are associated with changes in daily death rates (Stieb *et al*., 2002). Heavy metals cause serious environmental risks and, therefore, its effect has been examined extensively.

In most cities, airborne metals are not routinely monitored with fully or semi-automatic gauges commonly used in current pollution monitoring programs, owing to elevated costs and technical difficulties. This is a grave drawback because vehicular traffic, which is a great source of fine particulate and airborne metals in urban environments has risen dramatically in recent years (Cadle *et al*., 1997; Janssen *et al*.,1997). To complete the information on trace element deposition obtained from automatic gauges, increasing attention has recently being paid to plants as passive biomonitors. This reliable, versatile and inexpensive method can assist decision-makers on the subject of health and environmental protection against potentially hazardous trace elements. Providing a high density of sampling points, biomonitors are very effective for tracing maps of airborne metal contamination in the urban environments (Monaci *et al*., 1997).

The presence of trace elements in ecosystems is due to both natural and anthropogenic causes. While natural forms are usually found in relatively low concentrations, in recent decades the number and intensity of anthropogenic sources especially vehicle emissions, agricultural have increased overall environmental trace element concentrations. Accumulation of trace elements in the stand biomass over large areas and during long periods of time causes chronic damage to living organisms. Accumulative biomonitors are indispensable for evaluating long range tropospheric transport and deposition of trace elements on regional and global scales (Sardans and Penuelas, 2005). Over the last 20 years mosses, lichens and higher plants have been used as biomonitors and bioindicators (Loppi and Bonini, 2000; Fernandez and Carballeira, 2002) for drawing reliable pollution maps of the areas to which these persistent pollutants have been transported, but where instrument recordings would be impossible or at best very difficult (Steinnes *et al*., 1992). Among plants, bryophytes are the most extensively used biomonitors and bioindicadors of trace element pollution (Weiss *et al*., 1999; Fernandez *et al*., 2000; Carballeira *et al*., 2002). Due to their lack of root systems, mosses are dependent on aerial uptake.

Concentrations of metals in many environments have increased as a direct result of anthropogenic activities. Concern over the potential ecotoxicological hazards posed by elevated levels of metals in the environment has prompted a search to find reliable, low cost methods of assessing the extent of metal contamination at a locality and the exposure risk to indigenous biota (Keane *et al*., 2001).

In recent years, it has been shown that lead levels in soil and vegetation have increased considerably due to traffic pollution, i.e. usage of leaded petrol and exhaust combustion (Oztvos *et al*., 2003). This problem rises as daily traffic increases. Botanical materials such as fungi, lichens, tree bark, tree rings and leaves of higher plants have been used to detect pollution. The monitoring of the levels of atmospheric trace metallic concentration by using different types of biological monitors and various vegetation have been reported by different researchers (Rao and Dubey, 1992; Morselli *et al*., 2004).

Several studies have pointed out the importance of atmospheric input in the biogeochemical cycling of heavy metals, as reviewed by Markert (1993). The responses of plants to elevated concentrations of air contaminants are modified by environmental factors and by plant physiological status. Plant leaves are used as indicators of heavy metal pollution. Although higher plants are usually not as suitable biomonitors as lichens and mosses, in industrial and in urban areas, where lichens and mosses are often missing, higher plants can act as biomonitors. Also, in industrial and urban areas higher plants can give better quantifications for pollutant concentrations and atmospheric deposition than non-biological samples. Therefore, using plant leaves primarily as accumulative biomonitors of heavy metals pollution has a great ecological importance (Markert 1993).

The accumulation of heavy metals by higher plants depends on the binding and solubility of particles deposited on leaf surfaces, as well as on concentrations and bioavailability of elements in the soil. Considering that metal uptake in higher plants takes place through roots and leaves, it is difficult to distinguish whether the accumulated elements originate from the soil or from the air. In spite of difficulties in interpretation of the data, the use of the leaves of higher plants has been increasingly investigated for the purpose of heavy metals accumulation monitoring. Hence, studies are in progress in search for suitable tree species and approval of a validity of using their leaves as biomonitors (Tomasevic *et al*., 2004).

Analyzing plant tissues can give better results in terms of sensitivity and reproducibility (Lau and Luk, 2001). Heavy metals emitted into the environment in different ways, i.e. transportation, industry, fossil fuels, agriculture and other human activities (Aksoy and Ozturk , 1997). The most economical and reasonable method for monitoring the heavy metal levels in the atmosphere is using vegetation, other plants, and other organisms such as fishes have also been used for biomonitoring (Celik *et al*., 2005).

Uptake of elements into plants can happen via different ways. The elements can be taken up via roots from the soil and transported to the leaves. Trace elements may be taken up from the air, or by precipitation directly via the leaves. In addition, some plants even exhibit ion-exchange properties. Once deposited on the leaf surface some elements may also be taken up into the leaf via the stomata (Reimann *et al*., 2001). Considering that higher plants function as biomonitors in the monitoring of aerial metal contamination due to their accumulation properties. Correlations between metal concentration in fine particle and plants can be expected. Even several studies have been done on the contamination by particulate matter in a urban areas (Janssen *et al*., 1997; Monaci *et al*., 2000; Fernandez *et al*.,2001) and on the use of plant species as biomonitors (Sawidis *et al*., 1995; Aksoy and Ozturk, 1997; Bargagli *et al*., 2003; Oliva and Valdes, 2004; Rossini Oliva and Rautio, 2004). Nevertheless, relationships between PM10 composition and trace elements in plants have been insufficiently studied. This study is a first attempt to study the relationship between metals content in leaves in relation to that in the atmosphere in Bangkok environment.

In the case of metals, there is general agreement in the literature that a good biological monitor should be a species that is represented by large numbers of individuals over a wide geographic area, has a broad toxic tolerance, and accumulates metals at levels reflecting those in the environment so that their chemical composition will provide a quantitative measure of the magnitude of contamination when assessed against background values (Keane, *et al*., 2001; Falla *et al*., 2000). Since many plants have a tendency to assimilate metals from their surroundings, a variety of species are being evaluated for their utility as biological monitors of environmental metal pollution (Djingova *et al*., 1993; Markert, 1993; Wittig, 1993). With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wolterbeek, 2002). The large amount and wide ranges distributions of the Orange jasmine (*Murraya paniculata*) lead to the possibility of using this plant species as an attractive candidate for biological monitoring.

1.2 Objectives of the study

The objectives of the study can be summarized as follows:

1. To characterize and assess the distribution of airborne heavy metals in both inhalable atmospheric particles (PM10) and leaves of Orange jasmine *(Murraya paniculata)*
2. To investigate the possible source(s) of the airborne heavy metals in Bangkok area
3. To evaluate the reliability of using plant species Orange jasmine *(Murraya paniculata)* as bioindicators of airborne heavy metal pollution

1.3 Hypothesis

1. The main source of airborne heavy metals in Bangkok area has been the traffic.
2. Leaves of Orange jasmine can be good bioindicators for airborne heavy metal pollution.

1.4 Scope of the study

1. This study is conducted in three different sites in Bangkok area namely, Department of Land Transportation (DLT), Chulalongkorn Hospital (CHU), Ministry of Science and Technology (MST), and the Reference site at the Bangkok University, Rangsit campus, located in Pathumthani province.
2. The air particulate and soil samples at the same site of leaf samples are also simultaneously collected. The length of air sampling is at 24 hours. The soil sample is surface soil (0-10 cm. in depth from the surface).
3. The elements analyzed in this study are Cr, Cu, Fe, Mn, Ni, Pb and Zn. These metals are considered to be good representatives of metal pollutant indicators in urban environments. They can be found from both anthropogenic and natural sources. There are also potential toxicants to human health and the ecosystem.
4. Pearson's correlation is used to investigate the relationship between metals in the same and different sample matrices. Analysis of Variance (ANOVA) is used to detect significant differences between sites. Effects of washing, ages, and seasons on the variation of metal contents is evaluated by using statistical t-test. In order to identify the possible source(s) of metallic elements, multi-receptor model, Enrichment Factor (EF) and Principal Component Analysis (PCA) are used in this study.

1.5 Benefits of the study

The overall results from the study increase the understanding of the variations of atmospheric metals and their effects on Bangkok environments. In addition, this study also reveals the possible source(s) of airborne metal and the possibility of using plant species Orange jasmine *(Murraya paniculata)* as bioindicators of airborne heavy metal pollution.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Human activities, nowadays, cause the increasing concentrations of heavy metals in many environments. The fine particulate like particulate matter with its diameter less than 10μm (PM10) has been paid an attention by many researchers in decades because it is considered harmful for human health. In general known, the sources of airborne particulate matter can be from many sources such as agriculture, vehicle traffic, industrial processes and fuel combustion.

To assess the ecotoxicological hazards of metals, the reliable amount of toxicants must be known. However, the scientific equipment used in quantifying metal concentrations is very expensive. Concern over the potential posed by elevated levels of metals in the environment has prompted a search to find reliable, low cost methods of assessing the extent of metal contamination at a locality and the exposure risk to indigenous biota (Keane *et al*., 2001).

One avenue of research has been to identify organisms that could potentially be utilized as biological monitors for estimating levels of metal pollution. Biological monitors have commonly been defined as organisms that provide quantitative information on some aspect of their environment, such as how much of a pollutant is present (Keane, *et al*., 2001). Plants are well known to be used in biomonitoring studies of trace elements by its abilities to capture the metals (Oliva and Valdes, 2004; Sawidis *et al*., 1995). Several studies have been reported on the metal contamination by airborne particulate matter in urban areas (Janssen *et al*., 1997; Monaci *et al*., 2000; Fernandez *et al*., 2001), and on the use of plant species as biomonitors (Sawidis *et al*., 1995; Aksoy and Ozturk, 1997; Keane *et al*., 2001; Bargagli *et al*., 2003; Oliva and Valdes, 2004; Swaileh *et al*., 2004; Sardans and Penuelas, 2005; Onder and Dursun, 2006; Al-Khlaifat and Al-Khashman, 2007).

2.2 Airborne heavy metals in the environment

Emissions of anthropogenic air pollutants in northeastern Asia have been increasing drastically in the past decade (Lee *et al.*,2001; Querol *et al.*, 2001). Many researchers were interested in the distributions of particle matter and its chemical properties at different regions like urban, suburban, rural and industrial zones. The larger sized particles are greatly affected by gravity and fine particles are more affected by diffusion (Chan and Kwok, 2000). The concentration, composition, and particle size of suspended particulate matter at a given site are determined by such factors as meteorological properties of the atmosphere, topographical influences, emission sources, and by particle parameters such as density, shape, and hygroscopicity (Koliadima *et al.*, 1998). Most of the trip mileage is traveled by traffic vehicle; hence, PM10 and PM2.5 have become the major source of street-level air pollution. The larger size particles are greatly affected by gravity, and finer particles are more affected by diffusion (Chan and Kwok, 2000). Particulate matter standard of Asian cities are illustrated in Table 2.1 (Schwela *et al.*, 2006)

Average total suspended particulate (TSP) concentrations decrease from the urban and industrial zone to the residential area. The same behavior is observed for Pb, Zn, and Cd, but not for Cu, which has a relatively short residential time (Morawska *et al.*, 2001; Espinosa *et al.*, 2002). Urban populations are exposed to metals in suspended particles and these are often well above natural background levels owing to anthropogenic processes. This results in elevated metal concentrations that can pose an important risk to human health (Antonio *et al.*, 2001). Many investigations on heavy metals that were regulated by law in some countries, such as Pb, Cd, and Cr, have been made, regarding with their formation, enrichment in different size particles, removal by absorption and adsorption, reaction and so on. Combustion of waste and fuel generates particle matter which consists of inorganic matter including metal and unburned carbon mainly as soot (Yoo *et al.*, 2002). Ca, Mg and Mn indicate construction materials Al, K, Ti and Mn indicate wind-blown soil. Higher concentrations of Pb reflect the impact of vehicle emissions (Shu *et al.*, 2001). Mn also possesses multiple industrial, combustion and resuspension sources (Allen *et al.*, 2001).

Table 2.1: Particulate matter (PM) standards in Asian cities

		Total Suspended Particulate (TSP) (μg/m3)					PM10 (μg/m3)			PM2.5 (μg/m3)	
Country	Cities	1 hr	3 hrs	8 hrs	24 hrs	1 yr	1 hr	24 hrs	1 yr	24 hrs	1 yr
WHO								50	20	25	10
EU								50	40		
USEPA								150	50	65	15
Bangladesh								150	50	65	15
China (class II)					300	200		150	100		
Hong Kong, SAR, China					260	80		180	55		
India (Residential, rural & other areas)					200	140		100	60		
Indonesia					230	90		150			
	Jakarta				230	90		150			
	Surabaya				230	90		150			
Japan							200	100			
Republic of Korea								150	70		
	Busan							150	70		
	Seoul							120	60		
Nepal					230			120			
Philippines					230	90		150	60		
Singapore								150	50	65	15
Sri Lanka		500	450	350	300	100					
Taiwan, China								150	65		
Thailand					330	100		120	50		
Vietnam								150	50		

Source: Schwela *et al.*, 2006

The concentrations of Cd, Mn, Ni and Zn were significantly higher at the industrial sites and should be attributed to the pyrometallurgical processes (Pb and Zn smelters, non-ferrous metal industries, etc.) taking place in the area, as well as to the manganese ore treating plant. Relatively higher Pb concentrations were found at the urban sites due to higher traffic density (Voutsa and Samara, 2002). The possible releasing sources of fine particles emit to the atmosphere in urban area including traffic activities, wood and garbage burning, food cooking and others.

Motor vehicle exhaust is one of the most important sources of fine particulate matter in the polluted urban environment. The increase of atmospheric anthropogenic emissions of heavy metals is particularly damaging in rural areas. In fact these pollutants, carried by atmospheric particles, may settle on the superficial soil and their depositions may change the ratios among different chemical forms of heavy metals in the soil. In particular, the emissions from industrial sources may change the metal bioavailable concentrations in the soil, causing the introduction of some toxic metals in the alimentary chain (Ragosta *et al*., 2002). As expected, PM levels obtained at the different monitoring sites gradually increased from regional background stations to industrial sites as a result of the addition of local contributions (traffic, industry). The majority of the stations selected is subjected to the influence of close industrial and/or traffic PM sources (Viana *et al*., 2003).

2.3 Definitions and Principles for Bioindicator and Biomonitor

It has a lot of confusing in using terms related to bioindicator and biomonitor in the previous reports. Markert (2007) gave the best description of all confusing words as shown as follows.

"Due to the rapid development of this field, many terms have not been clearly defined and mathematically delimited. Therefore, ambiguous use of the terms bioindication and biomonitoring is possible. A bioindicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment). A biomonitor, on the other hand, is an organism (or part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment. The clear differentiation between bioindication and biomonitoring using the qualitative

/quantitative approach makes it comparable to instrumental measuring systems. Such effects (information bits) of bioindicators (biomonitors) may include changes in their morphological, histological or cellular structure, their metabolic–biochemical processes (including accumulation rates), their behaviour or their population structure. Accumulation indicators/monitors are organisms that accumulate one or more elements and/or compounds from their environment. Effect or impact indicators/monitors are organisms that demonstrate specific or unspecific effects in response to exposure to a certain element or compound or a number of substances. According to the paths by which organisms take up elements or compounds, various mechanisms contribute to overall accumulation (bio-accumulation), depending on the species-related interactions between the indicators/monitors and their biotic and abiotic environment. Biomagnification is the term used for absorption of the substances from nutrients via the epithelia of the intestines. It is therefore limited to heterotrophic organisms and is the most significant contamination pathway for many land animals except in the case of metals that form highly volatile compounds (e.g. Hg, As) and are taken up through the respiratory organs (e.g. trachea, lungs). Bioconcentration means the direct uptake of the substances concerned from the surrounding media, e.g. the physical environment, through tissues or organs (including the respiratory organs). Besides plants, which can only take up substances in this way (mainly through roots or leaves), bioconcentration plays a major role in aquatic animals. The same may also apply to soil invertebrates with a low degree of solarization when they come in contact with the water in the soil. Active bioindication (biomonitoring) is meant when bioindicators (biomonitors) bred in laboratories are exposed in a standardized form in the field for a defined period of time. At the end of this exposure time the reactions provoked are recorded or the xenobiotics taken up by the organism are analysed. In the case of passive bioindication (biomonitoring) organisms already occurring naturally in the ecosystem are examined for their actions. This classification of organisms (or communities of these) is in according to their ‘origin’. Various newer methods (biomarkers, biosensors, biotests in general) have been introduced into the application field of bioindication, besides the classical floristic, faunal and biocoenotic investigations that primarily record unspecific reactions to pollutant exposure at higher organismical levels of bioindication. Biomarkers are measurable biological parameters at the suborganismic (genetic, enzymatic, physiological, morphological) level in which structural or functional changes indicate environmental influences in general and the action of particular in qualitative and sometimes also in quantitative terms. Examples are enzyme or substrate induction of cytochrome P-450 and other Phase I enzymes by various halogenated hydrocarbons; the incidence of forms of industrial melanism as markers for air pollution; tanning of the human skin caused by UV radiation; changes in the morphological, histological or ultrastructure of organisms or monitor organs (e.g. liver, thymus, testicles) following exposure to pollutants. A biosensor is a measuring device that produces a signal in proportion to the concentration of a defined group of substances through a suitable combination of a selective biological system, e.g. enzyme, antibody, membrane, organelle, cell or tissue, and a physical transmission device (e.g. potentiometric or amperometric electrode, optical or optoelectronic receiver). Biomarkers and biosensors can be used as biotest (bioassay) which describes a routine toxicological–pharmacological procedure for testing the effects of agents (environmental chemicals, pharmaceuticals) on organisms, usually in the laboratory but occasionally in the field under standardized conditions (with respect to biotic and abiotic factors). In the broader sense the definition covers cell and tissue cultures when used for testing purposes, enzyme tests or tests using microorganisms, plants and animals in the form of single-species or multi-species procedures in model ecological systems (e.g. microcosms and mesocosms). In the narrower sense, the term only covers singlespecies and model system tests, while the other procedures may be called suborganismic test. Bioassays use certain biomarkers or – less often – specific biosensors and can be used in bioindication or biomonitoring. The term tolerance can be described as desired resistance of an organism or community by unfavourable abiotic (climate, radiation, pollution) or biotic factors (parasites, pathogens), where adaptive physiological changes (e.g. enzyme induction, immune response) can be observed. Unlike tolerance, resistance is a genetically derived ability to withstand stress. This means that all tolerant organisms are resistant, but not all resistant organisms are tolerant. Sensitivity of an organism or community means its susceptibility to biotic or abiotic changes. Sensitivity is low if the tolerance or resistance to an environmental stressor is high, and sensitivity is high if the tolerance or resistance is low.”

2.4 Orange jasmine (*Murraya paniculata*)

The tree species, Orange jasmine *(Murraya paniculata)* (Figure 2.1) was selected to evaluate the level of trace elements accumulation for a number of reasons. It is widespread in any areas of Bangkok and it is one of the most frequent tree species found in Thailand. Furthermore, it has shown in the first rough review to be a possible useful bioindicator of airborne trace elements. Because of its long-lived leaves, the easy identification, and its widespread occurrence in urban areas and natural ecosystems make this species adequate tool for trace elements bioindicator studies.

Figure 2.1: Orange jasmine (*Murraya paniculata*)

Source:http://www.desert-tropicals.com/Plants/Rutaceae/Murraya_paniculata.html

Orange jasmine is also known as mock orange, satin wood, honey bush. It is an evergreen shrub or occasionally a small tree, usually 2 to 3 m in height but reaching 7.5 m and 13 cm in stem diameter. Stem bark is gray, becoming fissured and rough. Orange jasmine branches and twigs are slender and abundant at all heights. The alternate leaves are pinnately compound with three to nine leaflets alternating on the rachis. The 1 to 5 cm., leaflets are dark-green, stiff, ovate, and smell of citrus when crushed. The shrub produces fragrant, five-petaled, white flowers borne in small clusters near the branch ends. Later, shiny, red elliptic fruits about 1 cm long develop. One or two light green seeds are embedded in the bitter, watery pulp. The seeds are tear-drop shaped, rounded or flattened on one side depending on whether there are one or two seeds per fruit. The native range of orange jasmine includes China, India, Sri Lanka, the Andaman Islands, Myanmar, Thailand, Cambodia, Viet Nam, Malaysia, northeastern Australia, New Caledonia, and Taiwan. It grows on most well-drained soils derived from both sedimentary and igneous rocks, although it is said to favor limestone areas. Plants survive temperatures to about –4 °C. Orange jasmine plants can live at least 15 years. Once established as an ornamental, they need little care. The species has not yet been reported to be a weed in any area. Orange jasmine flowers irregularly throughout the year. (Desert-Tropicals 2002; Gardner, 2000).

2.5 Tissue Organization of Leaves.

The tissue organization of leaves is described by Solomon *et al* (2005) as "Foliage leaves are the most variable of plant organs, so much so that plant biologists developed specific terminology to describe their shapes, margins (edges), vein patterns, and the way they attach to stems. Because each leaf is characteristic of the species on which it grows, many plants can be identified by their leaves alone. Leaves may be round, needle-like, scalelike, cylindrical, heart-shaped, fan-shaped, or thin and narrow. They vary in size from those of the raffia palm *(Raphia ruffia),* whose leaves often grow more than 20 m (65 ft) long, to those of water-meal *(Wolfia),* whose leaves are so small that 16 of them laid end-to-end measure 2.5 cm (1 in). The broad, flat portion of a leaf is the blade; the stalk that attaches the blade to the stem is the petiole. Some leaves also have stipules, which are leaflike outgrowths usually present in pairs at the base of the petiole (Figure 2.2). Some leaves do not have petioles or stipules."

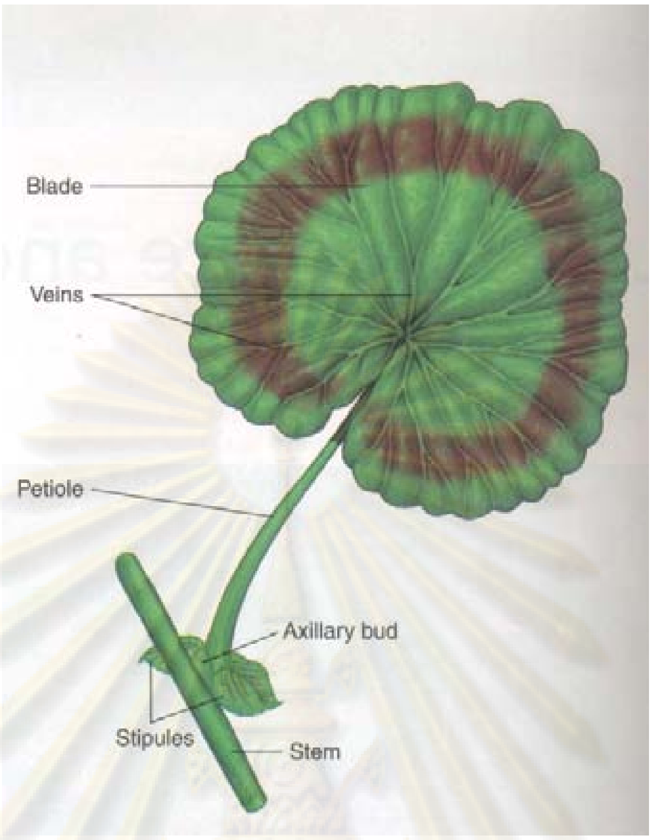


Figure 2.2: Part of leaf

Source: Solomon *et al*., 2005

Campbell *et al*., (2002) explain the leaf organization as “the leaf epidermis is composed of cells tightly interlocked like pieces of a puzzle (Figure 2.3). Like our own skin, the leaf epidermis is a first line of defense against physical damage and pathogenic organisms. Also, the waxy cuticle of the epidermis is a barrier to the loss of water from the plant. The epidermal barrier is interrupted only by the stomata, tiny pores flanked by specialized epidermal cells called guard cells. Each stoma is actually a gap between a pair of guard cells (Figure 2.4). The stomata allow gas exchange between the surrounding air and the photosynthetic cells inside the leaf. Stomata are also major avenues for the evaporative loss of water from the plant—a process called transpiration. The ground tissue of a leaf is sandwiched between the upper and lower epidermis in the region called mesophyll (from the Greek *mesos,* middle, and *phyll,* leaf). It consists mainly of parenchyma cells equipped with chloroplasts and specialized for photosynthesis. The leaves of many dicots have two distinct regions of mesophyll. On the upper part of the leaf are one or more layers of palisade parenchyma, made up of cells that are columnar in shape. Below the palisade region is the spongy parenchyma, which gets its name from the labyrinth of air spaces through which carbon dioxide and oxygen circulate around the irregularly shaped cells and up to the palisade region. The air spaces are particularly large in the vicinity of stomata, where gas exchange with the outside air occurs. The vascular tissue of a leaf is continuous with the xylem and phloem of the stem. Leaf traces, which are branches from vascular bundles in the stem, pass through petioles and into leaves. Within a leaf, veins subdivide repeatedly and branch throughout the mesophyll. This brings xylem and phloem into close contact with the photosynthetic tissue, which obtain water and minerals from the xylem and loads its sugars and other organic products into the phloem for shipment to other parts of the plant. The vascular infrastructure also functions as a skeleton that reinforces the shape of the leaf".

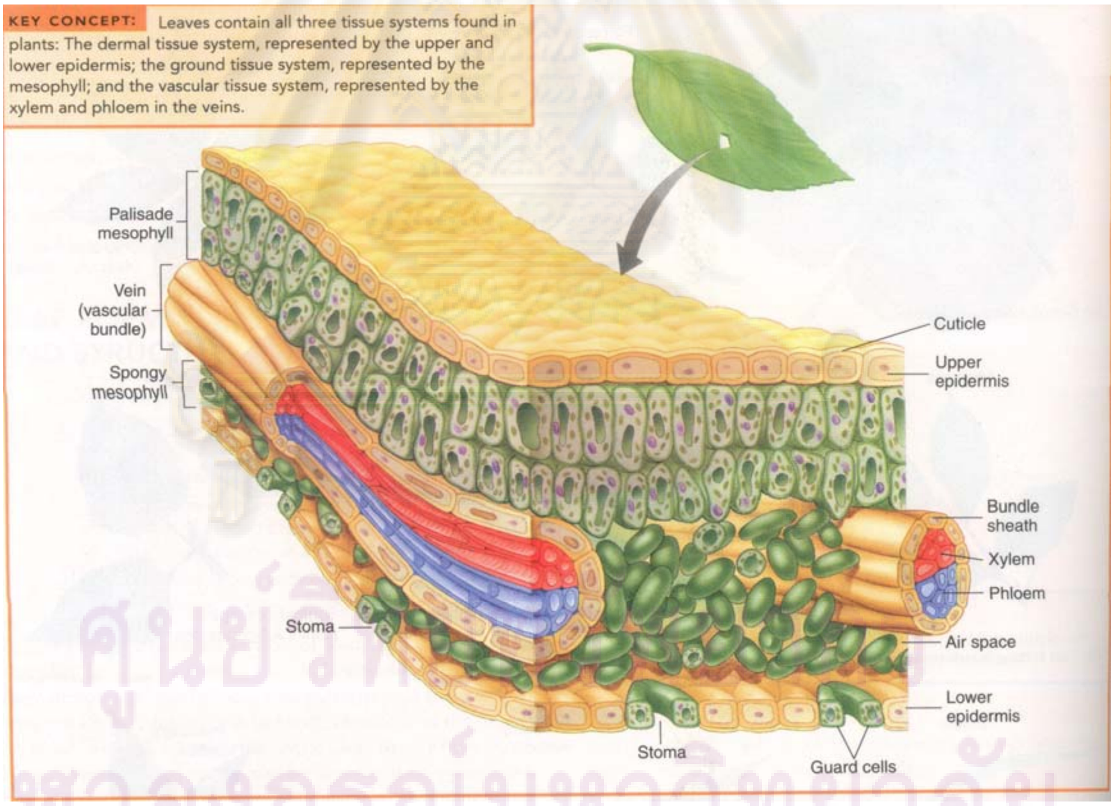


Figure 2.3: Leaf organization

Source: Solomon *et al*., 2005

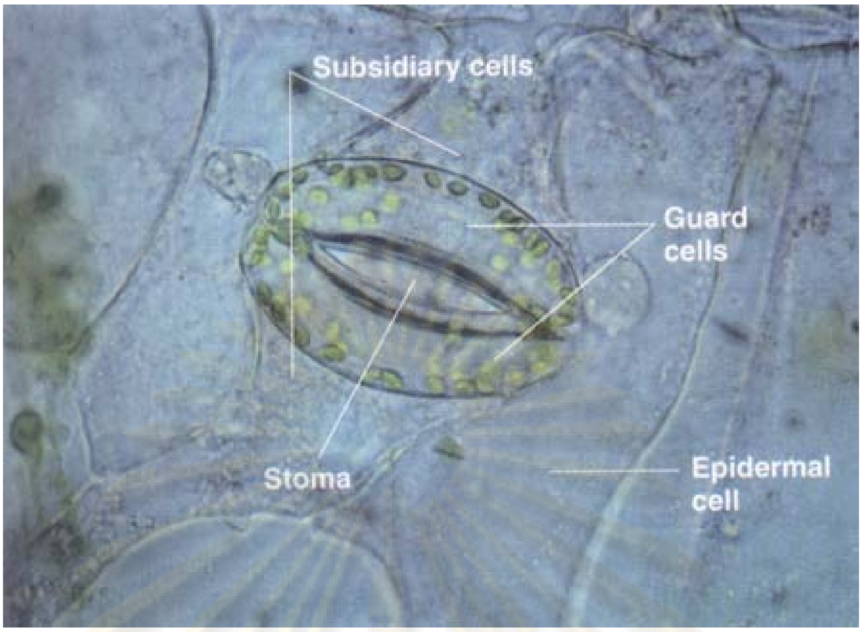


Figure 2.4: Stoma

Source: Solomon *et al*., 2005

2.6 Principal Component Analysis

Principal Component Analysis (PCA) is a multivariate statistical technique that reduces the dimensions of a data matrix with n variables by transforming the data to a new set of m variables, where m << n; the new variables are the Principal Components (PCs), and the PCs contain as much of the original variation in fewer variables. To begin PCA, the original concentrations are normalized by subtracting the mean and dividing by the standard deviation, resulting in a matrix of normalized variables, Z. The PCs are linear combinations of these original variables. In matrix form, this is expressed as PC = BZ, where the columns in B contain the coefficients for each PC. PCA calculates these coefficients.

The coefficients for the first PC are calculated so that the variance of PC 1 is maximized. In deriving the coefficients, the correlation matrix of Z, R, is used as follows: B^TRB, where B^T is the transpose of B. The use of Lagrange multipliers and differentiation results in an eigenvalue equation: $(R- \lambda_1 I)\ B = 0$, where I is the identity matrix, λ_1 is an eigenvalue of R, and B_1 is the corresponding eigenvector coefficient for PC 1. Because the maximization procedure begins with the first PC, λ_1 is the largest eigenvalue, and the subsequent eigenvalues decrease. The variance of the second PC is maximized in the same way but is subject to the restriction that PC_2 and PC_1 are uncorrelated (the covariance is zero). Accordingly, all PCs are uncorrelated, or orthogonal. The correlation matrix is used because it contains normalized data. By normalizing the data, the larger variables in the original data cannot dominate the variability. PCA gives PC*s* for the n original variables. However, the objective of PCA is to reduce the dimensions of the data such that fewer variables, m, contain as much of the original variation as possible. Several methods exist for choosing the appropriate number of PCs. Mostly, the number of PCs was chosen based on the eigenvalue of each PC: the PCs with eigenvalues greater than one were retained. If all of the entries in the original data matrix are independent, then all of the eigenvalues would be one, and the number of PCs would be the same as the number of original variables. Therefore, a PC with an eigenvalue less than one must contain less information than any of the original variables and is not worth retaining (Jolliffe, 1986).

2.7 Previous Works Review

Airborne heavy metal pollution is widely concerned in many places of the world especially in the city. It has several studies on this avenue; for example, Fernandez *et al*. (2001) studied on size distribution of metals in urban aerosols in Seville, Spain and found that potentially toxic metals, such as nickel, lead and cadmium are mainly accumulated in the smaller particles, with percentages of 72.6, 69.4 and 63.8%, respectively. Lead has a concentration of 63.7 ngm^{-3}, more than copper and manganese (26.7 and 16.5 ngm^{-3}) and above all more than nickel, cobalt and cadmium (1.97, 0.54 and 0.32 ngm^{-3}) with regard to the size distribution of metals.

. Valavanidis *et al*. (2006) studied on characterization of atmospheric particulates, particle-bound transition metals of urban air in the centre of Athens (Greece). The concentrations of trace metals adsorbed to total suspended particulate (TSP) and finer fractions of airborne particulate matter (PM) were determined from a site in the centre of Athens (Greece), which is characterized by heavy local traffic and is densely populated, during the winter and summer periods in 2003–2004. A seasonal effect was observed for the size distribution of aerosol mass, with a shift to larger fine fractions in winter. The most commonly detected trace metals in the TSP and PM fractions were Fe, Pb, Zn, Cu, Cr, V, Ni and Cd and their concentrations were similar to levels observed in heavily polluted urban areas from local traffic and other anthropogenic emissions.

It has been several researches conducted on the using of plant as bioindicators; for example, *Bauhinia blakeana* was used as a biomonitor to monitor the air quality in Hong Kong. Equations were set up to relate the ambient iron, copper, zinc and lead concentrations with those in leaves of the biomonitor and good correlations were observed. Using these equations the ambient pollutant levels in different districts of Hong Kong were determined quantitatively according to the concentrations of pollutants in leaves (Lau and Luk, 2001).

Bargagli *et al* (2003) studied on oak leaves as accumuluators of airborne elements in an area with geochemical and geothermal anomalies. In this study, leaves of the widespread oak *Quercus pubescens* and surface soils were collected from 90 sampling sites in the area and their elemental composition was compared. The results showed that the composition of oak leaves was not significantly affected by the presence of mineral deposits (metal sulphide ores) or soils with high concentrations of Cr, Mg, and Ni (ultramafic). Arsenic was the only element showing higher concentrations in leaves from sites with deposits of metal sulphide ores or As-polluted soils around abandoned smelting plants. Compared to the composition of epiphytic lichens and epigeic mosses from the same sites in the Colline Metallifere, the elemental composition of *Q. pubescens* leaves was less affected by element contributions from adsorbed soil particles.

Oliva and Valdes (2004) assessed the possibility of using *Ligustrum lucidum* Ait. F. leaves as a bioindicator of the air-quality in a Mediterranean city. In their study the concentration of 11 elements, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn and V, have been determined in leaves of *Ligistrum lucidum* Ait. f. growing in Palermo, by simultaneous inductively coupled plasma mass spectrometry. A total of 72 samples from six different sites were investigated to deduce the bioindicator ability of this species. Differences were found in the element concentrations in the leaves from the different sites and, in general, anthropogenic pollutant patterns match traffic levels. Results demonstrate that this species is useful as a bioindicator in a Mediterranean climate, especially as it has the added advantage of being an evergreen tree.

Monaci *et al*. (2000) investigated on the biomonitoring of airborne metals in urban environments of Italy. In this study, samples of *Quercus ilex* leaves and of the inhalable fraction of atmospheric particulate (PM10) were collected along a busy road and in a park in Florence (Italy). Quantitative comparisons and correlations of element concentrations in PM10 collected by air samplers at two sites showed that Ba, Cu, Fe, Mn, Pb and Zn were the main metal pollutants emitted by vehicles in Florence. Very similar results were obtained by the analysis of *Q. ilex* leaves which were found to accumulate airborne metals as a function of the exposure time (i.e. their age). One-year-old leaves showed the highest rate of metal accumulation. The results show that the progressive phasing-out of leaded petrol in Italy has resulted in a decrease of about 20% per year in the Pb concentrations in PM10. Both PM10 and *Q. ilex* analysis singled out Ba and Zn as valid tracers of automotive traffic instead of Pb.

Oliva and Rautio (2004) conducted a research to answer the question of that could ornamental plants serve as passive biomonitors in urban areas? Their study intended to search for cheap and efficient method to monitor atmospheric particulates in city centers. In their study, leaf samples of Golden dewdrop (*Duranta repens* L.) collected in Palermo (South of Italy) between 1998 and 2000 were analysed to study the possible use of this shrub as a passive biomonitor for atmospheric pollution in urban areas. Concentrations of Ba, Cd, Cu, Fe, Mg, Mn, Pb and Zn were determined from leaf samples collected from six sampling sites representing either (a) areas of high traffic density or (b) areas not directly affected by the city traffic (e.g. gardens). Most of the elements showed a significant temporal variation but no consistent trends could be seen, i.e. the highest (or lowest) values were not detected consistently at some particular time of the year. Furthermore temporal changes were of same magnitude in polluted versus less polluted areas; no statistically significant interaction between pollution level and collection period was detected. Pollution level (traffic density) was the primary factor to explain spatial variation only in the case of foliar Mg concentrations.

Tomasevic *et al*. (2004) studied on heavy metals accumulation in tree leaves from urban areas. The results show that the highest concentrations of heavy metals were found in horse chestnut leaves at Student ski Park site, amounting to 110.2, 20.3 and 4.9 m/g dry weight for Cu, Pb and Cd, respectively, which are considered above toxic levels for plants.

Caggiano *et al*. (2005) investigated heavy metals in ryegrass species versus metal concentrations in atmospheric particulate measured in an industrial area of southern italy. In the study, evaluation of the reliability of ryegrass species as active biomonitors by assessing atmospheric metal concentrations is also tested. The results reveal that statistical analysis of measured data suggests the Cd, Cr and Ni are suitable to be monitored by means of ryegrass species in the investigated site. For the other metals, their emission patterns in atmosphere make it difficult to identify the correlation structure between plants and particulate, and as a result the interpretation of the biomonitoring data is complex.

Oliva and Rautio (2005) conducted a research on the spatiotemporal patterns in foliar element concentrations in *Ficus microcarpa* L. growing in an urban area. They found that the elements showed a significant temporal variation but no consistent trends in this were present; the highest (or lowest) values were not detected at some particular time of the year. Results show that the progressive phasing-out of leaded petrol in Italy has resulted in a decrease of Pb, whereas they detected some indications to increased foliar V in the course of time. Even though some spatial patterns were seen in some of the elements, concentrations were not significantly higher in areas representing high traffic density than in samples collected from gardens. Hence, their results do not give unreserved support to use *F. microcarpa* as a biomonitor.

Tomasevic *et al*. (2005) characterized the trace metal particles deposited on some deciduous tree leaves in an urban area. This study revealed that morphological and chemical composition indicated the most abundant particles were soot and dust with minor constituents such as Pb, Zn, Ni, V, Cd, Ti, As and Cu. Using an electrochemical technique (DPASV), it was possible to measure trace metal concentrations (Pb, Cu, Zn) in a water-soluble fraction of deposits on each single leaf. Trace metal contents in the leaf deposits, increased during the vegetation period for both species and were considerably higher in *A. hippocastanum* due to different epidermal characteristics. The higher trace metal concentrations in deposits reflected greater atmospheric pollution in the Belgrade urban area.

El-Hasan *et al*. (2002) studied on Cypress tree (*Cupressus semervirens* L.) bark as an indicator for heavy metal pollution in the atmosphere of Amman City, Jordan. They found that Cypress barks were found to be a good bio-indicator for air pollution in arid regions. Variation in Pb, Zn, Mn, Cr, Ni, Cd, and Cu contents between sites was observed due to different types of activities. Traffic emissions were found to be the main source of heavy metal pollution in the atmosphere of Amman. Lead content was found to be the highest in highly traffic density areas. The industrial part of the city was characterized by high Zn, Mn, Cr, Ni, and Co contents. No significance variations were found in pH values of the bark between the sites. This was attributed to buffering effect of carbonate in the atmosphere originated from soil of the area.

Valerio *et al*. (1989) investigated the metals in leaves as indicators of atmospheric pollution in urban areas. They found the concentration of lead, chromium, copper, nickel and manganese in "*Quercus Ilex*" leaves may be used to classify urban areas according to the level of their atmospheric pollution. Particular emphasis was placed on reproducibility of sampling and the analytical method. Preliminary results show a linear correlation between concentration of metals in atmosphere (particularly lead) and in leaves collected in the same sampling areas.

Lorenzini *et al*. (2006) studied on the leaves of *Pittosporum tobira* as indicators of airborne trace element and PM10 distribution in central Italy. Leaves of the evergreen ornamental shrub species *Pittosporum tobira* were used as a passive monitor to describe the distribution of selected elements in three coastal cities of central Italy (namely Livorno, Rosignano Marittimo and Piombino) differing for number of inhabitants and economical activities. Factor analysis allowed to identify three main source groups of elements, namely crustal components, sea-salt spray and anthropogenic sources (vehicular traffic, industrial activities). SEM–EDX analyses were performed on 556 PM10 samples collected from the 88 sampling sites. High contributions of geological elements and marine aerosols were detected. Pb is no longer of environmental concern in the area.

Caselles *et al*. (2002) studied on the evaluation of trace element pollution from vehicle emissions in petunia plants. Effects of environmental pollution from road traffic on trace element accumulation and deposition were examined in washed and unwashed petunia leaves. The plants were grown in an urban and in a suburban area. Substantial amounts of elements were removed simply by washing with demineralised water, which removed at least 45% of Al, Fe and Pb and 15% of Mn, Cu and Zn in urban areas, where the aerial deposition took place. Throughout the growing season, the concentrations of Fe, Cu, Al, Ni and Pb increased in the washed leaves of the petunia plants grown in urban areas. However, the plant very specifically controlled the contents of Mn and Zn. Concentrations of elements were significantly higher in washed leaves from the urban area than those from the surburban area, indicating that this ornamental plant is able to absorb Fe, Al, Ni and Pb through its root and leaves. Periodic assessment of the accumulation of trace elements in urban areas with intense traffic is important in order to evaluate the rate of environmental pollution.

Al-Shayeb *et al*. (1995) studied on the date palm (*Phoenix dactylifera* L.) as a biomonitor of lead and other elements in arid environments. The metal content (Pb, Zn, Cu, Ni, Cr and Li) was determined for washed and unwashed leaflets collected from a wide range of sites with different degrees of metal pollution (urban, suburban, highway and industrial areas) and from a rural (control) site. Differences between washed and unwashed samples revealed that metal pollutants exist as superficial contaminants, especially levels of Pb and Zn, which varied according to the metal source. However, *P. ductylifera* leaflets were found to be suitable biomonitors for metal pollution in Riyadh and similar arid and semi-arid environments.

Kakulu (2003) studied on trace metal concentration in roadside surface soil and tree bark in abuja, Nigeria and found that elevated concentrations of some of cadmium, copper, lead, nickel and zinc were observed in the soil and tree bark samples from the commercial/high traffic areas of the city compared to background values. In soil samples, the average concentration of the metals were 0.6±0.4, 18.0±4.0, 281±39, 16±4 and 66±23 μg/g dry weight for Cd, Cu, Pb, Ni and Zn, respectively, whilst the average concentrations in tree bark were 0.3±0.2, 12±4, 133±32, 13±3 and 61±10 μg/g dry weight for Cd, Cu, Pb, Ni and Zn, respectively. The trend in trace metal levels suggested that automobile emissions are a major source of these metals as the highest concentrations of Pb and Zn were recorded in the commercial areas of the city known for their high traffic densities. The levels of metal in the study area were relatively low compared to levels found in some larger and older cities in various countries worldwide.

Al-Khlaifat and Al-Khashman (2007) researched on Atmospheric heavy metal pollution in Aqaba city, Jordan, using *Phoenix dactylifera* L. leaves. This study investigated the concentrations of iron (Fe), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), and chromium (Cr) using a flame atomic absorption spectrophotometer. Samples of unwashed leaves for testing were collected from different locations with different degrees of metal pollution (urban, suburban, industrial, highway, and rural sites). Separate leaves were taken from outside the city to be used as a control sample. Samples collected from industrial areas were found to have high contents of all metals except for nickel, copper, and lead, which were found at high concentrations in the samples collected from highway sites. Significant correlations between the heavy metal concentrations in date palm trees in unwashed leave samples were obtained. The principle component analysis (PCA) along with correlation analysis provides significant information about the origin of heavy metals in palm tree samples.

CHAPTER III

METHODOLOGY

3.1 Sampling

Samples of airborne particles smaller than 10^{-6} m. in diameter length (PM10), topsoil samples (0-10 cm. from surface soil) and leaf samples were collected at three different sites in the central area of Bangkok (Figure3.1). The first sampling site was at the Department of Land Transportation (DLT) which is located close to heavily traveled road. This site is opposite the Chatuchak Park which is one of the most popular parks in Bangkok, and the Chatuchak weekend market which is the biggest open-air market in Thailand. The area is very crowded with people and all types of vehicles especially on Saturday and Sunday. This site is also close to the biggest bus terminal in Thailand— Mor-Chit (Northeastern route bus terminal) which is crowded with all types of vehicles all the time. The second sampling site was at the Chulalongkorn Hospital (CHU) which is close to Silom, the central business area of Bangkok, and is opposite to the Lumpini Park, the oldest city park in the country. This site is located in a commercial area with high traffic on the working day. The third site was at the Ministry of Science and Technology (MST). The areas around site are government offices, a huge hospital, and a university campus. This area is crowded with people and vehicles, especially on the working day. The main road in front of the sampling site is covered by the elevated expressway and high buildings around the road. The fourth site, a relatively clean area considered as a reference site, was the Bangkok University, Rangsit campus, located in Pathumthani province approximately 40 km. north of Bangkok. The reference area is a control site and far from the traffic and major anthropogenic activities.

Figure 3.1: Location map of three sampling sites in Bangkok: DLT (Department of Transportation), MST (Ministry of Science and Technology), CHU (Chulalongkorn Hospital), and Pathumthani (Reference site at Pathumthani province).

Samples of old and young leaves in similar sizes of Orange jasmine as shown in Figure 3.2 were collected simultaneously in three sampling sites and one reference site from September to October 2006 and from December 2006 to January 2007. The tree species, Orange jasmine *(Murraya paniculata)* was selected to evaluate the level of trace elements accumulation for a number of reasons. It is widespread in any areas of Bangkok and it is one of the most frequent tree species found in Thailand. Furthermore, it has shown in the first rough review to be a possible useful bioindicator of airborne trace elements. Because of its long-lived leaves, the easy identification, and its widespread occurrence in urban areas and natural ecosystems make this species adequate tool for trace elements bioindicator studies.

The sampling was randomly carried out from two to three trees at each site. In each site, fully developed leaf samples were obtained by removing leaves from healthy-looking plants at the end of branch. Fully developed leaves from four directions were detached from the tree at 1.2-1.5 m. above the ground. The leaf samples were separated into two groups according to their age (old and young leaves).

The criteria for judging the age of leaf is based on the leaf color not sizes. The separation between old and young leaves is considered by the obvious different color with the same sizes of old and young leaves (dark green and pale green) as shown in Figure 3.2. The topsoil samples (0-10 cm. in depth from the surface) nearby the tree samples were also collected using the garden spade. The soil samples were collected at least four points around the area and then mixed into one to provide a composite sample.

Figure 3.2: Old and young Orange jasmine (*Murraya paniculata*) leaves

During the sample period, the airborne particulate samples were collected simultaneously leaf and soil samples with a standard high-volume sampler equipped with a PM10 inlet (as shown in Figure 3.3) followed by the Pollution Control Department (PCD) standard procedure. The airborne particulate samples were eventually obtained once a week at the same period of plant and soil sampling. Sampling periods for airborne particles were 24-hour sampling. Before analysis of airborne samples, filters used for particle collection were dried, before and after weighing, in a humidity control chamber (Figure 3.4).

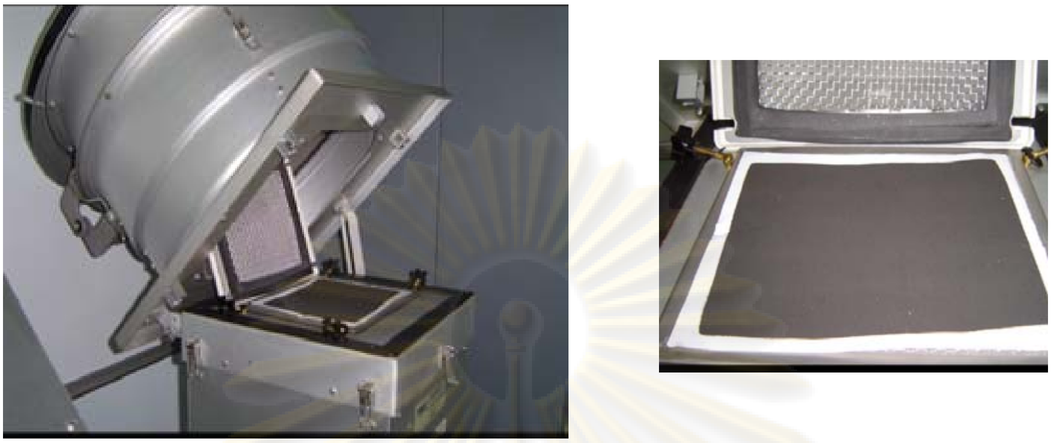


Figure 3.3: The high volume air sample equipped with PM10 air inlet and filter holder

Figure 3.4: The filter storage chamber

3.2 Analysis

Upon collection, soils and leaves were placed in plastic bags and transported to the laboratory where they were stored at 4 ^{o}C for the next step of elemental analysis preparation. When old and young sampling leaves at each sampling sites were taken to the laboratory, each of leaves were separated into two groups for further treatment. The first group was unwashed leaves without washing to quantify the total deposition. The rest was washed leaves which were first washed by the running tap water and then washed thoroughly with distilled water at least three times to remove dust particles from leaf surfaces.

Then samples were dried at 105 ^{o}C in an oven to constant weight and then ground with a blender to a size of less than 1.0 mm for leaf samples. The soil samples were sieved through a 212 μm-mesh sieve. The crushed dried samples were stored in a labeled plastic bag for further chemical analysis. Approximately one gram of each dried sample was digested with 20 ml. of 65% Nitric acid (HNO_3), and dilute to 50 ml. with deionized water (Sawidis *et al*., 1995; Gjengedal and Steinnes, 1994; Voutsa, *et al*., 1996; Sriyaraj and Shutes, 2001). A known portion of the exposed filter paper sample was taken and digested in 30 ml 65% pure nitric acid and then diluted to 50 ml with distilled-deionized water (Fang, 2004). The concentrations of Cu, Fe, Pb, Mn, Ni, Cr, and Zn in all samples were determined by using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) HORIBA model type ULTIMA2C as shown in Figure 3.5. Analytical quality was checked with the Certified Reference Material BCR-038 Fly ash from pulverised coal and BCR-100 Beech leaves.

Parts of the filter and leaf in both adaxial (upper) and abaxial (lower) surfaces were mounted on aluminium stubs and analyzed the physical and chemical characteristics of particles deposited on the leaves with a scanning electron microscope (SEM) JEOL model type JSM 6480LV. The microanalysis was carried out by an Energy-Dispersive X-ray (EDX) system (Figure 3.6).

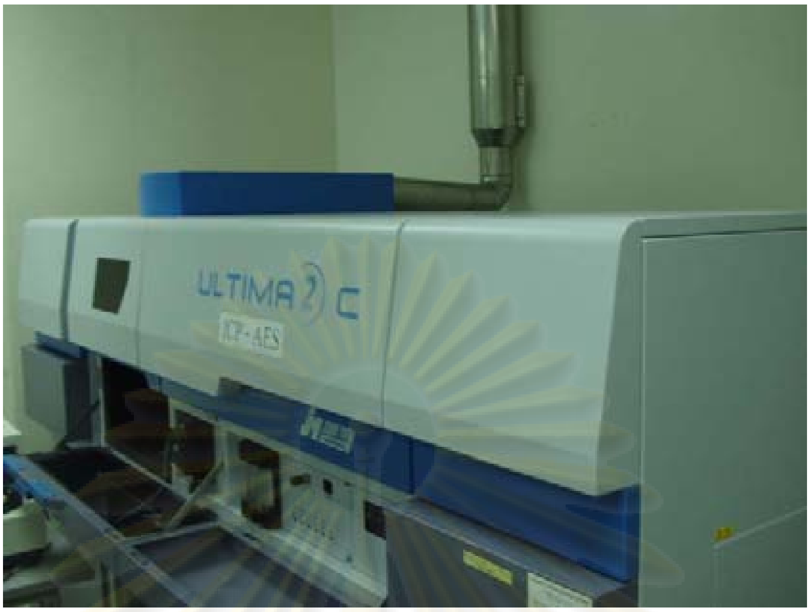


Figure 3.5: Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

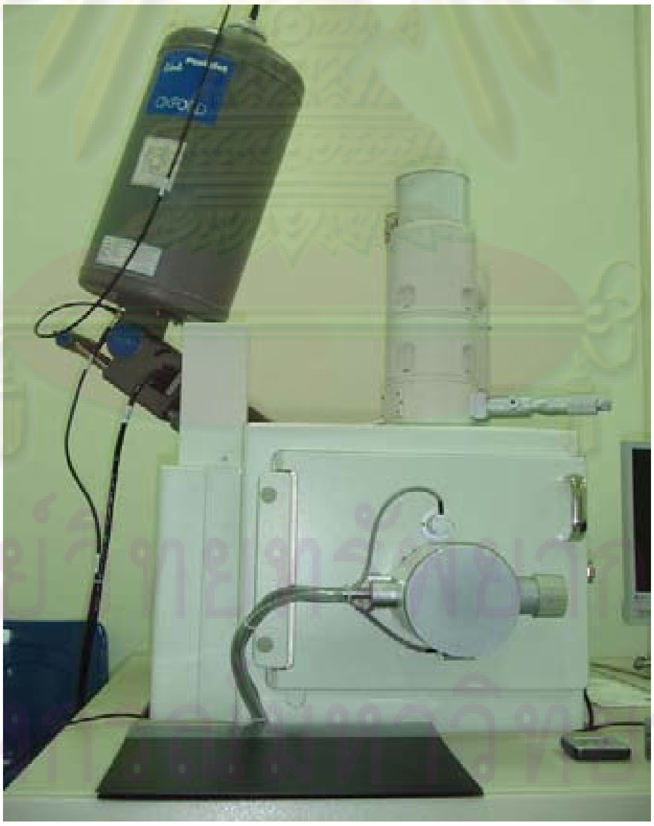


Figure 3.6: Scanning Electron Microscope (SEM)

3.3 Statistics

Statistical analyses in the study were conducted using SPSS software package. Relationships of metal concentrations between leaves – soil, leaves – PM10, and PM10 – soil were examined using Pearson's correlation analysis. Summary statistics were used to calculate the mean values and standard deviation of metal concentration in each matrix. Analysis of Variance (ANOVA) was used to detect significant differences between sites. The significant differences of metal concentrations between young – old leaves, washed – unwashed leaves, and dry – wet seasons were evaluated by independent t-test.

Enrichment factor (EF) was used in the obtained data, the metallic concentrations in order to provide an indication on the extent of the contribution of anthropogenic emissions to atmospheric elemental levels. The enrichment factor (EF) has been calculated for each element, using Fe as reference element and the crustal composition (Taylor, 1964). By convention, the average elemental concentration of the natural crust is used instead of the continental crust composition of the specific area, as detailed data for different areas are not easily available. The enrichment factor EF of an element E in total deposition (TD) relative to crustal reference material R is defined as:

$$EF = \frac{[E]_{TD} / [R]_{TD}}{[E]_{crust} / [R]_{crust}}$$

If the EF approaches unity, the crustal material is likely the predominant source for element; if the EF is higher than 10, the element has a significant fraction contributed by non-crustal sources (anthropogenic) (Tomasevic, *et al*., 2004).

In addition, elemental composition of samples were subjected to varimax rotated Principal Component Analysis (PCA), which is a multivariate statistical treatment frequently widely used in many atmospheric pollution researches to obtain the information on pollution sources. PCA separates a given set of elements into groups or factors based on their fluctuation and common variance, provided that each association of chemical species found is related to an identifiable source type.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The accuracy of the determinations

The accuracy of the determinations was previously checked by analysis of a BCR reference material. BCR is provided by Institute for Reference Materials and Measurements (IRMM) and is a registered trademark of the supported production by research funding of the European Commission (EC). A reference material BCR-038 Fly ash from pulverised coal and BCR-100 Beech leaves were used in the study. Table 4.1 shows the results of the certified reference materials analyses. These data show that the recovery range was from 78.7 % to 121.1 %, which proved the validity of the methodology used, but only for the elements measured in the reference materials.

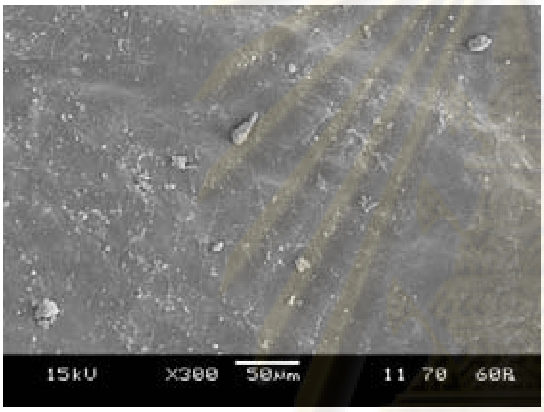
Table 4.1: Measured and certified element concentrations (mg/kg) in reference materials

Element	Certified value (mg/kg)	Experimental value (mg/kg)	Recovery (%)
BCR-038 Fly ash from pulverised coal			
Cr	192 ± 10	199.8 ± 48.7	104.1
Cu	176 ± 9	162.4 ± 20.3	92.3
Fe	33800 ± 700	34264.0 ± 1481.5	101.4
Mn	479 ± 16	579.9 ± 65.4	121.1
Ni	(194)	172.9 ± 19.6	89.1
Pb	262 ± 11	210.8 ± 18.4	80.5
Zn	581 ± 29	493.7 ± 35.0	85.0
BCR-100 Beech leaves			
Cr	8 ± 0.6	6.57 ± 0.36	82.1
Cu	(12)	9.44 ± 1.66	78.7
Fe	(550)	445.30 ± 11.96	81.0
Mn	(1300)	1280.28 ± 276.20	98.5
Ni	-	-	-
Pb	(16.3)	14.37 ± 1.99	88.2
Zn	(69)	64.35 ± 8.65	93.3

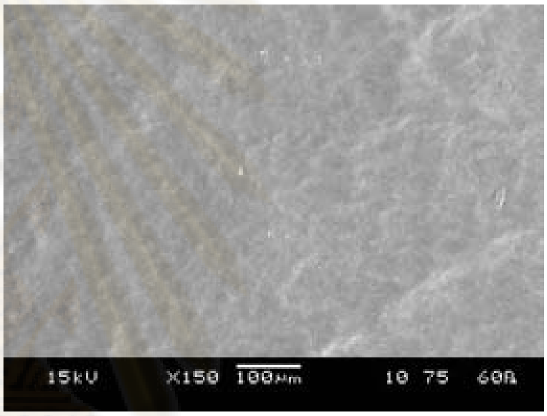
Value in bracket is not certified

4.2 SEM investigations

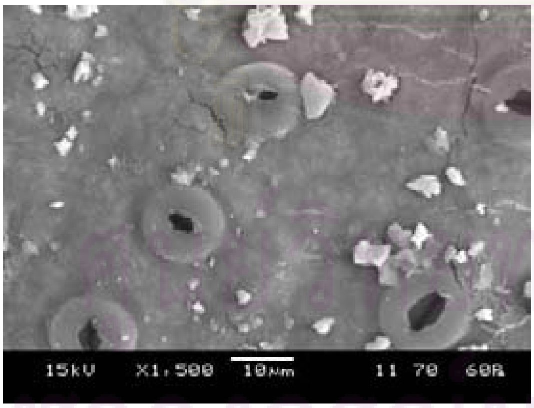
In this study, the pictures of adaxial (upper) and abaxial (lower) surfaces of unwashed and washed Orange jasmine leaves taken from Scanning electron microscope are presented in Figure 4.1. Approximately, 5-10 % of the leaf surface was covered with deposited particles. Particles are presented in a higher density on the adaxial leaf surfaces than abaxial. The particles are heterogeneously distributed and have many kinds of shapes and forms. There are presented in a wide range of diameters up from fine (less than 10 µm) to coarse (greater than 10 µm) particles.



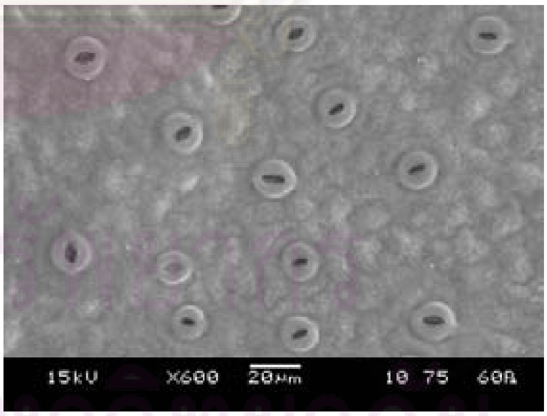
(a) Unwashed upper surface leaf



(b) Washed upper surface leaf



(c) Unwashed lower surface leaf



(d) Washed lower surface leaf

Figure 4.1: Scanning electron micrographs of particles deposited on the adaxial (a) and abaxial (c) leaf surfaces of unwashed Orange jasmine comparing with the adaxial (b) and abaxial (d) leaf surfaces of washed Orange jasmine

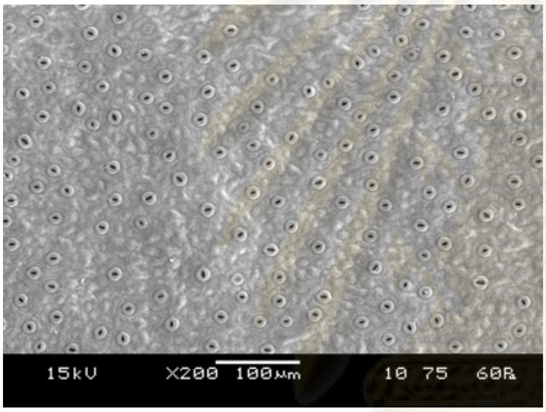
The particles with a diameter less than 2 μm (fine particles) are mainly originating from anthropogenic activities (Tomasevic *et al*., 2005). The particles can be divided into two main particle categories: particles of natural sources include materials of organic origin such as pollen, bacteria, fungal spores etc. This category also includes suspended soil dust (primarily soil mineral) such as the angular-shaped material. Particles from anthropogenic sources mostly emitted from high temperature combustion processes are characterized by their spherical shapes and smooth surfaces. This type of particles occur as individual particles but also in aggregate form as agglomerates of similar-sized particles and individual large particles caring several smaller attached particles (Tomasevic *et al*., 2005).

Fine particles are also observed around the stomata in the study. The diameter length of the opening stomata is approximately 10 μm. The stomata are the minute openings in the epidermis of a plant (as a leaf) through which gaseous interchange takes place. The stomata of plants are commonly found on the lower surface of leaf (abaxial). Thus, they have no signs of stomata on the upper surface (adaxial) of Orange jasmine leaf in the study. Because the length of diameter of stomata is wide enough, it may possibly be entered by the smaller than 10 μm particles via respiration process. Plants take oxygen and other gases through the stomata. Figure 4.1 (c) illustrated the fine particle at the entrance of opening stomata. Figure 4.1 is also showed the effects of washing leaf on particles deposition. The amounts of particles deposited on the both upper and lower surfaces of washed leaves significantly decrease comparing with the unwashed leaves.

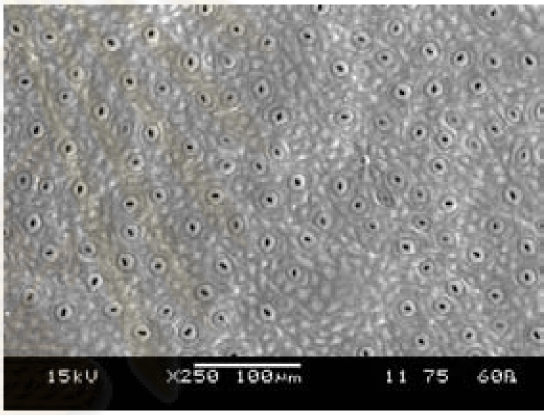
Figure 4.2 illustrated the characteristics of lower surface (abaxial) of old leaf and young leaf, and also exhibited the forms and sizes of stomata of old and young leaves. It can be said that there are not different in numbers, forms and sizes of old and young leaf.

The chemical compositions of the three spot particles deposited on both surface sides suggested that the most abundant elements on lower surface leaf are Ca, O and S (Figure 4.3). Al and Si are also found in the particles. These elements are main composition of soil dust. Therefore, the abundant chemical compositions of the three spot particles on upper surface leaf are Ca and O (Figure 4.4 and 4.5). The other elements, Al, Si, Na and Mg are also found in the particles as shown in Figure 4.4. The elements show the possibility sources of particles from soil dust and sea salt. Besides, the micro element, Cu found in the particle as shown in Figure 4.5 (c) indicates the possible source of particle from vehicle emission. All these findings may imply that the possible sources of the particles found on upper side of the young leaf the old leaf are quite similar.

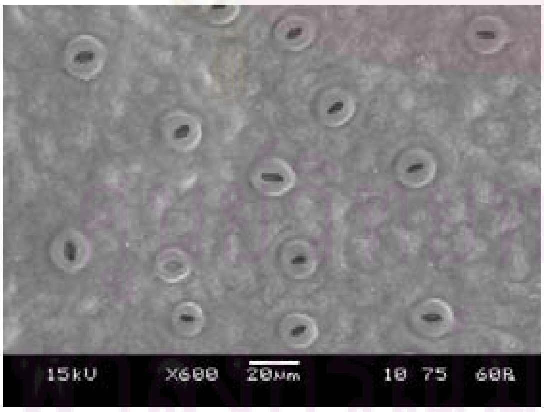
The picture of particles deposited on the filter is also exhibited in Figure 4.6. The enriched elements found in the particle are Si, O and Al, which are main components of soil. The Ca, Fe, and Na are also found in the particles which indicate the soil and sea salt sources. The results of elements found on leaf and filter indicate the possibility of the same origin sources of the particles.



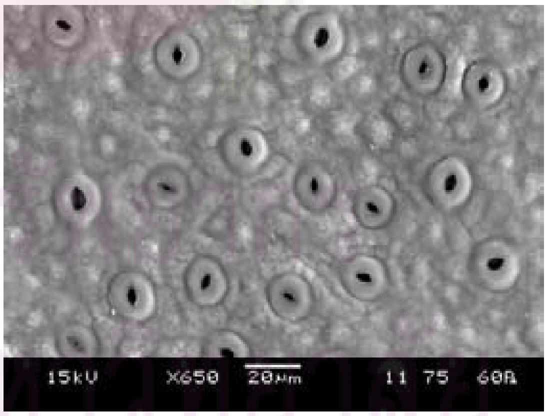
(a) Old lower surface leaf



(b) Young lower surface leaf

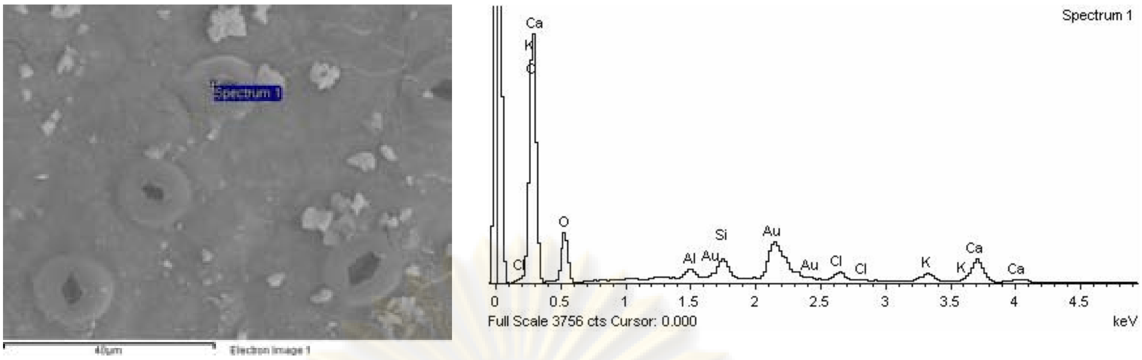


(c) Stomata of old leaf

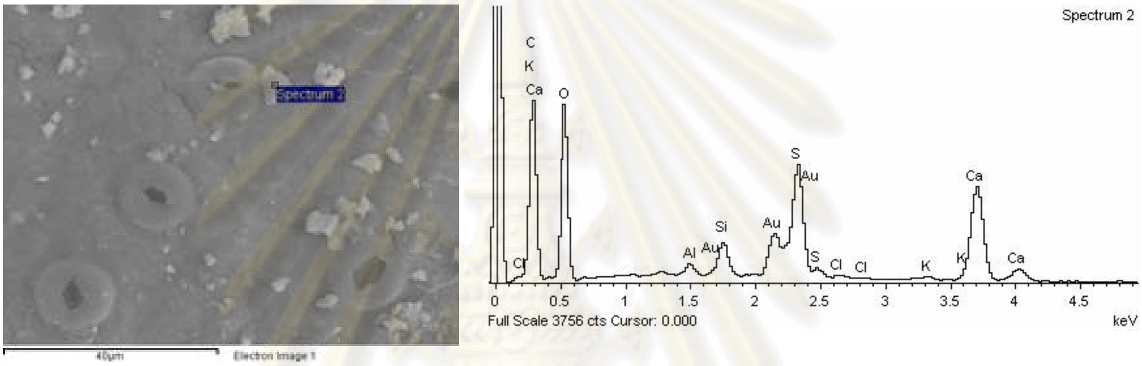


(d) Stomata of young leaf

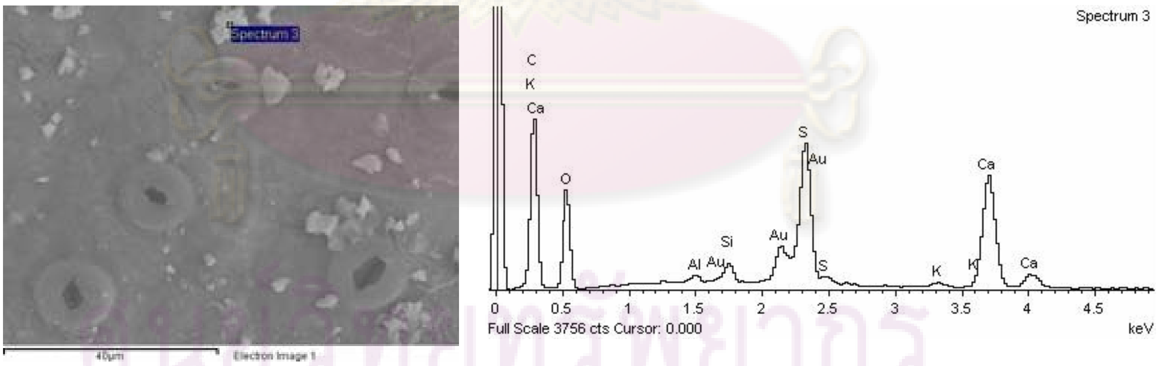
Figure 4.2 Characteristics of lower surface (abaxial) of old leaf (a) and young leaf (b), and size of stomata of old leaf (b) and young leaf (d)



(a)

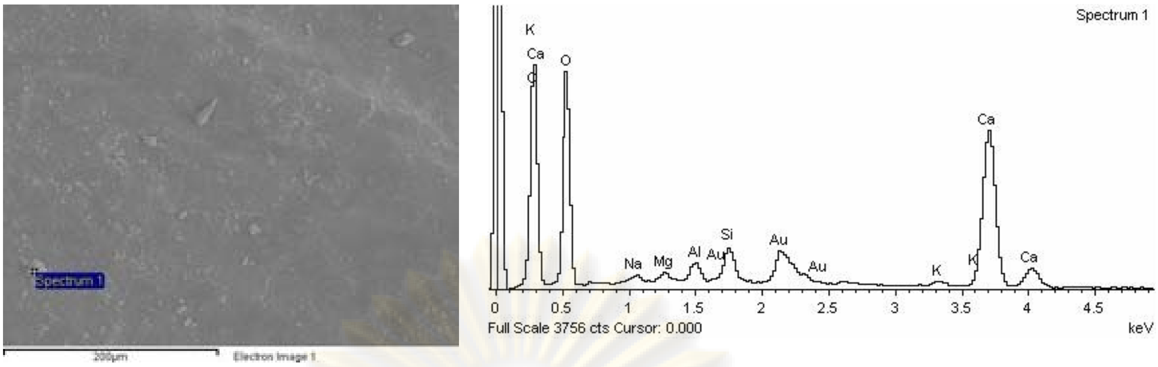


(b)

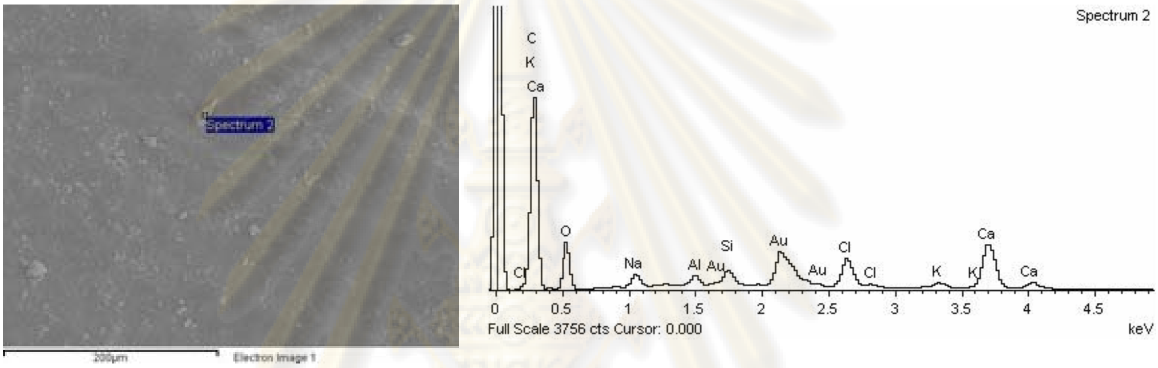


(c)

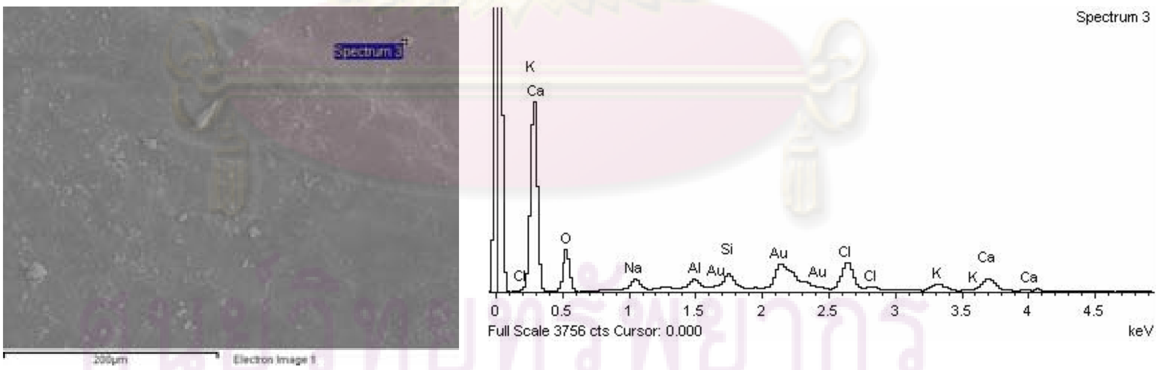
Figure 4.3: Particles deposited on lower surface (abaxial) and the X-ray spectrum



(a)

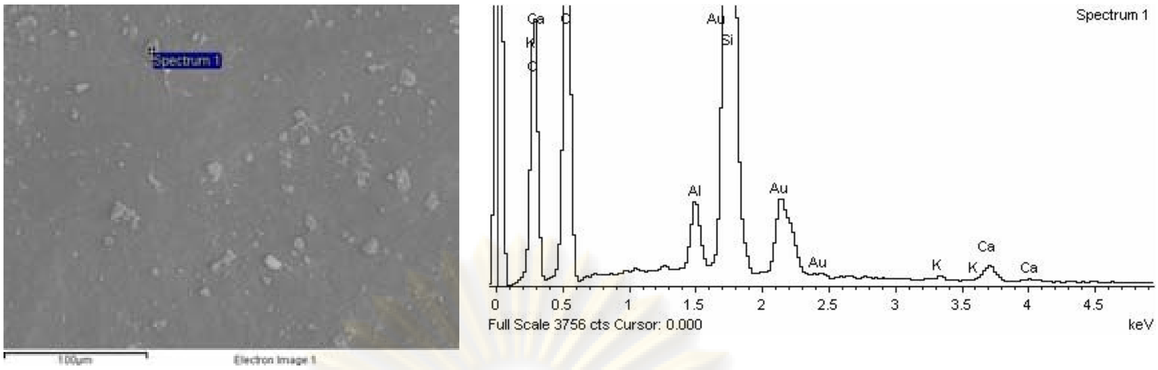


(b)

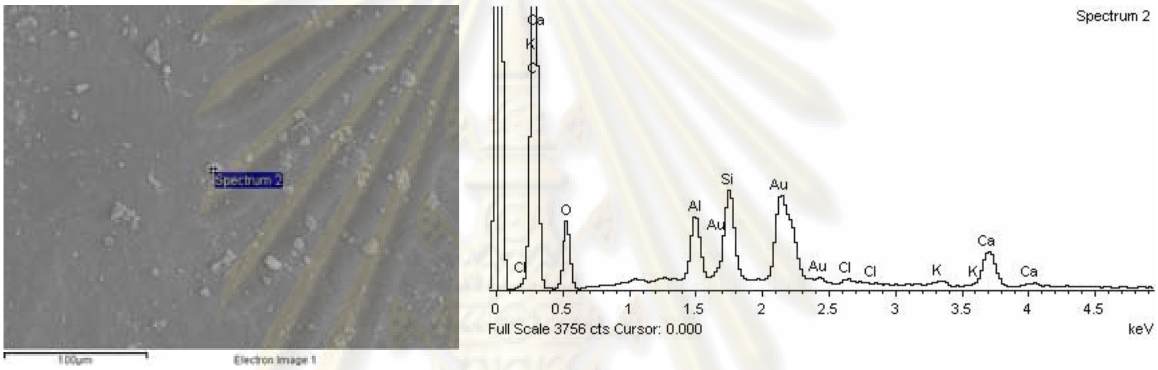


(c)

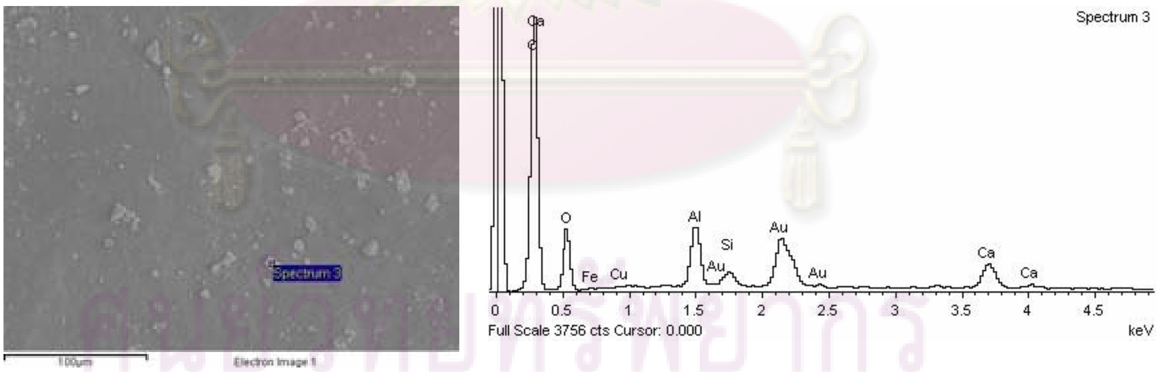
Figure 4.4: Particles deposited on upper surface (adaxial) of old leaf and the X-ray spectrum



(a)



(b)



(c)

Figure 4.5: Particles deposited on upper surface (adaxial) of young leaf and the X-ray spectrum

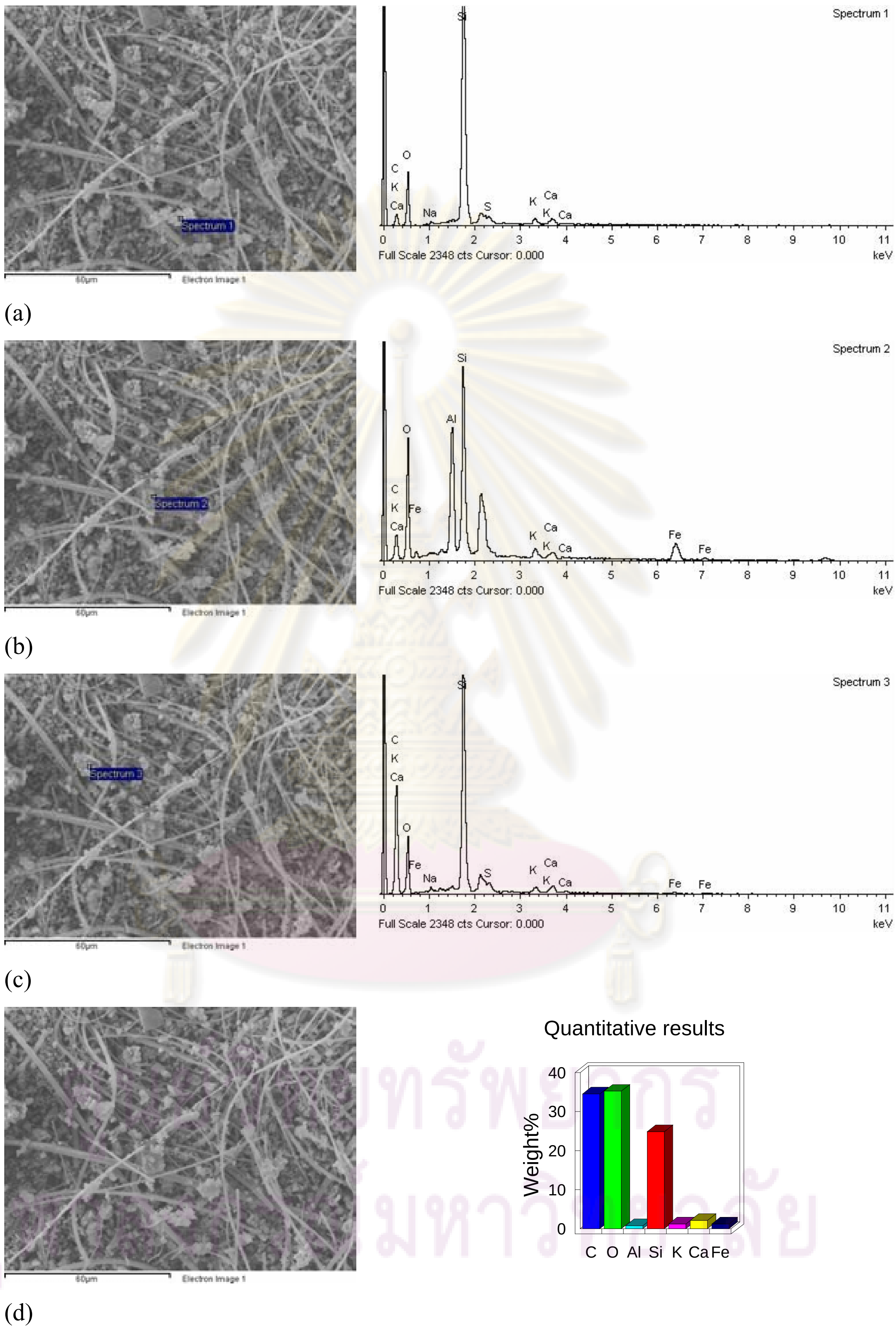


Figure 4.6: Particles deposited on the filter and the X-ray spectrum

4.3 Elemental concentrations

The elemental contents in PM10, soil, washed leaf, and unwashed leaf samples are shown in Table 4.2 and Figure 4.7. Heavy metal concentrations in the PM10 samples from the three sampling sites in addition to the reference samples indicate that they may not be significantly different between sites. Surprisingly, the Cu and Zn are both found at reference sites in higher amounts than other sites. The Cu and Zn can be from many sources like vehicular emissions, oil and waste combustion, incinerators, coal burning in electricity generation plants (Fang *et al*., 2006; Manoli *et al*., 2002; Wang *et al*., 2006). The area of reference site is located at Pathumthani province which surrounded by green university campus and rice field, and is away from factories and traffic activities. However, there is other source of Cu and Zn that may cause the high level of both metals in the site. The previous study revealed that biomass burning emissions contributed to more than 90% of the measured concentrations of P, Cl, S, K, Cu and Zn (Gaudichet *et al*., 1995). Farmers in the area around the sampling site tend to burn their organic waste like straw, grass etc. after harvesting. This can be the explanation of why the high level of Cu and Zn are found in the reference site.

According to Table 4.2 and Figure 4.7 (b), the most abundant heavy metals in soil are found at MST sampling site which is heavy traffic area of Bangkok. The results indicate that all heavy metals may be higher in the dense and heavy traffic areas than the reference area. Fe is the highest metal concentration found in all sites while Cr is the lowest. At the sampling site DLT, CHU, MST and reference site, the mean metallic concentrations are in the following order: Fe (8203.86) > Mn (311.79) > Zn (202.74) > Cu (19.61) > Ni (18.55) > Pb (13.79) > Cr (6.75), Fe (7175.40) > Mn (216.99) > Zn (152.02) > Pb (26.10) > Ni (16.63) > Cu (15.06) > Cr (6.75), Fe (14096.79) > Zn (249.18) > Mn (241.75) > Cu (79.48) > Pb (62.13) > Cr (18.73) > Ni (14.54), Fe (7236.38) > Mn (168.29) > Zn (40.91) > Ni (15.37) > Pb (12.41) > Cr (3.97) > Cu (3.91) in mg/kg dry weight, respectively. The comparison of each metallic element at all sites reveals that the highest concentrations of elements: Cr, Cu, Fe, Pb and Zn are found at MST site, which is the heavy traffic area.

Table 4.2: Mean concentrations and standard deviation of PM10 samples (ng/m^3), Soil samples (mg/kg dry weight), Unwashed leaf samples (mg/kg dry weight), and Washed leaf samples (mg/kg dry weight), at sampling sites.

PM10 (ng/m^3)

Sites	Conc.	Cr	Cu	Fe	Mn	Ni	Pb	Zn
DLT	Mean	13.3	260.2	1308.3	33.6	266.9	59.5	221.2
	SD	3.2	58.4	578.8	29.1	328.0	45.0	83.7
CHU	Mean	14.8	123.2	1448.0	39.6	286.6	89.9	279.6
	SD	4.5	46.0	460.4	20.3	307.2	65.2	89.2
MST	Mean	14.0	555.0	1440.9	43.5	284.1	66.7	273.1
	SD	4.6	211.0	696.8	33.2	482.4	46.0	91.6
Reference	Mean	13.7	571.4	909.1	30.9	250.7	82.8	400.2
	SD	10.3	123.3	297.2	12.6	286.8	45.9	306.4

Soil (mg/kg dry weight)

Sites	Conc.	Cr	Cu	Fe	Mn	Ni	Pb	Zn
DLT	Mean	6.75	19.61	8203.86	311.79	18.55	13.79	202.74
	SD	2.32	6.75	1084.84	79.34	10.40	3.12	73.71
CHU	Mean	6.75	15.06	7175.40	216.99	16.63	26.10	152.02
	SD	0.91	3.02	2360.63	44.65	16.86	8.11	87.07
MST	Mean	18.73	79.48	14096.79	241.75	14.54	62.13	249.18
	SD	3.40	15.52	2196.47	33.10	2.25	8.96	29.99
Reference	Mean	3.97	3.91	7236.38	168.29	15.37	12.41	40.91
	SD	1.95	1.79	846.50	58.50	11.84	3.20	7.06

Unwashed leaves (mg/kg dry weight)

Sites	Conc.	Cr	Cu	Fe	Mn	Ni	Pb	Zn
DLT	Mean		11.80	133.88	52.00		2.11	30.29
	SD		3.45	27.69	13.73		0.77	9.64
CHU	Mean		9.88	142.94	16.06		1.96	20.65
	SD		0.97	31.80	3.70		0.57	2.97
MST	Mean		10.97	324.80	24.73		1.85	29.92
	SD		2.84	147.97	5.67		0.54	6.15
Reference	Mean		6.76	157.12	14.05		1.82	20.67
	SD		0.96	37.02	1.88		0.56	3.64

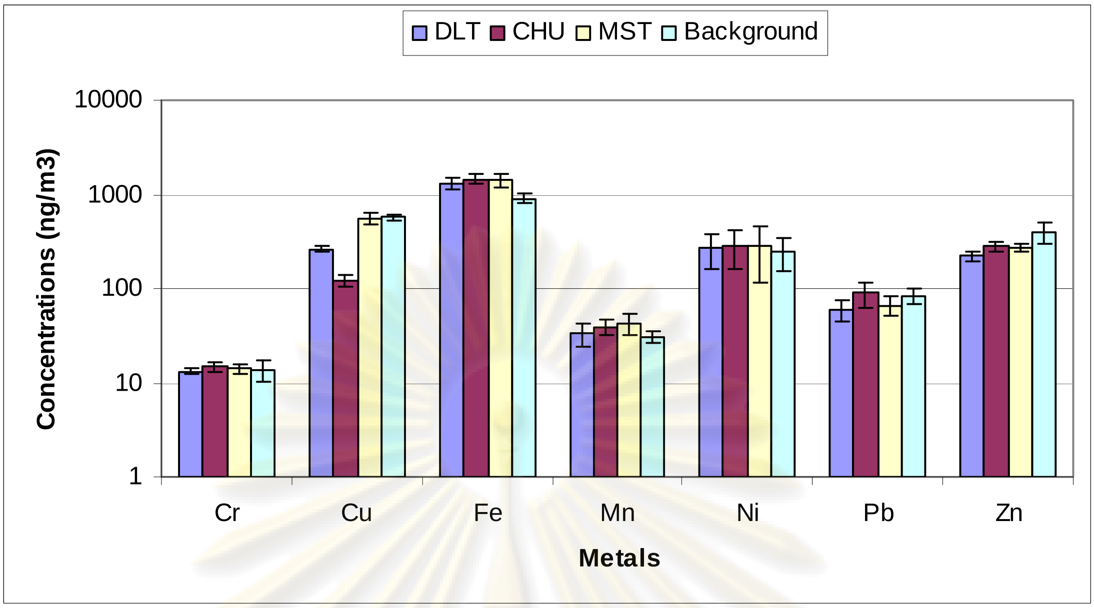
Washed leaves (mg/kg dry weight)

Sites	Conc.	Cr	Cu	Fe	Mn	Ni	Pb	Zn
DLT	Mean		8.69	44.22	38.54			20.12
	SD		2.18	10.93	12.99			5.98
CHU	Mean		7.70	80.30	12.19			16.05
	SD		0.92	28.26	2.95			2.14
MST	Mean		7.42	162.89	17.39			24.04
	SD		1.78	34.55	2.72			2.62
Reference	Mean		4.59	77.30	11.35			15.78
	SD		2.22	13.87	1.46			1.93

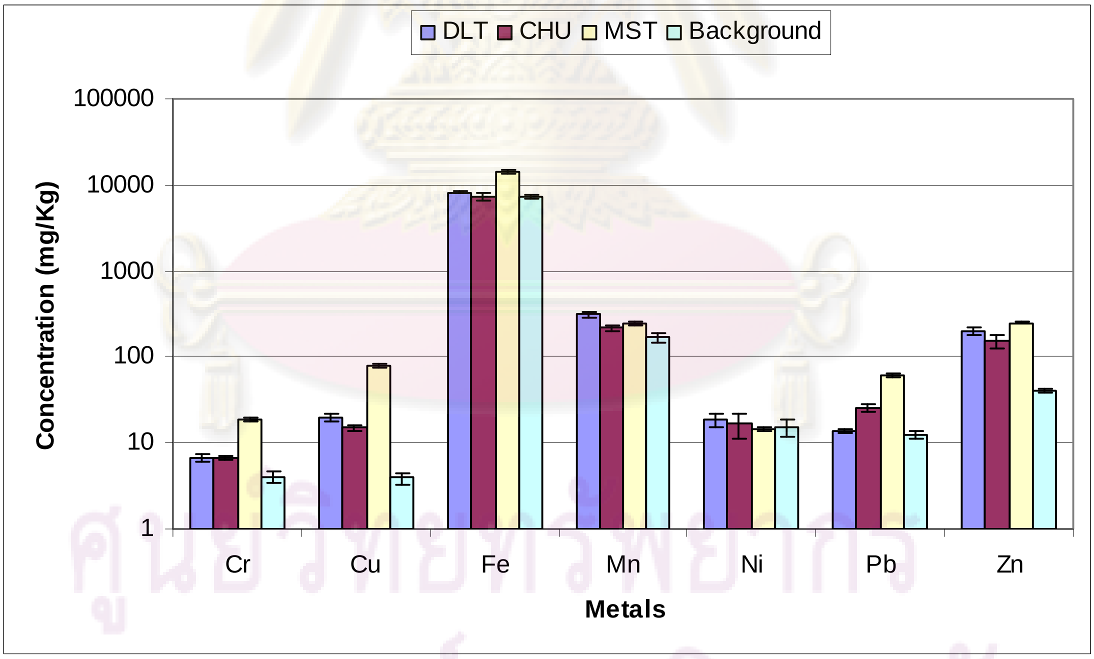
All sites average concentrations of four metals in soil in the present study are 29.51, 234.71, 28.61, and 161.21 mg/kg for the Cu, Mn, Pb and Zn, respectively whereas the previous metals in soil study conducted in Bangkok showed the metal concentrations of 41.7, 340, 47.8, and 118 mg/kg for the Cu, Mn, Pb and Zn (Wilcke *et al*., 1998). All metals found in the present study are noticeably lower than the previous report except for the Zn.

Heavy metal concentrations in leaf samples in both washed and unwashed conditions from the three sampling sites and the reference site are also presented in Table 4.2 and Figure 4.7 (c) and (d). Only five elements, the Cu, Fe, Mn, Pb and Zn in unwashed samples could be detected by ICP-AES, while only the Cu, Fe, Mn, and Zn in washed samples could be detected. The amounts of the Cr, Ni and Pb found in the study are lower than the detection limit of analytical instrument. The results, as shown in Figure 4.7 (c) and (d), indicate that all heavy metals are higher in sampling sites than the reference. The DLT and MST sites are found the highest metal levels in both types of leaves. Fe is again the highest metal concentration found in all sites while Pb is the lowest. At the sampling site DLT, CHU, MST and reference site, the mean metallic concentrations of washed samples are in the following order: Fe (44.22) > Mn (38.54) > Zn (20.12) > Cu (8.69), Fe (80.30) > Zn (16.05) > Mn (12.19) > Cu (7.70, Fe (162.89) > Zn (24.04) > Mn (17.39) > Cu (7.42), and Fe (77.30) > Zn (15.78) > Mn (11.35) > Cu (4.59) in mg/Kg dry weight, respectively.

The results also show that the highest concentrations of elements: Cu, and Fe are found at DLT site, while Fe and Zn are found at MST. The pattern of metallic concentrations found in unwashed leaf samples is noticeably similar to washed samples. The metal concentrations are in the order Fe > Mn > Zn > Cu > Pb at DLT site. In addition, the metallic order at CHU, MST and reference are in the order Fe > Zn > Mn > Cu >Pb.

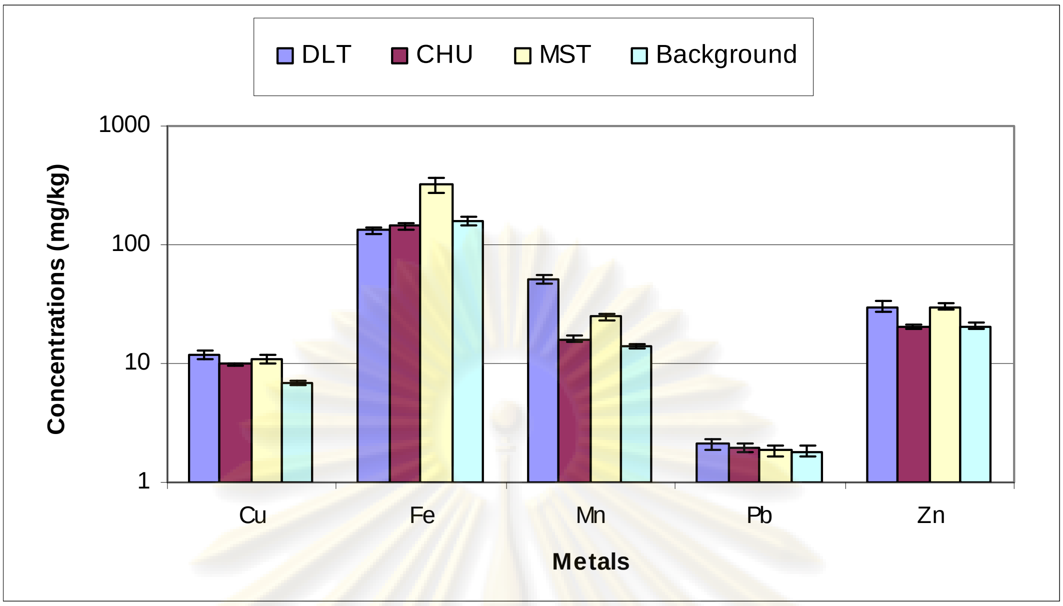


(a) PM10

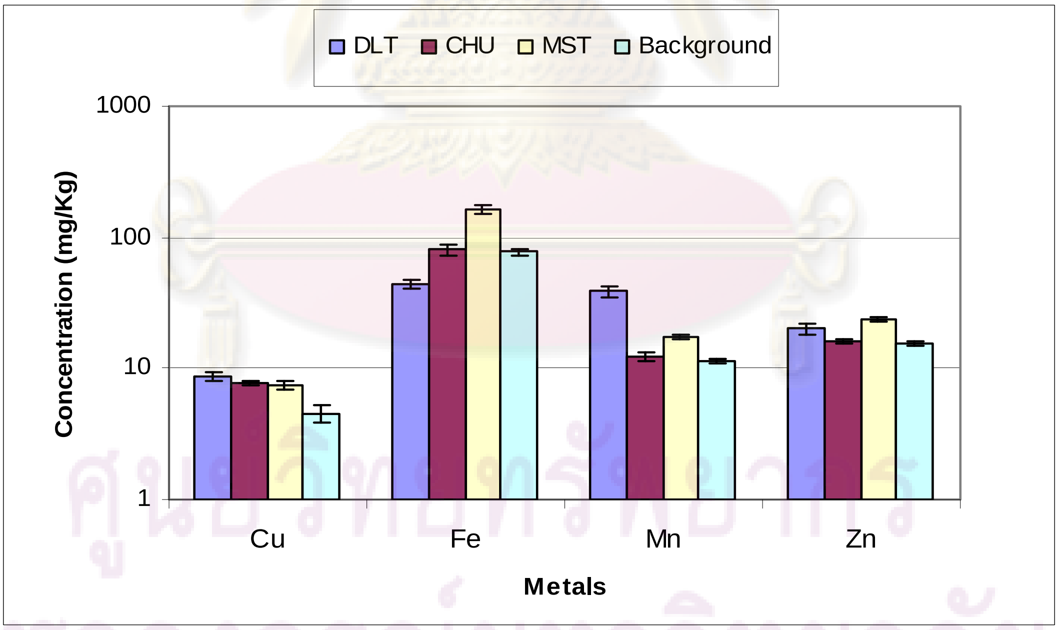


(b) Soil

Figure 4.7: Mean concentrations and standard error (SE) of (a) PM10 (ng/m^3), (b) Soils (mg/kg dry weight), (c) Unwashed leaves (mg/kg dry weight), and (d) Washed leaves (mg/kg dry weight), at sampling sites.



(c) Unwashed leaves



(d) Washed leaves

Figure 4.7: Mean concentrations and standard error (SE) of (a) PM10 (ng/m^3), (b) Soils (mg/kg dry weight), (c) Unwashed leaves (mg/kg dry weight), and (d) Washed leaves (mg/kg dry weight), at sampling sites. **(Continued)**

The differences in metal concentrations of airborne particles and soils between sites are assessed by one-way analysis of variance (one-way ANOVA). The normality and homogeneity of variance must be observed before selecting the methods of ANOVA testing. The result from normality and homogeneity of variance testing showed that all metals in both types sample should be analyzed by non-parametric method. The results of ANOVA testing are demonstrated in Table 4.3.

Table 4.3: Statistical data of one-way analysis of variance (ANOVA) of elements in PM10 and soil between three sampling and reference sites.

Non-parametric Method ($\alpha = 0.05$)

(a) PM10

Test Statistics(a,b)	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Chi-Square	1.689	25.795	6.797	1.405	0.529	2.364	19.540
df	3	3	3	3	3	3	3
Asymp. Sig.	0.639	0.000	0.079	0.704	0.913	0.500	0.000
Results		*					*

(b) Soil

Test Statistics(a,b)	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Chi-Square	28.843	33.841	23.647	19.864	2.170	32.178	27.760
df	3	3	3	3	3	3	3
Asymp. Sig.	0.000	0.000	0.000	0.000	0.538	0.000	0.000
Results	*	*	*	*		*	*

a Kruskal Wallis Test
b Grouping Variable: Place
* Significantly different between sites at $\alpha = 0.05$

There is a significant difference in heavy metals found in PM10 between the sites for Cu, and Zn whereas no difference is found for the others at significant level (α) 0.05. This may be attributed to different anthropogenic activities between the sites for these two metals. Highlighted observation should be paid to the Cu and Zn, which are only two metals found in differences between sites. Both of these are dominant metal emitted from vehicular emissions (Fang *et al*., 2006; Monaci *et al*., 2000).

The difference between traffic activities in each site may cause the different metals concentrations found in PM10. Even though only two metals, the Cu and Zn of PM10 are found in different in concentrations between sites, almost metals consisted in soil are found in difference in their concentrations between sites except the Ni.

The different results found between two matrices (air and soil) might be from the difference of aerial deposition sampling. The PM10 samples are collected in 24 hours of sampling which means the metals found in PM10 are the suspended metals in the air at the present time of only 24 hours. Therefore, the air metal concentrations reflect the air pollution situation within the hours of sampling only. The variations in activities in the short period of time may impact directly on the amounts of particles found in PM10. Meanwhile, the metal deposition in soil reflects the long term accumulation and takes in the longer times than the sampling period of PM10. In general, the metal concentrations found in soil indicate the impact of air pollution on the area in the longer time. The more times in accumulation means more influence on the pollutant numbers. The results from ANOVA testing in soil in all sites indicate significant differences in most heavy metals between the sites. The difference in contents might be from the different activities between sites. The significant different metals in soil reflect the fact of that soil is a good sink for pollutants and the differences in activities in the area can influence the quantity of metals. Due to metals accumulations in urban soil can come from many sources for example soil itself, road infrastructures or aerial deposition, the assessment of airborne metal by using soil is improper. By the way, long-term changes in the elemental balance of soils and ecosystems are also related to concentrations in the atmospheric input and to the total cumulative deposition over long periods of time (Gjengedal and Steinnes, 1994). The direct measures of air pollutant sometimes are limited by the sampling period and the sensitivity of responses. The accumulation of pollutants in plants or their reaction to them is a better indication of a system's pollution stress than direct pollution measurement (Gjengedal and Steinnes, 1994).

4.4 Metallic correlations

Pearson's correlation coefficient is used to measure the degree of correlation between washed leaves and soil metal concentrations in the study. The relationships between metals in the same matrix are also illustrated. Table 4.4 indicates the high correlation between Fe and Mn in soil samples (r = 0.795, 0.713, 0.749 and 0.839) at DLT, CHU, MST and reference sampling sites respectively. High correlations between Fe and Zn in soil are observed at DLT and reference sites (r = 0.813 and 0.704). However, there appeared to not be significant correlations of the same metal concentrations between washed leaves and soils in the study.

The findings above indicate that it has no effects of metal levels in soil upon metal concentrations in leaves. It can be said that the studied metals accumulation in leaves are not mainly directly from soil in the present study. The present finding is not in line with the previous studies. The study conducted in Turkey using *Nerium oleander* leaves as a biomonitor of heavy metals revealed that it has relationship between metal concentration in surface soil and the washed leaves for Pb, Cd, Zn and Cu (Aksoy and Ozturk, 1997). The other study in Palestine described the statistically significant correlations between the concentration of some metals (Pb, Cu, Zn) in soil and plant samples, while other metals (Cd, Cr, Fe, Mn, Ni) did not exhibit any significant correlation (Swaileh *et al*., 2004). The study done in the USA showed that concentrations in common dandelion (*Taraxacum officinale*) leaves of four metals (Cr, Mn, Pb and Zn) found to increase significantly as the soil levels of these metals increased, but the percentage of the total variation explained by the relationship in theses cases was generally low. However, the other four metals (Cd, Cu, Fe, Ni) had no significant relationship between leaf and soil. This study also stated that along with the lack of a significant relationship between leaf and soil concentrations for the four other metals, indicate the factors affecting metal absorption from the soil by dandelions are complex and that, aside from soil metal concentrations, other soil, plant and/or other environmental factors affect metal uptake (Keane *et al*., 2001).

Table 4.4: Correlation analysis of washed leaves and soils metal concentrations at four sites

DLT	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_soil	Fe_soil	Mn_soil	Zn_soil
Cu_wl	1							
Fe_wl	.469	1						
Mn_wl	.664(*)	.426	1					
Zn_wl	.754(*)	.334	.562	1				
Cu_soil	-.405	-.134	-.467	-.509	1			
Fe_soil	-.480	-.259	-.606	-.600	.853(**)	1		
Mn_soil	-.135	-.059	-.569	-.329	.630	.795(**)	1	
Zn_soil	-.550	-.019	-.452	-.618	.929(**)	.813(**)	.556	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

CHU	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_soil	Fe_soil	Mn_soil	Zn_soil
Cu_wl	1							
Fe_wl	.584	1						
Mn_wl	-.205	.050	1					
Zn_wl	.032	-.234	.602	1				
Cu_soil	.193	-.235	-.376	.081	1			
Fe_soil	-.119	-.182	-.350	.228	-.009	1		
Mn_soil	.014	.332	-.163	.058	-.330	.713(*)	1	
Zn_soil	-.028	.025	-.295	.149	.622	.415	.392	1

* Correlation is significant at the 0.05 level (2-tailed).

MST	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_soil	Fe_soil	Mn_soil	Zn_soil
Cu_wl	1							
Fe_wl	.408	1						
Mn_wl	.070	-.065	1					
Zn_wl	.616	.243	.239	1				
Cu_soil	-.154	-.135	-.120	-.077	1			
Fe_soil	.238	.228	.554	.080	-.153	1		
Mn_soil	.157	.082	.352	.185	.251	.749(*)	1	
Zn_soil	-.397	-.365	.109	.158	.576	-.398	-.041	1

* Correlation is significant at the 0.05 level (2-tailed).

Reference	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_soil	Fe_soil	Mn_soil	Zn_soil
Cu_wl	1							
Fe_wl	.370	1						
Mn_wl	-.050	.036	1					
Zn_wl	.295	.047	.284	1				
Cu_soil	.614	.073	-.506	.026	1			
Fe_soil	-.257	-.336	.077	-.095	-.181	1		
Mn_soil	-.435	-.574	.177	-.283	-.454	.839(**)	1	
Zn_soil	.298	.044	.331	.079	.034	.704(*)	.560	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Even though many previous studies exhibited the relationship of metal concentrations between soil and leaf which implied the effect of metal uptake to leaf from soil, it has not been the general rule. Heavy metal uptake by plants grown in polluted soils has been studied to a considerable extent. All findings have shown that elevated levels of metals in soil may lead to increased uptake by plants. However, there is not generally a strong relationship between the concentrations in soil and plants, because it depends on many different factors, such as soil metal bioavailability, plant growth and metal distribution to plant parts (Vousta *et al*., 1996). The plant composition only partly reflects the composition of the soil or the atmosphere, but it is a function of compound selective processes, such as uptake, translocation and enzymatic actions that take place on the root and foliar surfaces, or in the plant cells. (Vousta *et al*., 1996).

The correlations of metal contents in samples of washed leaf and PM10 are shown in Table 4.5. The correlations of metals in the same matrix are also stated in Table 4.5. High correlation coefficients are found between Fe and Mn in PM10 samples at all sites (r = 0.745, 0.731, 0.752, and 0.674 for DLT, CHU, MST, and reference respectively). The significant correlation between Fe and Zn in PM10 is observed at CHU (r = 0.756), while the significant correlation between Mn and Zn is found (r = 0.797) at DLT site. The finding of Fe-Mn relationship pattern in PM10 is rather similar to the relationship pattern found in soil. From the present finding, there are not significant correlations of the same metal concentrations between washed leaves and PM10 in the study like washed leaf and soil samples.

The finding above may imply of that it has no effects of metal levels in PM10 on metal concentrations in leaves as well. The relation of metals contents in PM10 and plant leaves are recondite. Therefore, there is also no evidence that leaf metal concentrations were positively correlated with PM10 in the previous study (Keane *et al*., 2001). Both the present study and Keane *et al*., (2001) study are conducted using washed leaves as samples.

Table 4.5: Correlation analysis of washed leaves and PM10 metal concentrations at four sites

DLT	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_PM10	Fe_PM10	Mn_PM10	Zn_PM10
Cu_wl	1							
Fe_wl	.469	1						
Mn_wl	.664(*)	.426	1					
Zn_wl	.754(*)	.334	.562	1				
Cu_PM10	.116	-.504	.191	-.126	1			
Fe_PM10	-.840(**)	-.428	-.747(*)	-.814(**)	.153	1		
Mn_PM10	-.828(**)	-.290	-.386	-.767(**)	.125	.745(*)	1	
Zn_PM10	-.590	-.182	-.354	-.632	.159	.545	.797(**)	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

CHU	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_PM10	Fe_PM10	Mn_PM10	Zn_PM10
Cu_wl	1							
Fe_wl	.584	1						
Mn_wl	-.205	.050	1					
Zn_wl	.032	-.234	.602	1				
Cu_PM10	-.261	.181	.360	-.122	1			
Fe_PM10	.678	.609	-.207	-.190	.243	1		
Mn_PM10	.765(*)	.722(*)	-.246	-.053	-.189	.731(*)	1	
Zn_PM10	.228	.339	.213	.170	.601	.756(*)	.499	1

* Correlation is significant at the 0.05 level (2-tailed).

MST	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_PM10	Fe_PM10	Mn_PM10	Zn_PM10
Cu_wl	1							
Fe_wl	.408	1						
Mn_wl	.070	-.065	1					
Zn_wl	.616	.243	.239	1				
Cu_PM10	-.257	-.551	.447	.143	1			
Fe_PM10	-.158	-.534	-.446	-.137	.341	1		
Mn_PM10	-.668(*)	-.532	-.224	-.373	.507	.752(*)	1	
Zn_PM10	-.488	.337	-.207	.020	-.026	.098	.434	1

* Correlation is significant at the 0.05 level (2-tailed).

Reference	Cu_wl	Fe_wl	Mn_wl	Zn_wl	Cu_PM10	Fe_PM10	Mn_PM10	Zn_PM10
Cu_wl	1							
Fe_wl	.370	1						
Mn_wl	-.050	.036	1					
Zn_wl	.295	.047	.284	1				
Cu_PM10	-.298	-.020	.353	.228	1			
Fe_PM10	.448	.003	.689(*)	.172	-.165	1		
Mn_PM10	.307	.312	.559	-.234	-.321	.674(*)	1	
Zn_PM10	.080	-.088	.296	-.423	.302	.366	.177	1

* Correlation is significant at the 0.05 level (2-tailed).

Many previous reports reveal the uptake of metals in plants can happen via different ways. The trace element concentrations in inner plant tissues can have soilborne or airborne origin (Sardans and Penuelas, 2005). Plants are fairly sensitive to the presence of trace metals given their uptake capacity through roots from soils or by direct contact, mainly between leaves and air or water. Plants have trace element uptake capacity from soils due to mechanisms such as ion exchange (Sardans and Penuelas, 2005; Crist *et al*., 1996; Onder and Dursun, 2006) and chelation between macromolecules and bioavailable trace elements ions (Sardans and Penuelas, 2005). Once deposited on the leaf surface some elements may also be taken up into the leaf via the stomata (Onder and Dursun, 2006; Reimann *et al*., 2001). As shown on Figure 4.1, the length of stomata of Orange jasmine leaf is approximately 10 µm which is the maximum diameter length of the PM10. The scanning electron micrograph confirms the possibility of particle penetrating through stomata and accumulating of elements in inner tissue.

By the way, the finding of those metal levels in PM10 did not relate to washed leaves in the study can infer that metal accumulation in inner leaf tissue did not come directly from airborne fine particles although trace elements can be taken up from the air, or by dry and wet precipitation directly via the leaves (Onder and Dursun, 2006; Al-Khlaifat and Al-Khashman, 2007). Otherwise, airborne metal pollution may be in the forms of metal bound with coarse particulate in place of fine particles. The airborne particle collected in the present study is the PM10, which is only part of total airborne particles might be the reason of the insignificant relationship between leaf and air particle. In other word, the penetration of metals attached on fine particles through the stomata via respiration process is not the main cause of metal accumulation in leaves in the present study.

4.5 Enrichment factors (EF)

The enrichment factor (EF) is employed in the study for each element. Fe is selected as reference element and the crustal composition according to Tomasevic *et al*., (2004). The concentrations of crustal elements used in the calculation are taken from Taylor (1964) as shown in Table 4.6.

Table 4.6: Chemical elements in the continental crust (Taylor, 1964).

Element	Crustal average
Cr	100 ppm
Cu	55 ppm
Fe	5.63 %
Mn	950 ppm
Ni	75 ppm
Pb	12.5 ppm
Zn	70 ppm

If the EF approaches unity, the crustal material is likely the predominant source for element; if the EF is higher than 10, the element has a significant fraction contributed by non-crustal sources (anthropogenic) (Tomasevic *et al*., 2004). The enrichment factor values of the washed leaves, unwashed Leaves and PM10 are illustrated in Figure 4.8. Because some metal contents in washed and unwashed leaves are lower than the limit of detection. Four and five elements are available for washed and unwashed leaves, respectively. In the meantime, all seven metals can be quantified for air particulate. The EF values of Cu, Zn and Mn (only at DLT site) are above than 10 for washed leaves, while Cu, Pb, Zn and Mn (only at DLT site) are greater than 10 for unwashed leaves. Meanwhile, Cu, Ni, Pb and Zn are the most enriched elements in the atmospheric depositions (PM10). Noticeably, the patterns of EF values of all sample types are similar. These findings reveal that the dominant sources for these elements at all locations are not from natural. Human activities or anthropogenic are the dominant sources of these elements and a variety of pollution emissions might contribute to their loading in the ambient air. The other metals, Cr and Mn (except for DLT site), represent the insignificant of anthropogenic influence.

The EF values for Mn in PM10 is close to 1 in all samples indicate that almost of Mn in PM10 collected in this study are presumed to be soil in origin. Meanwhile, Mn in both washed and unwashed leaves is almost reach to 10, especially Mn found at DLT site is greater than 10. These can be concluded that there are other sources than natural influencing on Mn contents in leaf samples. All finding of these suggests that using plant leaves as biomonitor is more effective in revealing the accumulation of metals in the environment than using air sampling equipment in terms of long range accumulation. As stated above, the patterns of EF values of all matrices are similar but the leaf samples highlight in reflecting the continuous metal accumulation better than air sampling filter. Using air sampler in collecting air pollutants is limited by its sampling duration. The sampling period for air sampler can not be long sometimes it is not enough to assess the real situation of problem. Hence, the data obtained from scientific equipments sometimes do not reflect the real impact of pollutant on the ecosystem even as human health especially the chronic impact.

Considering all three methods of metal accumulation (washed leaf, unwashed leaf and PM10) and all scientific findings from the present study plus all other aspects of effective sampling such as cost of operating, reliability, short and long term assessment and etc., the most effective tool for air metal pollution assessment should be unwashed leaves as biomonitor. This conclusion is in line with previous suggestion of that analyzing plant tissues can give better results in terms of sensitivity and reproducibility (Lau and Luk, 2001). Botanical materials like leaves of higher plants have been used to detect pollution including air metal pollution. The monitoring of the levels of atmospheric trace metallic concentration by using different types of biological monitors and various vegetation have been reported by different researchers (Rao and Dubey, 1992; Morselli *et al*.,2004; Ondrer and Dursun, 2006). The most economical and reasonable method for monitoring the heavy metal levels in the atmosphere in the view of many researchers is using plants (Yılmaz and Zengin, 2003; Aksoy and Ozturk, 1997; Monaci and Bargagli, 1997; Tomasevic *et al*., 2004; Espinosa and Oliva, 2006; Sawidis *et al*., 1995, Al-Alawi and Mandiwana, 2007).

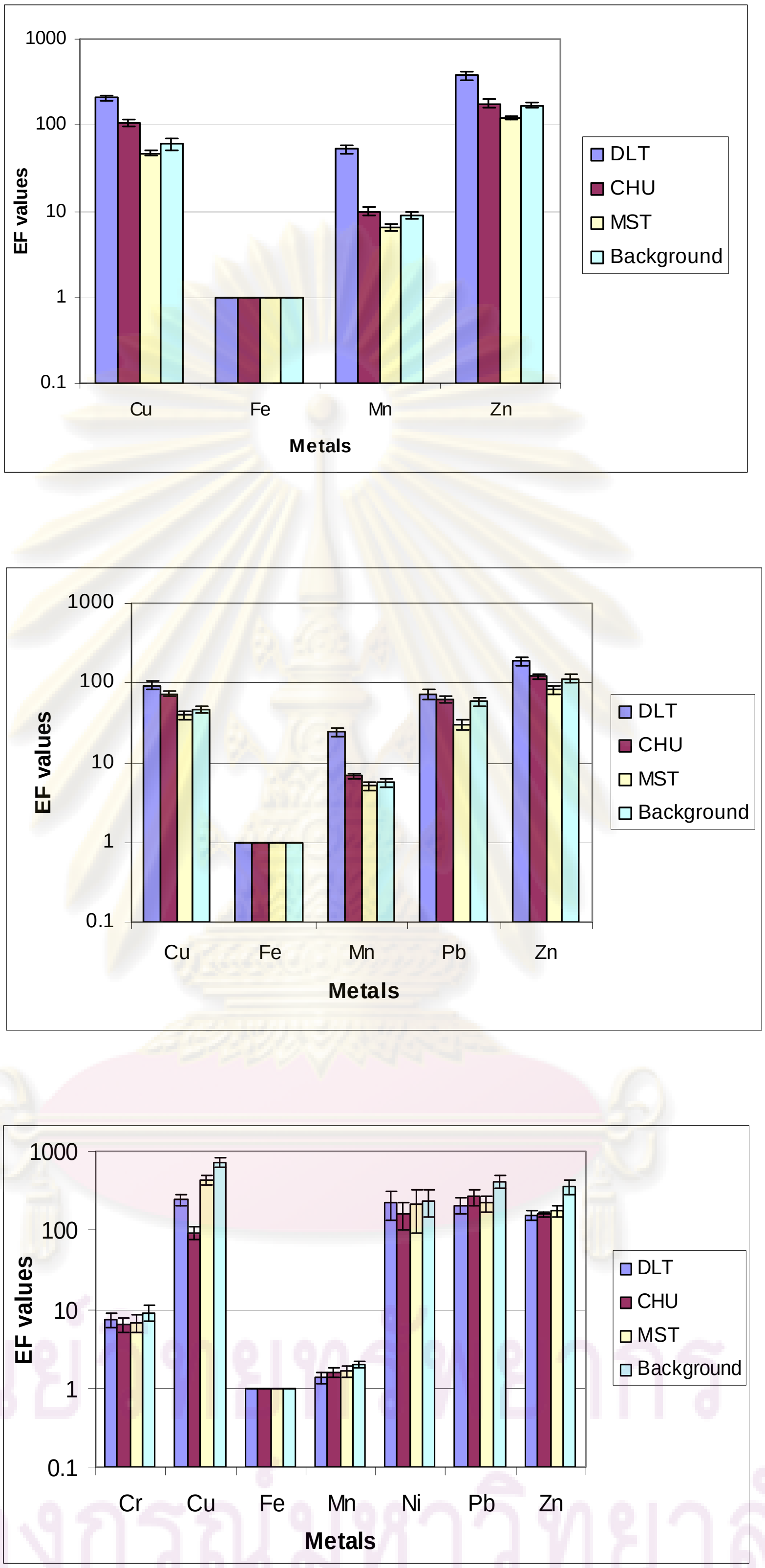


Figure 4.8: EF values and standard error (SE) of (a) Washed leaves (b) Unwashed Leaves and (c) PM10

4.6 Effect of washing on metal accumulation

The previous studies explained the metal accumulation in plants depends not only on environmental availability, but also on other physical condition such as seasonal variations, soil type, temperature, moisture, light, rainfall, age of plant, and height (Loppi *et al*., 1997; Oliva and Valdes, 2004). Many researchers investigated the effects of washing on metal accumulation in leaves (De Nicola *et al*., 2008; Swileh *et al*., 2004, Aksoy *et al*., 1999; Aksoy and Ozturk, 1997; Tam *et al*., 1987; Celik *et al*., 2005). The comparing washed and unwashed leaves can be used to distinguish between airborne and soilborne contamination (Caselles *et al*., 2002; Al-Alawi and Mandiwana, 2007). The effect of washing treatment on metal concentrations in unwashed and washed leaves is evaluated in the study using statistical t-tests with significance ($p<0.05$) of the differences. The effects of washing are presented in Figure 4.9 and Table 4.7.

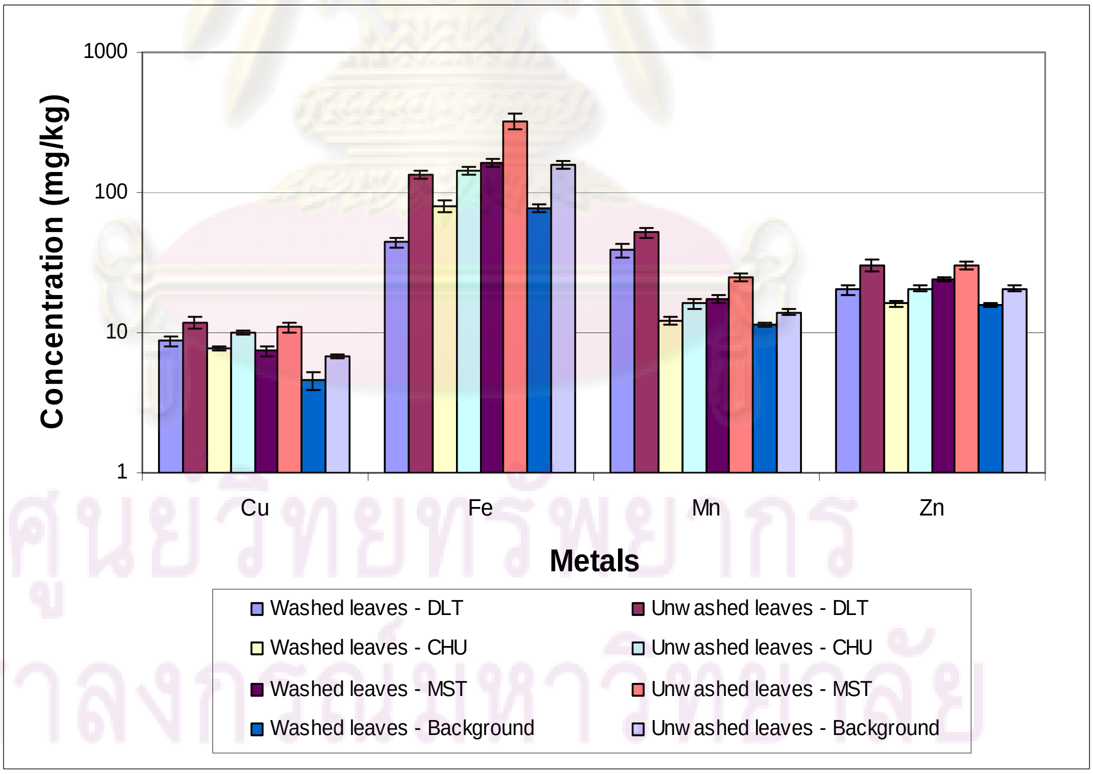


Figure 4.9: Elemental concentrations in washed and unwashed leaves

The results of the determination of metals in washed and unwashed leaves exhibit that washing the leaves significantly reduces the concentrations of contaminants in Orange jasmine from all sites. This indicates that metals bound with air particulate deposition on leaves surface is crucial to the metal accumulation on leaves. Moreover, the finding of significant metal amount on surface leaves confirms that the metals pollution originated from anthropogenic sources via the atmosphere not a function of soil type. Some researchers in the previous reports pointed that the total concentration of an element present in the vegetation means adding up the amount of the element taken up by the plant from the soil and the amount deposited on the plant surface by atmospheric fallout. But there is also an additional soil content as a result of the fallout from the air and the flow from the stem of the plant to the soil and the uptake by the leaf via the cuticle or living tissue from particles deposited on the leaf (Caselles *et al*., 2002). Surface deposits of trace elements may be inferred from differences in the concentration between washed and unwashed samples. This consideration is very important in order to distinguish between aerial deposition and the concentration of the element within the plant. In other word, the ability to distinguish between airborne and soilborne contamination was assessed by washing the leaves (Aksoy *et al*., 1999; Caselles *et al*., 2002). The statistical t-test found in the present study reveals that the washing effect is most noticeable in all sites for all elements (Table 4.7).

Comparing the amount of metal found in unwashed and washed leaves leads to the fact of that the amount of metals would be removed noticeably from the leaf by water washing. Fe is the most metal removed by washing in both rural and urban sites. Meanwhile, the others vary between metals. The removal efficiency by water washing (%) at sites and overall average are illustrated on Table 4.8. The removal efficiency of water washing on leaves in the study is in line with the report on total percent removed from the leaflets of *Phoenix dactylifera* collected in urban area by washing procedure for Cu and Zn which are 25.84 and 25.89 (Al-Shayeb *et al*., 1995). The report on washing an ornamental species *Petunia hybrida* L. showed removal efficiency in percent for Fe, Mn, Cu, and Zn are 53, 23, 19, and 15 respectively (Caselles *et al*., 2002). The washing can remove 34.2 %, 49.7 % and 25.6 % for Cu, Fe and Zn deposited on *Quercus ilex* leaves in urban area respectively.

Table 4.7 Mean concentrations of metals (mg/kg dry weight) of washed and unwashed leaves and statistical t-test.

Sites	Cu			Fe		
	Washed	Unwashed	*t*-test	Washed	Unwashed	*t*-test
DLT	8.69	11.80	*	44.22	133.88	*
CHU	7.70	9.88	*	80.30	142.94	*
MST	7.42	10.97	*	162.89	324.80	*
Reference	4.59	6.76	*	77.30	157.12	*
Average all	7.10	9.85	*	91.18	189.69	*

Sites	Mn			Zn		
	Washed	Unwashed	*t*-test	Washed	Unwashed	*t*-test
DLT	38.54	52.00	*	20.12	30.29	*
CHU	12.19	16.06	*	16.05	20.65	*
MST	17.39	24.73	*	24.04	29.92	*
Reference	11.35	14.05	*	15.78	20.67	*
Average all	19.87	26.71	*	19.00	25.38	*

Significance: * $p < 0.05$

Table 4.8: Removal efficiency (%) of metal accumulation on leaves by water washing

Plant leaves	**Site**	**Cu**	**Fe**	**Mn**	**Zn**	**Source**
Orange jasmine (*Murraya paniculata*)	DLT	26.33	66.97	25.88	33.57	Present study
	CHU	22.09	43.82	24.09	22.30	
	MST	32.43	49.85	29.69	19.67	
	Reference	32.12	50.80	19.22	23.65	
	Average all	28.24	52.86	24.72	24.80	
Quercus ilex	Urban Remote	34.2 1.95	49.7 48.5		25.6 4.26	De Nicola *et al.* (2008)
Phoenix dactylifera	Urban Suburban Highway Industrial Rural	25.84 46.52 22.35 32.16 9.70			25.89 21.74 27.98 39.72 9.86	Al-Shayeb *et al.* (1995).
Petunia hybrida	Urban	19	53	23	15	Caselles *et al.* (2002).

Similar results are reported by previous studies, for example, washing the leaves of *Capsella bursa-pastoris* can reduced the Pb, Cu, Zn and Cd concentrations significantly (Aksoy *et al*. 1999). The study of washing effect on the leaves of perennial herb inula (*Inula viscos*a L., Compositae) showed the notable reduction of the Pb and Fe (Swaileh *et al*., 2004). Washing leaves of *Robinia pseudo-acacia* L. (Fabaceae) significantly reduced heavy metal concentrations of the Fe, Pb, Zn, Cu, Cd, and Mn (Celik *et al*., 2005). In contrast, the washing of the leaves of *Ficus microcarpa* (Oliva and Valdes, 2004), *Inula viscose* (Swaileh *et al*., 2004) and *Citrus limon* (Caselles, 1998) reduces the concentration of Pb while the concentrations of Zn, Cu and Cd between washed and unwashed leaves remains the same. It can be concluded that the contents of remained metals after washing vary according to plant species. This also shows that plant species that retain constant metal concentration in washed and unwashed treatment could be a more suitable biomonitor of metal pollution (Al-Alawi and Mandiwana, 2007). It can be concluded from all finding above that Orange jasmine could be a good biomonitor of airborne metal accumulation.

4.7 Effect of age on metal accumulation

To eliminate the influence of air particulate deposition on leaf surface, washed leaf is only type tested in the study. Only four elements namely Cu, Fe, Mn and Zn are available in this leaf type because of the limit of detection of the equipment. The results on metal level accumulated in different ages of leaves are illustrated on Figure 4.10 and Table 4.9. The statistical t-test result reveals that Cu contents found in old and young leaves in each site is not significantly different except for DLT site. Results from almost sites point that it has increasing tendency in Cu contents in urban sites in the elapsed time while the opposite is found at reference which is rural site. On the contrary, the differences of Fe are significant at all sites except DLT. Comparing between old and young leaves, Fe is found in high contents in old leaves at all sites.

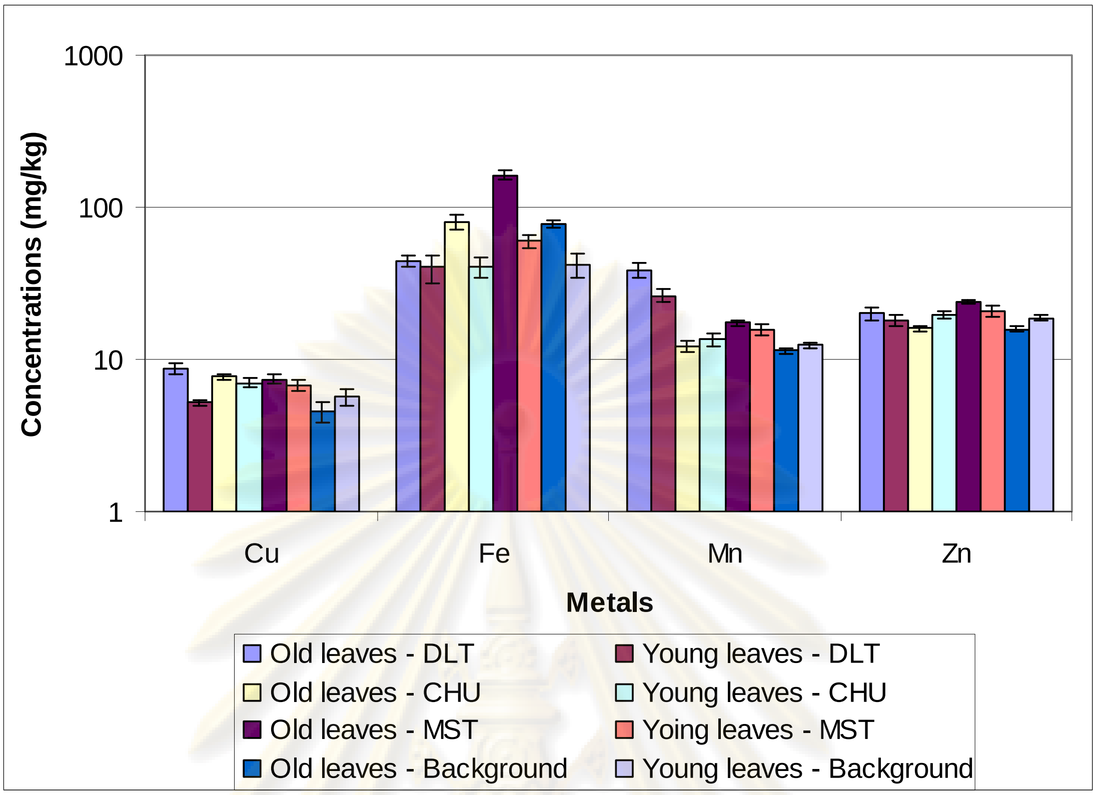


Figure 4.10: Elemental concentrations in old and young leaves

The increasing of Mn in old leaves is found at DLT and MST sites but the decreasing of Mn is found at CHU and reference sites. The significant difference of Mn in old and young leaves is only observed at DLT. Meanwhile, notable differences of Zn concentrations between old and young leaves are found at CHU and reference sites. Like the Mn, the increasing of Zn in old leaves is found at DLT and MST sites while the decreasing of Zn is found at CHU and reference sites.

The present finding gets along well with the previous reports. Caselles *et al* (2002) reported that the concentrations of trace elements in washed petunia plant leaves at the beginning (0 weeks) and at the end (9 weeks) of the experiment in the different sampling areas are significantly different. In urban areas, the levels of Fe, Cu in leaves increased significantly in the course of time. The opposite happened with Mn and Zn. When the plants grew in the suburban areas the concentrations of Mn, Cu and Zn in the leaves decreased in the course of time. The other previous study on the washed leaves of perennial herb inula (*Inula viscos*a L., Compositae) revealed that the statistical significant difference in Pb and Fe contents between old and young leaves is found though Cu, Mn and Zn are not noticeably different (Swaileh *et al*., 2004).

The high levels of Fe detected in old washed leaves in all areas compared with young washed leaves may be logically explained by the metal uptake. The increasing rate of Fe is higher than other metals. The average concentration of Fe in the old leaf is much two times higher than the young one. Meantime, the other metals are slightly increased or decreased. Some researcher reveals that Fe can be absorbed both by the leaf cuticle and by the roots (Caselles *et al*, 2002). This may lead to the high amount of Fe in the older leaves of Orange jasmine plants. Fe is not only the most abundant elements found in the crustal but also the metal industry and construction sites (Fang *et al*., 2006). The Fe contents found in leaf can be from both soil and air sources while the other metals like Cu and Zn are mainly from the aerial depositions which are not in high levels comparing with Fe. This is why Fe is found in the high level at the time elapsed.

Table 4.9: Mean concentrations of metals (mg/kg dry weight) of old and young leaves and statistical t-test.

Sites	Cu			Fe		
	Old	Young	*t*-test	Old	Young	*t*-test
DLT	8.69	5.23	*	44.22	40.17	
CHU	7.70	7.00		80.30	40.67	*
MST	7.42	6.76		162.89	59.78	*
Reference	4.59	5.70		77.30	41.81	*
Average all	7.10	6.17	*	91.18	45.61	*

Sites	Mn			Zn		
	Old	Young	*t*-test	Old	Young	*t*-test
DLT	38.54	26.31	*	20.12	18.24	
CHU	12.19	13.58		16.04	19.46	*
MST	17.39	15.76		24.03	20.76	
Reference	11.35	12.41		15.78	18.75	*
Average all	19.87	17.02		19.00	19.30	

Significance: * $p < 0.05$

4.8 Effect of seasons on metal accumulation

In the study, the significant differences of metals contents accumulated in leaves in wet and dry season are investigated. The period of study is conducted in rainy season and winter season. Because rainfall is a very important factor, since parts of the pollutants are deposited on the leaf surface and raining can wash contaminants off. Therefore, considerable concentration variations can be expected when rain falls during the period of exposure (Oliva and Valdes, 2004). The results of different concentrations between wet and dry season in all sites of washed leaves are illustrated in Figure 4.11.

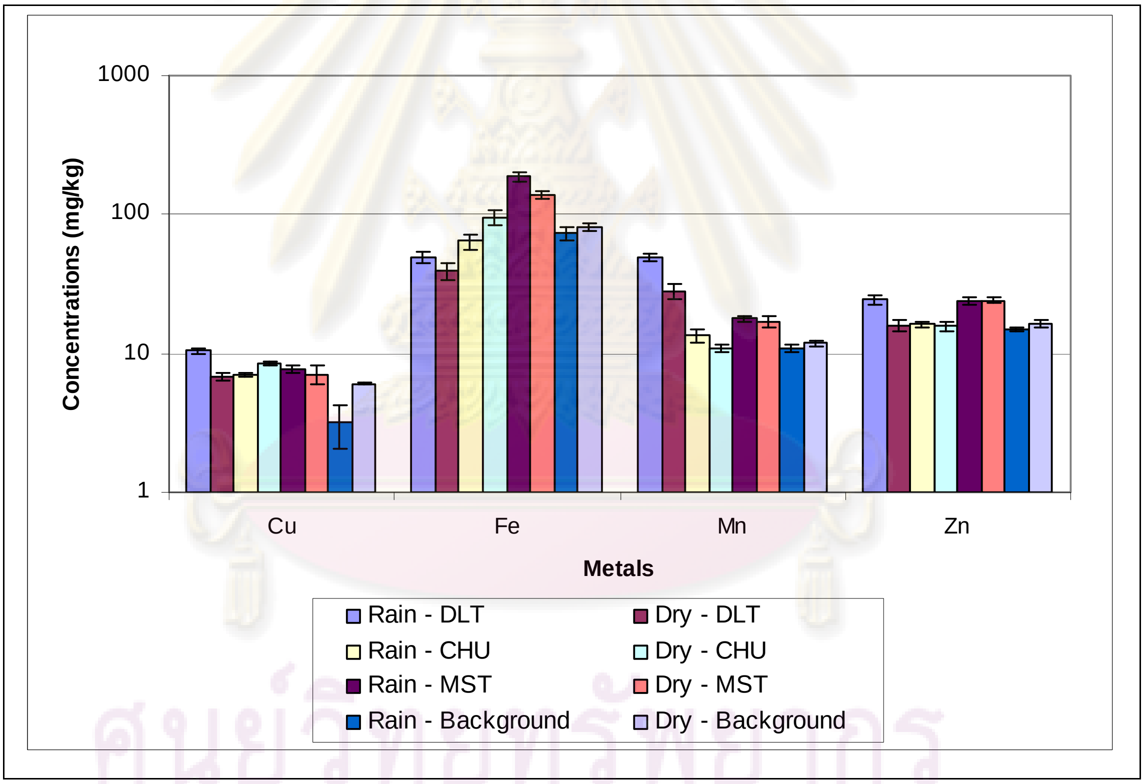


Figure 4.11: Elemental concentrations in washed leaves in rain and dry seasons

The statistical t-test is employed to distinguish the significant different of metal accumulated in washed leaves in different seasons (Table 4.10). The statistical result reveals that most of the significant differences in metal concentrations are found at DLT. By the way, the average concentration of all sites highlights the insignificant differences in all metals. This can be implied that the washing out effects of rain does not impact on metal accumulation in inner leaf tissue.

Table 4.10: Mean concentrations of metals (mg/kg dry weight) of washed leaves in rain and dry seasons and statistical t-test.

Sites	Cu			Fe		
	Rain	Dry	*t*-test	Rain	Dry	*t*-test
DLT	10.53	6.85	*	49.31	39.13	
CHU	7.00	8.39	*	64.36	96.23	
MST	7.73	7.10		186.41	139.37	*
Reference	3.16	6.01		73.20	81.39	
Average all	7.10	7.09		93.32	89.03	

Sites	Mn			Zn		
	Rain	Dry	*t*-test	Rain	Dry	*t*-test
DLT	48.85	28.23	*	24.44	15.80	*
CHU	13.44	10.94		16.25	15.85	
MST	17.77	17.02		23.92	24.15	
Reference	10.87	11.84		15.02	16.55	
Average all	22.73	17.00		19.91	18.09	

Significance: * $p < 0.05$

The significant differences of metals contents accumulated in leaves in wet and dry season of unwashed leaves are also investigated in the study. The results of different concentrations between wet and dry season in all sites of washed leaves are not a surprise. Because rain can wash out only the particles deposited on the surface of leaf, the rain water can not penetrate into the inner plant tissue and take the metals inside out with it. However, the result found in the unwashed leaves amazes the researcher how the indifferent of metals contents is found in the study. The information of metal concentrations in unwashed leaves in different seasons is shown in Figure 4.12.

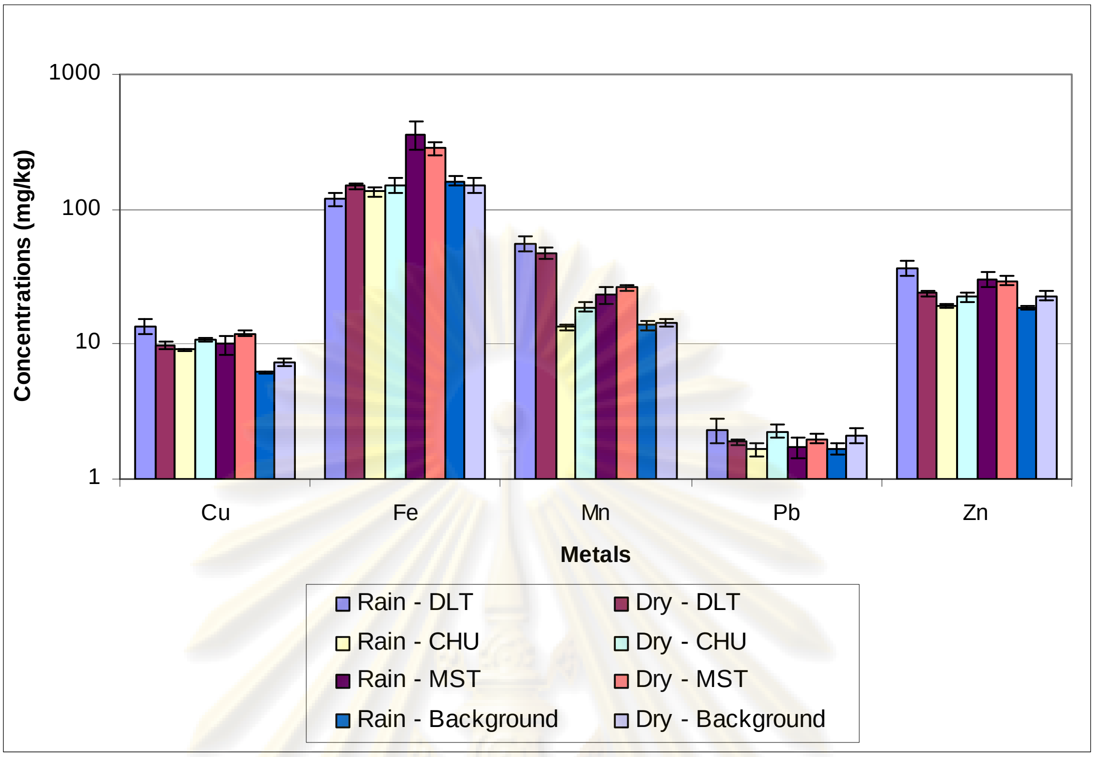


Figure 4.12: Elemental concentrations in unwashed leaves in rain and dry seasons

The statistical t-test is also used to determine the significant difference of metal accumulated in unwashed leaves in different seasons as shown in Table 4.11. The significant differences between rain and dry seasons are hardly found in the study. Like the washed leaves, the average concentration of all sites points the insignificant differences in all metals. This can be implied that the washing out effects of rain does not influence on metal accumulation in unwashed leaves as well as washed leaves.

Metals accumulation of plant leaves can come from many routes: up-taking from soil, penetrating through the stomata, or aerial depositing on leaf surface (Aksoy *et al*. 1999; Swaileh *et al*., 2004; Oliva and Valdes, 2004; and Celik *et al*., 2005). The investigation of washing effects on metal content in the topic 4.6 in the present study shows that there is significant different between leaves in all metal at all sites of sampling after washing. It can be said that at the moment, the previous finding is not agree with the present finding.

The accumulations of metals on leaf are depended on several factors. Different in shapes and sizes of leaves can cause the different particle retention capabilities (Smith, 1981). When the contaminants transported through air mass movements and are deposited by dry and wet deposition and intercepted by plant canopies: the leaves absorb gaseous compounds or accumulate airborne particulates by interception, impaction or sedimentation. The particulate accumulation on the leaf surface depends on particle size, speed of deposition and leaf surface properties (De Nicola *et al*., 2008). For instance, the hairy leaf surface appeared to be the most active accumulators of atmospheric elements compared to smooth leaf species (Caselles *et al*., 2002; Espinosa and Oliva, 2006). In addition, the chemistry of different waxes in different plant species can affect on airborne particle retention on the leaf surface including the accumulation of contaminants. (De Nicola *et al*., 2008; Komp and McLachlan, 1997; Jouraeva *et al*., 2002; Monteith and Unsworth, 1990).

Unlike the washing leaf in the laboratory nor heavy rain in the field, the common rain is probably not strong enough to wash out all over the particles deposited on the leaf surface. In other word, the ordinary rain can wash out only part of deposited particulate because of the attachment force of surface waxes on particles. The explanation of this is in line with the previous studies. It has a report on leaf waxes of different plant species have different physicochemical properties, which result in a higher retention of atmospheric air pollutants by certain species. Moreover, in the same study also revealed that the presence of other biological entities on the surface of leaves may play an important role in the processes of accumulation and retention of fine atmospheric particles (Jouraeva *et al*., 2002). The other previous study on leaf accumulation of trace elements reported that the influence of rain on washing off or leaching particulate from the *Q. ilex* leaf surface was negligible and a conspicuous load remained (De Nicola *et al*., 2008).

Table 4.11: Mean concentrations of metals (mg/kg dry weight) of unwashed leaves in rain and dry seasons and statistical t-test.

Sites	Cu			Fe		
	Rain	Dry	*t*-test	Rain	Dry	*t*-test
DLT	13.66	9.93		119.42	148.34	
CHU	9.12	10.64	*	134.82	151.06	
MST	9.85	12.00		363.06	286.53	
Reference	6.17	7.35		162.67	151.57	
Average all	9.73	9.98		194.99	184.38	

Sites	Mn			Pb		
	Rain	Dry	*t*-test	Rain	Dry	*t*-test
DLT	56.06	47.95		2.33	1.89	
CHU	13.39	18.72	*	1.65	2.26	
MST	23.30	16.17		1.72	1.98	
Reference	13.79	14.31		1.68	2.11	
Average all	26.64	26.79		1.85	2.06	

Sites	Zn		
	Rain	Dry	*t*-test
DLT	36.84	23.73	*
CHU	19.05	22.26	
MST	30.20	29.65	
Reference	18.62	22.72	
Average all	26.18	24.59	

Significance: * $p < 0.05$

4.9 Source identification of air metal pollution using leaves

According to all previous summaries on the topic 4.3 to 4.8 points that unwashed leaves of Orange jasmine is suitable to be a good bioindicator for airborne metals comparing with the conventional method. Therefore, the unwashed leaves are used in the study to investigate the possible sources of air metal pollution. The metal levels in leaf indicate the significant difference between sampling and reference sites (Figure 4.7) while the metals found in PM10 are slightly different. This shows the better metal accumulation capacity of leaf. Moreover, the EF values as shown on Figure 4.8 illustrate that both the conventional method (using PM10 as indicator for airborne particulate) and the bioindicator method (using leaf as indicator for airborne particulate) are similar in pattern. The finding points that the bioindicator method can be used to separate the metal emission sources as well as the conventional method.

4.9.1 Metal concentrations

Heavy metal concentrations in unwashed leaf samples from the three sampling sites in addition to the reference samples are presented in Table 4.12. Only Cu, Fe, Mn, Pb and Zn could be detected by ICP-AES. The amounts of Cr and Ni found in the study are lower than the detection limit of the analytical instrument.

Table 4.12: Mean concentrations (mg/kg dry weight) in unwashed leaf samples collected from different sites.

Site		Cu	Fe	Mn	Pb	Zn
DLT	Mean	**11.80**	**133.88**	**52.00**	**2.11**	**30.29**
	S.D	3.45	27.69	13.73	0.77	9.64
	Max	16.56	177.81	70.97	3.47	48.64
	Min	6.64	92.94	29.82	0.95	20.71
CHU	Mean	**9.88**	**142.94**	**16.06**	**1.96**	**20.65**
	S.D	0.97	31.80	3.70	0.57	2.97
	Max	11.35	216.57	22.90	2.91	26.64
	Min	8.24	115.43	11.04	0.97	17.70
MST	Mean	**10.97**	**324.80**	**24.73**	**1.85**	**29.92**
	S.D	2.84	147.97	5.67	0.54	6.15
	Max	13.96	602.89	31.21	2.49	37.15
	Min	3.42	160.44	10.26	0.98	16.61
Reference site	Mean	**6.76**	**157.12**	**14.05**	**1.82**	**20.67**
	S.D	0.96	37.02	1.88	0.56	3.64
	Max	8.45	217.41	17.56	2.98	27.07
	Min	5.85	76.92	12.33	1.39	16.59

The results, as shown in Table 4.12, indicate that all heavy metals are higher in dense and heavy traffic areas relative to reference area. Fe is the highest metal concentration found in all sites while Pb is the lowest. At the sampling site DLT, CHU, MST and reference site, the mean metallic concentrations are in the following order: Fe (133.88) > Mn (52.00) > Zn (30.29) > Cu (11.80) > Pb (2.11), Fe (142.44) > Zn (20.65) > Mn (16.06) > Cu 9.88) > Pb (1.96), Fe (324.80) > Zn (29.92) > Mn (24.33) > Cu (10.97) > Pb (1.85), and Fe (157.12) > Zn (20.67) > Mn (14.05) > Cu (6.76) > Pb (1.82) in mg/kg dry weight, respectively. The comparison of each metallic element at all sites reveals that the highest concentrations of elements: Cu, Mn, Pb and Zn are found at DLT site, which is the heavy traffic area. Fe, a crustal element mostly found in crustal rock and soil is the highest at MST (Fang *et al*., 2004). This indicates the metallic deposition on leaves in this site is influenced by soil re-suspension. Even though DLT and CHU are located near the main roads, both are still opposite the big parks. Land covered with grasses in the park can reduce the rate of soil re-suspension from the ground. Meanwhile, the MST site is located on the heavy traffic road with high buildings around and roofed with express way along the road. The trapped condition could be the reason of high concentration of Fe from soil re-suspension particles.

Generally, lead is accepted worldwide to be a good indicator for traffic emission source. In this study, however, lead could not be a good metallic tracer of traffic emission in Bangkok environment because Thailand has completely banned leaded gasoline since 1996. It has been a steady decrease in the amount of Pb in the Bangkok atmosphere and surrounded environment (Pollution control department, 2004). However, the air particulate and biomonitoring data in the previous study suggested that Zn is a reliable tracer for vehicle traffic emissions, instead of Pb (Monaci *et al*., 2000). High concentrations of Zn found in DLT and MST suggested that these areas are influenced by traffic emissions. As all three sampling sites are situated in areas of with heavy traffic roads, a vehicular emission is possible main source of metals in the atmosphere.

4.9.2 Correlation coefficients of trace metals

Correlation coefficients of trace metals at the monitoring sites are shown in Table 4.13. The correlation could be used to impute the possible sources of the trace metals in samples. In other words, correlation is a first insight into the sources of the toxic metals. A moderate to weak correlation among toxic metals could be the result of the concentrations of different correlation behavior in urban atmosphere, especially in a metropolitan city, where various emission sources coexist (Karar *et al*., 2006). Copper has strong correlation with Mn, Pb and Zn (r = 0.70, 0.68, and 0.76, respectively) at DLT site. In addition, this strong correlation pattern is also found in CHU site (r = 0.88, 0.66, and 0.78, respectively), and MST site (r = 0.91, 0.66, 0.85 respectively).

There is also high significant correlation coefficient between Cu and Mn, Pb, Zn in all sampling sites (r = 0.58, 0.63, and 0.77, respectively). At MST site, Zn has good correlation with Cu, Fe, and Mn (r = 0.85, 0.63, and 0.81, respectively), while good correlation between Zn and Fe is not found in other sites. The high contents of Cu, Mn, and Zn metals in sampling sites and high correlation of Cu-Mn, and Cu-Zn (Table 4.12 and 4.13) could be related to metal processing in the industrial area but may also indicate vehicular activity as a possible originator (Garty *et al*., 2001). Besides, human populations in urban areas are generally exposed to manganese released into the air by the combustion of fuels in automobiles, steel production, dry-cell batteries, matches, power plants, coke ovens, etc (Karar *et al*., 2006) All of these show the influence of anthropogenic activities over the atmosphere. Thus, it can be said that the airborne metals in sampling area originated from human activities.

Table 4.13: Correlation coefficients in site 1, 2, 3 and all sites.

DLT	Cu	Fe	Mn	Pb	Zn
Cu			0.70	0.68	0.76
Fe					
Mn				0.66	0.57
Pb					0.74
Zn					

CHU	Cu	Fe	Mn	Pb	Zn
Cu			0.88	0.66	0.78
Fe					
Mn				0.69	0.75
Pb					0.77
Zn					

MST	Cu	Fe	Mn	Pb	Zn
Cu			0.91	0.66	0.85
Fe					0.63
Mn					0.81
Pb					
Zn					

All	Cu	Fe	Mn	Pb	Zn
Cu			0.58	0.63	0.77
Fe					
Mn					0.60
Pb					0.54
Zn					

Only values above 0.50 are shown

4.9.3 Analysis of variance of metals

Analysis of variance (ANOVA) was used to compare the significant difference in the mean concentration of heavy metals between all sampling sites including the reference site (Table 4.14).

Testing of normality and homogeneity of variance were used to each group of elements before selecting the method of one-way analysis of variance. The result from normality and homogeneity of variance testing showed that only Pb could be analyzed by parametric method while Cu, Fe, Mn, and Zn could be analyzed by non-parametric method. Table 4.14 demonstrates that there is a significant difference in heavy metals between the sites for Cu, Fe, Mn and Zn whereas no difference is found for the Pb at significant level (α) 0.05. This may be attributed to different anthropogenic activities between the sites.

Table 4.14: Statistical data of one-way analysis of variance (ANOVA) of elements between three sampling and reference sites.

(a) Parametric Method (α = 0.05)

Pb	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.508	3	.169	.444	.723
Within Groups	13.731	36	.381		
Total	14.239	39			
Result					/

(b) Non-parametric Method (α = 0.05)

Test Statistics[a,b]	**Cu**	**Fe**	**Mn**	**Zn**
Chi-Square	20.575	21.751	29.447	17.022
df	3	3	3	3
Asymp. Sig.	.000	.000	.000	.001
Results	*	*	*	*

a Kruskal Wallis Test
b Grouping Variable: Place

Results * Significant difference at α = 0.05
/ Insignificant difference at α = 0.05

The result also confirms the finding from the correlation coefficients of trace metals that different human activities in the area cause the different impact on local air quality. The non-significant difference of Pb in the atmosphere between sites might come from the measure of phasing lead out of gasoline since 1991 to an absolute ban of leaded gasoline in 1996. Bangkok and the whole country, nowadays, have had no leaded gasoline. The previous studies indicate a significant reduction of lead content in air particulate of Bangkok atmosphere (Oanh *et al*., 2006; Cheevaporn *et al*., 2004). There is no significant difference in Pb concentration, for this reason, in all sampling and reference sites. The level of Pb found at all sites could be a reference level. The previous biomonitoring study done in Italy revealed that Ba, Cu, Fe, Mn, Pb and Zn were the main metal pollutants emitted by vehicles in Florence. In the same study, very similar results were obtained by the analysis of *Quercus ilex* leaves which were found to accumulate airborne metals as a function of the exposure time (i.e. their age) (Monaci *et al*., 2000). The results from ANOVA testing in all sites indicate a significant difference in most heavy metals between the sites. This might be from the different traffic activity between sampling and reference sites. In other words, traffic activity is influential on the metal contents of airborne particles.

4.9.4 Factor analysis and source identification

In the present study, principal component analysis (PCA) was performed in the data set. A total of 5 metallic variables at each sampling site were considered for factor analysis. Varimax rotated factor analysis was carried out using statistical software (SPSS) with measured metals as variables for sampling sites. The results from SPSS processing are illustrated in Table 4.15.

Table 4.15: Factor analysis on elemental data at three sampling sites

Elements	DLT		CHU	MST	
	Factor 1	Factor 2	Factor 1	Factor 1	Factor 2
Cu	**.901**	.026	**.907**	**.953**	-.220
Fe	-.144	**.971**	.431	.546	**.764**
Mn	**.841**	-.248	**.932**	**.910**	.066
Pb	**.872**	.260	**.862**	.615	-.671
Zn	**.879**	.111	**.887**	**.947**	.154
Eigenvalue	3.074	1.085	3.408	3.310	1.111
Variance(%)	61.484	21.705	68.157	66.208	22.217
Cumulation(%)	61.484	83.189	68.157	66.208	88.425
Source type	Anthropogenic	Soil	Anthropogenic	Anthropogenic	Soil

At the DLT site, two factors are selected based on the criteria of cumulative percentage variance higher than 80% and eigenvalue higher than one. Varimax rotated factor analysis reveals two possible factors (based on factor loading higher than 0.70) representing two different contributing sources for the trace metals. The factor 1 represented by Cu, Mn, Pb and Zn with 61% variance can be attributed to traffic activities. The previous study showed that it has a significant correlation between traffic density and Pb, Cu and Zn concentrations (Tam *et al*., 1987). Zinc can be considered a reliable tracer for vehicle traffic emissions instead of Pb (Monaci *et al*., 2000) Zn, Cu, Fe and Mn levels are also good tracers of traffic emissions (Querol et., 2007; Manoli *et al*., 2002; Fang *et al*., 2003). Particulate Zn in the urban atmosphere may have its origin from automobile sources, for example, wear and tear of vulcanized rubber tires, lubricating oil and corrosion of galvanized vehicular parts (Banerjee, 2003). Manganese is used as an additive in vehicular fuel (Karar *et al*., 2006). Factor 2 at DLT comprises only one metal, Fe with a variance of 22%. This factor represents soil re-suspension (Fang *et al*., 2006; Lorenzini *et al*., 2006). At CHU, only one factor is found at the site. The factor consists of Cu, Mn, Pb, and Zn with a variance of 68%. Like factor 1 of DLT, it represents the traffic activities. There are two factors at the MST site. The total percentage variance explained by these factors is 88%. Factor 1 at MST has higher loading of Cu, Mn, Zn with 66% variance. This factor can be attributed to automobile sources. Factor 2 is uniquely associated with Fe and 22% variance and is likely to be from soil re-suspension like factor 2 of DLT site. The results from all sites show that anthropogenic activity (traffic emission) is more influential in the airborne particulate than the nature activity (soil and dust re-suspension) in the areas.

4.10 Comparative with other studies

The metals concentrations found in leaves and soil samples in the present study are compared and showed in Table with other studies (Table 4.16 – 4.17). The previous studies as shown in Table 4.16 point that the application of using plant leaves as biomonitor are widely accepted in several places. The concentrations of metal found in their study varied to the plant species and sampling site characteristics including activities in each sampling site. It can be noticed that human activities are strongly influence on the metal pollution in the areas also.

Table 4.16: Comparison of mean metal concentrations (mg/kg dry weight) in plants with other studies

Country	Plants	Parts	Sites	Treatment	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Jordan Amman	Aleppo pine (*P. halepensis*)	Needles	Industry	Washed		15.5				45.0	118.0	Al-Alawi and Mandiwana (2007)
				Unwashed		29.5				90.0	262.0	
			Roadside	Washed		16.0				75.5	55.0	
				Unwashed		37.0				196.0	95.0	
			Suburban	Washed		7.50				15.0	36.0	
				Unwashed		16.5				40.0	55.0	
			Control	Washed		5.32				11.0	10.0	
				Unwashed		10.0				19.0	25.0	
Palestine West bank	Perennial herb inula (*Inula viscosa*)	Leaves	Roadside	Washed	7.03	10.6	730	140	4.87	7.25	47.6	Swaileh *et al*. (2004)
Italy Naples	*Quercus ilex*	Leaves	Urban	Washed	0.94	9.05	0.39			2.04	23.89	Nicola *et al*. (2008)
				Unwashed	1.88	13.75	0.77			3.34	32.13	
			Remote	Washed	0.30	3.82	0.09			0.18	22.5	
				Unwashed	0.37	3.90	0.17			0.20	23.5	
Spain Seville	*Nerium oleander*	Leaves	Low traffic	Unwashed		4.83	108.3	42.38	0.17	0.71		Espinosa and Oliva (2006)
			High traffic	Unwashed		7.04	109.5	18.26	0.09	0.91		
			Background	Unwashed		2.60	63.97	23.79	n.d.	0.27		
	Lantana camara	Leaves	Low traffic	Unwashed		11.88	222.1	17.75	0.13	1.28		
			High traffic	Unwashed		5.94	70.98	23.53	0.15	0.47		
			Background	Unwashed		7.79	119.0	33.47	0.04	0.77		

Table 4.16: Comparison of mean metal concentrations (mg/kg dry weight) in plants with other studies **(continued)**

Country	Plants	Parts	Sites	Treatment	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Saudi Arabia Riyadh	Date palm (*Phoenix dactylifera*)	Leaves	Urban	Washed	2.32	3.64			2.77	31.83	12.87	Al-Shayeb *et al.* (1995)
				Unwashed	2.77	4.88			4.05	101.09	17.46	
			Suburban	Washed	2.00	2.07			1.24	24.75	12.85	
				Unwashed	2.45	3.78			2.15	73.58	16.90	
			Highway	Washed	2.25	3.19			2.40	24.37	14.55	
				Unwashed	2.82	4.11			4.03	61.80	20.37	
			Industry	Washed	2.04	3.07			1.47	3.16	15.60	
				Unwashed	2.91	4.61			2.83	6.89	26.87	
			Rural	Washed	1.96	2.65			1.36	1.00	8.69	
				Unwashed	2.20	2.92			2.35	1.46	9.68	
Hong Kong	*Buhina variegata*	Leaves	Roadside Park	Washed		43	620	99		74	296	Tam *et al.* (1987)
				Unwashed		47	861	135		72	406	
			Control site	Washed		19	131	103		10	66	
				Unwashed		22	168	106		12	74	
Turkey Denizli	*Robinia pseudo-acacia*	Leaves	Industry	Washed		8.46	89.91	229.2			43.49	Celik *et al.* (2005)
				Unwashed		16.92	3087.0	349.2			89.91	
			Urban roadside	Washed		10.15	139.0	175.3			53.05	
				Unwashed		20.81	414.4	221.3			139.0	
			Suburban	Washed		8.13	33.2	95.4			21.01	
				Unwashed		12.22	255.0	147.8			33.20	
			Control	Washed		5.28	11.4	43.3				
				Unwashed		5.64	100.2	53.6				

Table 4.16: Comparison of mean metal concentrations (mg/kg dry weight) in plants with other studies **(continued)**

Country	Plants	Parts	Sites	Treatment	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Italy Siena	*Quercus ilex*	Leaves	Suburban	Unwashed	2.84	16.7	340	95.1	1.16	3.16	77.9	Monaci and Bargagli (1997)
			Urban with fast-moving traffic	Unwashed	2.89	13.0	531	281	1.92	14.0	41.8	
			Urban with slow-moving traffic	Unwashed	3.21	15.8	619	157	1.45	25.2	33.3	
Thailand Bangkok	Orange jasmine (*Murraya paniculata*)	Leaves	DLT	Washed		8.69	44.22	38.54			20.12	Present study
				Unwashed		11.80	133.88	52.00		2.11	30.29	
			CHU	Washed		7.70	80.30	12.19			16.05	
				Unwashed		9.88	142.94	16.06		1.96	20.65	
			MST	Washed		7.42	162.89	17.39			24.04	
				Unwashed		10.97	324.80	24.73		1.85	29.92	
			Reference site	Washed		4.59	77.30	11.35			15.78	
				Unwashed		6.76	157.12	14.05		1.82	20.67	

Table 4.17: Comparison of mean metal concentrations (mg/kg dry weight) in soil with other studies

Country	Place	Sites	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Paleastine	West Bank	Roadside	42.4	60.4	15700	224	18.9	87.4	82.2	Swaileh *et al*. (2004)
Thailand	Bangkok	Urban		41.7		340		47.8	118	Wilcke wt al. (1998)
Greece	Athens	Highway + Urban		232.5	2.6 %	502.8		577.7		Riga-karandinos *et al*. (2004)
		Highway		239.1	2.7 %	545.7		571.0		
		Urban		217.1	2.2 %	393.6		594.7		
		Suburban + Rural		38.1	2.4 %	475.9		93.8		
Hong Kong		Roadside Park		142	15020	758		268	1281	Tam *et al*. (1987)
		Control site		15	6584	509		40	399	
Turkey	Denizli	Industry		54.31	3939.3	786.47			456.88	Celik *et al*. (2005)
		Urban roadside		69.71	2554.5	428.46			506.43	
		Suburban		17.19	2892.7	337.36			81.23	Present study
		Control		8.69	2695.6	271.87			10.67	
Thailand	Bangkok	DLT	6.75	19.61	8203.86	311.79	18.55	13.79	202.74	
		CHU	6.75	15.06	7175.40	216.99	16.63	26.10	152.02	
		MST	18.73	79.48	14096.79	241.75	14.54	62.13	249.18	
		Reference site	3.97	3.91	7236.38	168.29	15.37	12.41	40.91	

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The results from the SEM study reveal that particles are presented in higher density on the adaxial (upper surface) than abaxial (lower surface). The particles are heterogeneously distributed in forms, shapes and diameter lengths. The diameter length of the opening stomata is approximately 10 μm which is a length that fine particles can penetrate into the inner tissue via plant respiratory process. The stomata of Orange jasmine plants are only found on the abaxial in the study. The characteristics of stomata appeared on abaxial of old and young leaf in forms, sizes, and numbers are not different. The chemical compositions of the particles deposited on both upper and lower surfaces investigated by EDS suggest that the most abundant elements are from soil/dust re-suspension. Trace metals found in the particles also indicate the possible source of particles from vehicle emission.

Heavy metal concentrations in PM10 samples from the three sampling sites in addition to the reference samples indicate that there is no significant difference between sites. Meanwhile, the soil results indicate that all heavy metals are higher in the dense and heavy traffic areas relative to the reference site. Fe is the highest metal concentration found in all sites while Cr is the lowest in both two matrices. Only five elements: Cu, Fe, Mn, Pb and Zn in unwashed samples could be detected by ICP-AES, while Cu, Fe, Mn, and Zn in washed samples could be detected. All heavy metals found in both types of leaves are higher in the sampling urban sites than the reference site. Fe is again the highest metal concentration found in all sites while Pb is the lowest. The pattern of metallic concentrations found in unwashed leaf samples is noticeably similar to that of washed samples.

Pearson’s correlation coefficient is used to measure the degree of relationship between washed leaves and soil metal concentrations in the study. The relationships between metals in the same matrix are also evaluated. The result indicates the high correlation between Fe and Mn in soil samples at all sites. However, there appeared to not be significant correlations of the same metal concentrations between washed leaves and soils in the study. It can be said that metal accumulation in leaves is not directly from soil in the present study. Even though some researchers described the statistically significant correlations between the concentration of metals in soil and plant leaves in some species, the other investigators found metals are not significantly correlated between soil and leaf in some species as well. The relationship of metal concentrations between soil and leaf can be implied the effect of metal uptake to leaf from soil but it is not the general rule. The relationship of soil and leaf depends on many different factors, such as soil metal bioavailability, plant growth and metal distribution to plant parts.

The Pearson's correlations of metal contents in washed leaf and PM10 is also used in the study. High correlation coefficients are found between Fe and Mn in PM10 samples at all sites. The pattern of Fe-Mn relationship in PM10 is rather similar to the soil. Nevertheless, there are no significant correlations of metal concentrations between washed leaves and PM10. The finding may imply that metal levels in PM10 have no effect on metal concentrations in leaves also. Therefore, there is also no evidence of that washed leaf metal concentrations were positively correlated with PM10 in the previous study. Once the air particles deposited on the leaf surface some elements may also be possible to take up into the leaf via the stomata. By the way, the finding of those metal levels in PM10 did not relate to washed leaves in the study can infer that metal accumulation in inner leaf tissue did not come directly from airborne fine particles. The airborne particle collected in the present study is PM10, which is only part of total airborne particles. This might be the reason of the insignificant relationship between leaf and air particle. It can be said that the penetration of metals through the stomata via respiration process is not the main cause of metal accumulation in leaves in the present study.

The enrichment factor (EF) is employed in the metal levels found in leaf and air particle samples to separate the anthropogenic source from the natural source. The findings show that the patterns of EF values of all sample types are similar. Four metals Cu, Ni, Pb and Zn are the most enriched elements and their EF values indicate the dominant sources of these elements are not from the natural. Besides, the Cr and Mn are found their origin from the natural.

The different metal amounts between washed and unwashed leaves can be used to distinguish between airborne and soilborne contamination. The effect of water washing treatment on metal concentrations in both unwashed and washed leaves is evaluated using statistical t-tests with significance ($p<0.05$) of the differences. The results show that washing the leaves reduces the concentrations of all metal contaminants at all sites significantly. This indicates that metals deposited on surface leaves play an important role in metal accumulation on leaves. The finding also confirms that the metals pollution originated from anthropogenic sources via the atmosphere not a function of soil type. Fe is the most metal removed by washing in both rural and urban sites. Meanwhile, the others vary between metals. The removal efficiencies by water washing are around 20% to 60%. The removal efficiency of washing on leaves in the study is in line with the other report. The contents of remained metals after washing vary according to plant species. The plant species that retain constant metal concentration in washed and unwashed treatment could be a more suitable biomonitor of metal pollution. It can be concluded that Orange jasmine could be a good biomonitor of airborne metal accumulation.

The investigation of metal level accumulated in different ages of leaves is evaluated in the study in addition. The statistical t-test reveals that Cu contents found in old and young leaves in each site are not significantly different except for the DLT site. The result also points that it has increasing tendency in Cu contents in the urban sites in the elapsed time while the opposite is found at the reference site. In contrast, the differences of Fe are significant at all sites except the DLT. Fe is found in high contents in old leaves at all sites. The high content of Mn in old leaves is found at the DLT and the MST sites but the opposite is found at the CHU and the reference sites. The significant difference of Mn in old and young leaves is only observed at the DLT. Meanwhile, notable differences of Zn concentrations between old and young leaves are found at the CHU and the reference sites. Like the Mn, the increasing of Zn in old leaves is found at the DLT and the MST sites while the decreasing of Zn is found at the CHU and the reference sites. The present finding gets along well with the previous reports which said that in the urban areas, the levels of Fe, Cu in leaves increased significantly in the course of time while the opposite happened to Mn and Zn.

The differences of metals contents accumulated in both washed and unwashed leaves in wet and dry season are investigated in the study as well. The statistical t-test reveals that most of the significant differences in metal concentrations are found at the DLT for washed leaves. By the way, the average concentration of all sites highlights the insignificant differences in all metals. This can be implied that the washing out effects of rain does not impact on metal accumulation in inner leaf tissue. The differences of metals contents accumulated in leaves in wet and dry season of unwashed leaves are also investigated in the study. The result is also found that there is no significant difference in all metals. This can be implied that the washing out effects of rain does not influence on metal accumulation in unwashed leaves as well as washed leaves. The accumulations of metals on leaf are depended on several factors. The particle deposited on leaf can be retained by the adhesive force of cuticle coated on the leaf surface. The chemistry of leaf surface wax in different plant species can affect on airborne particle retention including the accumulation of contaminants. Unlike the washing leaf in the laboratory nor heavy rain in the field, the common rain is probably not strong enough to wash out all over the particles deposited on the leaf surface. The finding of this gets along well with the previous report.

According to all above findings point that unwashed leaves of Orange jasmine is proper to be a good biomonitor of airborne metal, so the unwashed leaves are used in the study to investigate the possible sources of air metal pollution. The results of the source identification of air metal pollution using leaves study show that all three sampling sites are polluted with heavy metals compared with the reference site. The highest metal concentrations are found in the heavy traffic sites while the lowest metal concentrations were reported in the control site. The highest mean concentration of the studied metals is Fe, while Pb is the lowest. The mean values of metal concentrations are lower at the reference site compared to all other sampling sites. The correlation analysis shows that there is a high correlation coefficient between metals Cu-Mn, Cu-Zn, Cu-Pb, and Mn-Zn. However, Fe is not correlated to other metals. Correlation analysis is also effective in identifying the possible sources of metals in leaf samples. According to correlation analysis, traffic emissions pose the main source of metal pollution in the urban air of Bangkok. The ANOVA testing reveals that there is no a significant difference in Pb contents between sites. The phenomenon of the low level concentration of Pb at present might be from the Thai government's measure on phasing Pb out of gasoline since 1991. The significant difference in metals (Cu, Fe, Mn, and Zn) found in the study could be attributed to different anthropogenic activities between sites. The principal component analysis (PCA) shows two main factors according to the sources of metals found. Factor 1 and 2 are anthropogenic (traffic) and natural (soil) sources, respectively. The PCA study points that traffic emission is found to be the main source of metal pollution in the atmosphere.

Based on the overall study, it can be concluded that the airborne particles found in Bangkok are strongly impacted by anthropogenic sources especially traffic. The results also show that leaves of Orange jasmine *(Murraya paniculata)* can be used as bioindicator for metal air pollution.

5.2 Applications of using Orange jasmine for urban environmental management

Trees play an important role in being the air environment indicators. As non-mobile living, they are unavoidable to expose to the pollutants surrounding them. The nature of plant species and the properties of pollutants are both responsible for the toxicants accumulation. The using of higher plants for pollutants monitoring is recently popular because of its cost saving monitoring method as well as a direct assessment of pollutant contamination in the living. Only bioindicator techniques are able to accomplish both two requirements not a conventional method. Several bioindicator methods have been developed, tested, used and further refined, partly up to a high degree of standardization in some countries, for example, Germany (Weiss *et al.*, 2003). This present study recommends Orange jasmine as bioindicator plant for urban air quality monitoring. The standard procedure of using Orange jasmine as bioindicator should be further conducted. Even though the rigor protocol of using Orange jasmine as bioindicator can not be set from the present study, the guideline of using this species, which is higher plant, is recommended. The in-depth procedure and advices in the field of bioindicator can be found in several comprehensive articles, books and standard guidelines, for example, Markert (2007); Wittig (1993); and VDI 3957/2, 2003: *Method of Standardized Grass Exposure, Part 2,* Beuth Verlag GmbH, Berlin. Factors influencing pollutant concentrations in plant should be considered. Weiss *et al.* (2003) describe that the factors should include 1.type of deposition, 2.the uptake mechanisms, 3.accumulation, losses, degradation and metabolism of compounds. Further abiotic and biotic influences on the pollutant concentrations in plants should also be concerned. These include 1.indicator-related accumulation behavior, 2.external biotic influences, 3.external abiotic influences. The external abiotic influences cover the meteorological conditions, soil conditions, location of the plant and atmospheric pollution and pollution patterns. The methodical considerations and limitations are also important aspects to be concerned. Weiss *et al* (2003) also stated the brief overview on the relevant issues which are 1.selection of the appropriate bioindicator and bioindication technique. 2.selection and number of sites, 3.duration of exposure and sampling date, 4.guidelines, 5.adequate resources, 6.sampling, 7.sample treatment, 8.chemical analysis and 9.documentation and statistical analysis.

The benefit of growing plant like Orange jasmine in the urban area is not only pollutants bioindicator but also the effective filter out air pollutants. Several studies reveal that trees are effective in capture of particles from urban air to extent that they can significantly improve urban air quality (Freer-Smith *et al.*, 2005). Tree can act as biological filter, removing large numbers of airborne particles and hence improving the quality of air in polluted environments (Beckett *et al.*, 1998). It can be said that, the urban air quality can be improved by growing more trees in towns and cities.

5.3 Recommendation for further study

Airborne pollution is still a crucial problem not only in Bangkok but also other places. The present study is a beginning of using easy, reliable, inexpensive and effective biological tool to monitor the air quality in city. The more understanding about the spatial and temporal pattern and more types of pollutants should be further conducted. The more elemental components in airborne particulate should be identified in the next study for better understanding on the possible sources of airborne particles. Moreover, the other pollutants such as polycyclic aromatic hydrocarbons (PAHs) should be paid an attention in the next investigation.

In addition, the using of different plant parts like bark either the other plant species for being bioindicators should be conducted. Finding the other effective species not only plants should also be concerned. The short and long term toxicity of particle pollution and its components on living and human health should be further studied also to understand the specific impact well.

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APPENDICIES

APPENDIX A

Biology and distributions of Orange jasmine (*Murraya paniculata*)

Source: http://www.hear.org/pier/species/murraya_paniculata.htm

Accessed date 16 September 2008

Murraya paniculata (L.) Jack, Rutacea

Present on Pacific Islands? yes

Threat only at high elevations? no

Risk assessment results: Evaluate, score: 6

Other Latin names: *Chalca spaniculata* L., *Murraya exotica* L.

Common name(s):

English: Chinese box, cosmetic bark tree, Hawaiian mock orange, jasmine orange, mock orange, orange jasmine, orange jessamine, satinwood
French: bois de Chine, rameau
Maori (Cook Islands): tiare ‘ānani
Tahitian: tiare ‘ānani

Habit: shrub/tree

Description: "Small **tree** or **shrub** to 7 m tall, evergreen; **leaves** pinnately 3-7 foliolate, or unifoliolate, glabrous and glossy; elliptic to cuneate-obovate to rhombic; **inflorescence** terminal, corymbose, few-flowered, dense, fragrant; petals 12-18 mm long, recurved, white (fading cream); **fruit** oblong-ovoid, red to orange" (Welsh, 1998; p. 256).

Habitat/ecology: Favors limestone soils (Stone, 1970; p. 350).

Propagation: Seed, possibly bird-dispersed.

Native range: India to Malaya, cultivated and now pantropical.

Presence:

Pacific			
Country/Terr./St. & Island group	**Location**	**Cited status & Cited as invasive & Cited as cultivated & Cited as aboriginal introduction?**	**Reference & Comments**
Commonwealth of the Northern Mariana Islands (US) Northern Mariana Islands	Saipan Island	introduced	Raulerson, L. (2006) (p. 40)
Cook Islands Southern Cook Islands	‘Atiu Island	cultivated	McCormack, Gerald (2007) Uncommon.
Cook Islands Southern Cook Islands	‘Atiu Island	introduced cultivated	Space, James C./Flynn, Tim (2002) (p. 81)
Cook Islands Southern Cook Islands	Mangaia Island	cultivated	McCormack, Gerald (2007) Uncommon.
Cook Islands Southern Cook Islands	Rarotonga Island	cultivated	McCormack, Gerald (2007) Uncommon.
Ecuador (Galápagos Islands) Santa Cruz Group	Santa Cruz Island	introduced cultivated	Charles Darwin Research Station (2005)
Fiji Fiji Islands	Viti Levu Island	introduced cultivated	Smith, Albert C. (1985) (p. 513) Vouchers cited: Greenwood 8, DA 18046, DA 578, DA 1123, DA 3470
Fiji Fiji Islands	Viti Levu Island		Bishop Museum (Honolulu) (1972) (voucher ID: BISH 147560) Taxon name on voucher: Murraya paniculata (L.) Jack
French Polynesia Society Islands	Raiatea (Havai) Island	introduced cultivated	Welsh, S. L. (1998) (p. 256) Voucher cited: Moore 361

French Polynesia Society Islands	Tahiti Island	introduced cultivated	Welsh, S. L. (1998) (p. 256) Vouchers cited: Grant 4127, Florence 2459
French Polynesia Society Islands	Tahiti Island		Bishop Museum (Honolulu) (1982) (voucher ID: BISH 491731) Taxon name on voucher: Murraya paniculata (L.) Jack
Guam Guam Island	Guam Island	introduced cultivated	Stone, Benjamin C. (1970) (p. 350) Voucher cited: Stone 5091 (GUAM)
Guam Guam Island	Guam Island	introduced	Fosberg, F. R./Sachet, Marie-Hélène/Oliver, Royce (1979) (p. 125)
State of Hawaii Hawaiian Islands	Hawaiian Islands	introduced cultivated	Staples, George W./Herbst, Derral/Imada, Clyde T. (2000) (p. 29)
State of Hawaii Hawaiian Islands	Maui Island	introduced invasive	Starr, Forest/Starr, Kim/Loope, Lloyd L. (2003) (p. 32) East Maui. Voucher cited: Starr & Martz 010726-7 (BISH) Sparingly naturalized; all life stages present in pastures and along fence lines near Puka‘auhuhu.
State of Hawaii Hawaiian Islands	O‘ahu Island	introduced invasive	Starr, Forest/Starr, Kim/Loope, Lloyd L. (2003) (p. 32) Naturalized
Japan (offshore islands) Bonin (Ogasawara) Islands	Bonin (Ogasawara) Islands	introduced	Kato, Hidetoshi (2007)
Marshall Islands Ralik Chain	Kwajalein (Kuwajleen) Atoll	introduced	Fosberg, F. R./Sachet, Marie-Hélène/Oliver, Royce (1979) (p. 125)
Marshall Islands Ralik Chain	Kwajalein (Kuwajleen) Atoll	introduced	Whistler, W. A./Steele, O. (1999) (p. 102) Not seen on this survey and may no longer be present.
Marshall Islands Ratak Chain	Majuro (Mãjro) Atoll	introduced cultivated	Vander Velde, Nancy (2003) (p. 123)
Nauru Nauru Island	Nauru Island	introduced cultivated	Thaman, R. R./Fosberg, F. R./Manner, H. I./Hassall, D. C. (1994) (pp. 192-193) Voucher cited: Hassall 184 (SUVA)

New Caledonia New Caledonia	New Caledonia Islands		Bishop Museum (Honolulu) (1867) (voucher ID: BISH 178258) Taxon name on voucher: Murraya paniculata (L.) Jack
New Caledonia New Caledonia Archipelago	Île Grande Terre	introduced cultivated	MacKee, H. S. (1994) (p. 126) Vouchers cited: Baumann 9898, MacKee 12128, MacKee 38284
Niue Niue	Niue Island	introduced cultivated	Space, James C./Waterhouse, Barbara M./Newfield, Melanie/Bull, Cate (2004) (p. 44) Cult. and being propagated at Vaipapahi nursery.
Palau Palau (main island group)	Babeldaob Island	introduced cultivated	Space, James C./Waterhouse, Barbara/Miles, Joel E./Tiobech, Joseph/Rengulbai, Kashgar (2003) (pp. 16, 86)
Palau Palau (main island group)	Koror Island	introduced cultivated	Space, James C./Waterhouse, Barbara/Miles, Joel E./Tiobech, Joseph/Rengulbai, Kashgar (2003) (pp. 16, 81)
Palau Palau (main island group)	Ngerkebesang Island	introduced cultivated	Space, James C./Waterhouse, Barbara/Miles, Joel E./Tiobech, Joseph/Rengulbai, Kashgar (2003) (pp. 16, 81)
Philippines Philippine Islands	Philippine Islands	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Samoa Western Samoa Islands	Upolu Island	introduced cultivated	Space, James C./Flynn, Tim (2002) (p. 12)
Solomon Islands Solomon Islands	Solomon Islands	native	Hancock, I. R./Henderson, C. P. (1988) (p. 92)
Tonga Ha‘apai Group	Ha‘ano Island	introduced cultivated	Space, James C./Flynn, Tim (2001) (p. 10)
Tonga Ha‘apai Group	Lifuka and Foa Islands	introduced cultivated	Space, James C./Flynn, Tim (2001) (p. 10)
Tonga Tongatapu Group	‘Eua Island	introduced cultivated	Space, James C./Flynn, Tim (2001) (p. 10)
Tonga Tongatapu Group	Tongatapu Island	introduced cultivated	Space, James C./Flynn, Tim (2001) (p. 10)
Tonga Vava‘u Group	Vava‘u Island	introduced cultivated	Space, James C./Flynn, Tim (2001) (p. 10)

Vanuatu New Hebrides Islands	Malakula (Malekula) Island		Bishop Museum (Honolulu) (1988) (voucher ID: BISH 628581) Taxon name on voucher: Murraya paniculata (L.) Jack
Vanuatu New Hebrides Islands	Tanna Island		Bishop Museum (Honolulu) (1928) (voucher ID: BISH 178645) Taxon name on voucher: Murraya paniculata (L.) Jack
Vanuatu New Hebrides Islands	Tanna Island		Bishop Museum (Honolulu) (1978) (voucher ID: BISH 424732) Taxon name on voucher: Murraya paniculata (L.) Jack
Pacific Rim			
Country/Terr./St. & Island group	**Location**	**Cited status & Cited as invasive & Cited as cultivated & Cited as aboriginal introduction?**	**Reference & Comments**
Australia Australia (continental)	Northern Territory	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Australia Australia (continental)	Queensland	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Cambodia Cambodia	Cambodia (Kingdom of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
China China	China (People's Republic of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Indonesia Indonesia	Indonesia (Republic of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Malaysia Malaysia	Malaysia (country of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)

Taiwan Taiwan	Taiwan Island	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Thailand Thailand	Thailand (Kingdom of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)
Vietnam Vietnam	Vietnam (Socialist Republic of)	native	U.S. Dept. Agr., Agr. Res. Serv. (2007)

Indian Ocean

Country/Terr./St. & Island group	**Location**	**Cited status & Cited as invasive & Cited as cultivated & Cited as aboriginal introduction?**	**Reference & Comments**
Australia (Indian Ocean offshore islands) Christmas Island Group	Christmas Island	native	Orchard, Anthony E., ed. (1993) (p. 19)
La Réunion (France) La Réunion Island	La Réunion Island	introduced invasive cultivated	Lavergne, Christophe (2006) "Cultivé/subspontanée"
Mauritius Mautitius Islands (Mauritius and Rodrigues)	Mauritius Island	introduced	Lorence, D./Sussman, R. W. (1986) (p. 156)

Also reported from

Country/Terr./St. & Island group	**Location**	**Cited status & Cited as invasive & Cited as cultivated & Cited as aboriginal introduction?**	**Reference & Comments**
United States (continental except west coast) United States (other states)	USA (Florida)	introduced	U.S. Dept. Agr., Nat. Res. Cons. Serv. (2005)

Comments: "*Murraya exotica* L. is said to differ from *M. paniculata* in the larger leaves (less than 4, not 3-7) cm long and petals rarely over 2 cm long (not sometimes over 2 cm long)" (Welsh (1998). Smith (1985) and Stone (1970) list them as synonyms).

Extensively used as a hedge and ornamental plant in Tonga but no naturalization noted so far (Space & Flynn, 2001).

"In Asia, *M. paniculata* is the preferred host to the insect pest *Diaphorina citri*, the citrus psyllid. The psyllid is the vector for the serious disease "citrus greening" or 'huanglongbing'. Both the psyllid and the disease have recently been confirmed in Irian Jaya and it seems almost unavoidable that they will move into Papua New Guinea. The disease is very damaging to citrus in Asia and Australia is so concerned about both the psyllid and the disease moving in her general direction that we are supporting an international program exploring the potential for biological control of the psyllid." (Barbara Waterhouse, pers. com.)

Additional information: Additional online information about *Murraya paniculata* is available from the Hawaiian Ecosystems at Risk project (HEAR).

Taxonomic information about *Murraya paniculata* may be available from the Germplasm Resources Information Network (GRIN).

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APPENDIX B

Table of raw data used in the study

Abbreviation used in raw data table

oul = Old unwashed leaf at Department of Land Transportation site

owl = Old washed leaf at Department of Land Transportation site

yul = Young unwashed leaf at Department of Land Transportation site

sol = Soil at Department of Land Transportation site

PML = Particulate matter at Department of Land Transportation site

ouc = Old unwashed leaf at Chulalongkorn Hospital site

owc = Old washed leaf at Chulalongkorn Hospital site

yuc = Young unwashed leaf at Chulalongkorn Hospital site

soc = Soil at Chulalongkorn Hospital site

PMC = Particulate matter at Chulalongkorn Hospital site

oum = Old unwashed leaf at Ministry of Science and Technology site

owm = Old washed leaf at Ministry of Science and Technology site

yum = Young unwashed leaf at Ministry of Science and Technology site

som = Soil at Ministry of Science and Technology site

PMM = Particulate matter at Ministry of Science and Technology site

oub = Old unwashed leaf at Bangkok University site

owb = Old washed leaf at Bangkok University site

yub = Young unwashed leaf at Bangkok University site

sob = Soil at Bangkok University site

PMB = Particulate matter at Bangkok University site

ND = Non detectable

Metal concentrations (mg/kg dry weight) for leaf and soil samples

Metal concentrations (ng/m^3) for particulate matter samples

Wet season: 5 October 2006 to 29 October 2006

Dry season: 22 December 2006 to 15 January 2007

oul	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		14.39	115.64	70.97		3.47	48.64
11-Oct-06		15.30	116.21	62.17		1.43	35.87
17-Oct-06		16.56	104.26	69.67		2.92	33.13
23-Oct-06		6.64	92.94	33.19		0.95	23.71
29-Oct-06		15.41	168.05	44.30		2.89	42.85
22-Dec-06		9.86	138.08	54.74		1.97	20.71
28-Dec-06		8.95	157.07	29.82		1.49	20.88
3-Jan-07		8.96	129.38	49.76		1.99	22.89
9-Jan-07		9.44	177.81	53.64		1.99	28.31
15-Jan-07		12.44	139.36	51.76		1.99	25.88

owl	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		11.82	55.18	45.32			23.65
11-Oct-06		11.44	40.50	47.17			26.68
17-Oct-06		9.95	38.83	58.72			20.36
23-Oct-06		10.35	57.19	51.76			31.55
29-Oct-06		9.07	54.87	41.27			19.95
22-Dec-06		5.46	35.72	40.18			12.90
28-Dec-06		7.37	35.85	20.14			14.24
3-Jan-07		8.19	41.92	27.95			15.42
9-Jan-07		6.80	56.32	32.04			15.05
15-Jan-07		6.46	25.85	20.88			21.37

ywl	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		4.78	29.62	35.83			27.23
11-Oct-06		6.25	23.54	23.06			24.02
17-Oct-06		5.93	93.09	24.19			20.53
23-Oct-06		3.96	21.27	13.35			11.87
29-Oct-06		5.30	19.29	16.88			13.98
22-Dec-06		4.96	65.47	22.82			17.86
28-Dec-06		5.42	66.52	32.52			18.23
3-Jan-07		5.33	42.65	33.44			18.90
9-Jan-07		4.47	20.35	23.33			14.39
15-Jan-07		5.95	19.85	37.71			15.38

sol	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	4.01	9.03	7331.63	350.80	11.04	10.04	90.33
11-Oct-06	6.21	15.30	6935.95	209.37	39.20	11.95	97.99
17-Oct-06	5.34	18.93	8011.17	250.97	7.77	13.59	210.19
23-Oct-06	5.94	15.84	6782.53	263.42	8.42	10.40	174.29
29-Oct-06	7.39	23.16	9283.53	357.74	20.20	15.28	248.84
22-Dec-06	12.67	16.56	7804.46	249.90	27.77	11.69	186.57
28-Dec-06	7.44	21.83	8413.04	360.15	10.42	14.88	239.11
3-Jan-07	6.77	34.84	10251.14	474.69	15.00	20.81	331.46
9-Jan-07	6.49	21.95	8243.86	258.43	16.96	14.97	264.42
15-Jan-07	5.26	18.65	8981.25	342.45	28.70	14.35	184.14

PML	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	15.20	212.79	489.42	9.12	182.39	24.32	151.99
11-Oct-06	15.66	363.33	836.29	12.53	407.18	25.06	206.72
17-Oct-06	9.12	310.19	894.06	12.16	988.33	18.25	127.72
23-Oct-06	18.21	212.48	843.85	9.11	60.71	27.32	130.52
29-Oct-06	15.33	211.49	962.42	27.59	649.78	116.47	327.96
22-Dec-06	12.45	305.09	1874.13	102.73	267.73	152.55	376.69
28-Dec-06	12.52	291.02	2199.87	50.07	12.52	84.49	284.76
3-Jan-07	9.37	290.49	1864.79	31.24	81.21	56.22	206.16
9-Jan-07	15.72	194.87	1738.10	53.43	9.43	53.43	213.73
15-Jan-07	9.28	210.41	1380.04	27.85	9.28	37.13	185.66

ouc	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		8.98	140.66	12.97		2.00	21.45
11-Oct-06		9.32	118.78	14.44		1.86	17.70
17-Oct-06		8.64	119.07	11.04		1.44	18.72
23-Oct-06		8.95	172.59	13.93		1.99	18.90
29-Oct-06		9.72	123.02	14.59		0.97	18.48
22-Dec-06		9.90	154.41	13.86		1.48	17.82
28-Dec-06		11.14	144.78	18.40		2.91	23.24
3-Jan-07		9.85	124.09	18.22		1.97	20.19
9-Jan-07		10.95	216.57	22.90		2.49	23.40
15-Jan-07		11.35	115.43	20.22		2.47	26.64

owc	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		7.35	47.06	10.79			15.20
11-Oct-06		6.34	61.98	10.25			15.13
17-Oct-06		7.07	54.68	11.78			15.08
23-Oct-06		7.36	95.25	18.17			17.18
29-Oct-06		6.87	62.83	16.20			18.65
22-Dec-06		8.96	120.01	11.45			17.43
28-Dec-06		8.48	129.99	9.89			11.30
3-Jan-07		8.41	91.97	13.84			18.30
9-Jan-07		7.20	78.26	9.60			15.36
15-Jan-07		8.92	60.94	9.91			16.85

ywc	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		7.74	18.39	11.61			21.77
11-Oct-06		6.65	31.33	9.97			17.56
17-Oct-06		6.08	17.30	7.95			14.96
23-Oct-06		7.66	42.59	11.96			14.36
29-Oct-06		4.95	39.14	9.91			15.86
22-Dec-06		5.98	68.25	19.43			22.42
28-Dec-06		5.97	29.35	12.44			19.90
3-Jan-07		8.49	74.04	16.98			24.05
9-Jan-07		6.45	42.67	15.38			19.85
15-Jan-07		10.10	43.63	20.21			23.88

soc	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	7.05	13.17	9290.01	234.15	12.22	26.33	121.78
11-Oct-06	6.44	13.34	9726.67	229.16	14.72	28.53	138.97
17-Oct-06	5.81	15.97	5953.44	164.55	5.81	19.36	86.15
23-Oct-06	7.16	12.41	3227.65	165.68	62.55	21.01	95.49
29-Oct-06	7.38	12.80	7109.67	250.05	12.31	27.07	120.59
22-Dec-06	5.87	12.24	9909.43	292.76	22.52	43.08	176.74
28-Dec-06	6.87	13.29	4526.17	223.63	12.83	16.04	80.65
3-Jan-07	6.89	18.20	8863.14	215.47	9.35	29.52	194.31
9-Jan-07	5.48	20.10	7992.51	244.43	7.77	32.44	376.92
15-Jan-07	8.57	19.05	5155.30	150.06	6.19	17.63	128.62

PMC	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	18.18	151.54	1118.36	27.28	163.66	36.37	269.74
11-Oct-06	ND	ND	971.63	15.18	ND	ND	200.40
17-Oct-06							
23-Oct-06	18.35	159.01	1036.63	18.35	275.21	27.52	229.34
29-Oct-06	18.50	86.34	1242.68	37.00	339.19	117.18	302.19
22-Dec-06	8.86	100.37	1520.30	73.80	862.00	135.79	277.49
28-Dec-06	ND	ND	2065.05	58.56	ND	ND	314.38
3-Jan-07	15.13	178.48	2190.22	51.43	60.50	184.54	465.88
9-Jan-07							
15-Jan-07	9.51	63.39	1439.05	34.87	19.02	38.04	177.50

oum	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		11.29	160.44	24.47		1.88	29.64
11-Oct-06		11.78	553.93	25.44		2.36	34.86
17-Oct-06		11.38	266.45	25.13		2.37	32.71
23-Oct-06		11.89	602.89	31.21		0.99	37.15
29-Oct-06		3.42	231.60	10.26		0.98	16.61
22-Dec-06		9.91	263.50	25.26		1.49	23.77
28-Dec-06		11.96	268.12	23.92		1.99	27.91
3-Jan-07		13.96	410.31	28.92		2.49	34.90
9-Jan-07		11.87	270.03	23.74		1.98	33.14
15-Jan-07		12.29	220.73	29.00		1.97	28.51

owm	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		8.45	169.58	20.89			26.36
11-Oct-06		9.28	212.85	16.60			23.92
17-Oct-06		7.48	187.47	18.23			27.12
23-Oct-06		6.43	142.84	15.82			19.77
29-Oct-06		7.01	219.30	17.30			22.44
22-Dec-06		2.99	122.52	18.43			20.92
28-Dec-06		8.17	169.15	13.46			26.91
3-Jan-07		8.93	135.51	22.34			26.31
9-Jan-07		7.94	137.91	16.37			22.32
15-Jan-07		7.48	131.76	14.48			24.30

ywm	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		7.45	92.41	17.89			25.83
11-Oct-06		6.29	65.28	18.86			27.08
17-Oct-06		5.79	57.86	17.84			25.56
23-Oct-06		4.00	50.97	7.00			11.49
29-Oct-06		5.43	65.61	10.36			14.80
22-Dec-06		6.95	75.50	19.87			20.37
28-Dec-06		6.78	26.64	14.05			19.86
3-Jan-07		6.93	50.81	12.93			18.48
9-Jan-07		8.28	65.25	16.56			19.48
15-Jan-07		9.68	47.44	22.27			24.69

som	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	19.87	69.32	19652.03	313.77	17.10	70.70	226.43
11-Oct-06	17.11	98.51	14519.47	254.83	12.95	77.70	228.47
17-Oct-06	20.32	91.69	12971.45	264.68	18.43	50.10	303.43
23-Oct-06	24.20	97.77	15012.84	255.28	12.34	66.66	252.81
29-Oct-06	19.81	51.51	14426.01	200.08	16.34	51.51	215.43
22-Dec-06	21.13	89.33	12970.90	234.85	14.41	62.43	279.03
28-Dec-06	19.67	78.68	12247.25	222.76	14.75	56.55	235.05
3-Jan-07	18.36	77.29	14061.44	217.85	14.49	57.97	260.36
9-Jan-07	14.72	61.20	13150.19	244.34	11.04	69.94	215.35
15-Jan-07	12.12	79.53	11956.35	209.02	13.58	57.71	275.46

PMM	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	21.37	491.60	784.73	18.32	403.05	27.48	216.79
11-Oct-06	ND	ND	1057.55	21.40	ND	ND	278.14
17-Oct-06	15.26	396.73	1116.95	30.52	82.40	57.98	415.04
23-Oct-06	12.05	292.16	771.06	12.05	231.92	27.11	171.68
29-Oct-06	18.07	430.77	1093.49	33.14	1430.88	120.49	337.39
22-Dec-06	15.15	860.47	1757.29	109.07	15.15	154.52	384.79
28-Dec-06							
3-Jan-07	6.08	857.30	1270.75	24.32	36.48	48.64	145.92
9-Jan-07	12.14	473.60	2504.61	81.97	42.50	54.65	248.94
15-Jan-07	12.21	637.75	2612.03	61.03	30.51	42.72	259.37

oub	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		5.85	142.47	17.56		1.95	16.59
11-Oct-06		6.01	141.91	12.48		1.39	18.03
17-Oct-06		6.12	161.05	13.19		1.41	17.42
23-Oct-06		6.46	150.51	12.42		0.99	20.86
29-Oct-06		6.41	217.41	13.31		1.97	20.21
22-Dec-06		5.92	157.81	12.33		1.48	17.75
28-Dec-06		7.93	163.17	13.52		1.86	20.05
3-Jan-07		8.45	161.60	15.41		2.98	26.35
9-Jan-07		7.94	198.38	16.80		2.33	27.07
15-Jan-07		6.53	76.92	13.52		1.86	22.38

owb	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		0.50	51.20	11.93			13.92
11-Oct-06		4.93	74.41	11.83			16.75
17-Oct-06		0.49	85.01	11.79			15.72
23-Oct-06		4.95	91.13	8.91			13.87
29-Oct-06		4.94	64.25	9.88			14.83
22-Dec-06		5.94	77.24	9.90			17.82
28-Dec-06		5.92	90.80	13.82			18.75
3-Jan-07		5.87	66.54	11.25			18.10
9-Jan-07		5.92	95.24	11.35			13.82
15-Jan-07		6.43	77.15	12.86			14.24

ywb	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06		4.92	28.55	14.77			16.25
11-Oct-06		5.76	11.52	10.56			17.29
17-Oct-06		6.98	69.31	11.97			18.45
23-Oct-06		8.42	48.05	13.87			20.81
29-Oct-06		3.95	23.18	10.36			15.78
22-Dec-06		0.50	81.98	12.50			18.99
28-Dec-06		6.24	22.07	11.04			19.19
3-Jan-07		5.96	54.63	13.41			21.85
9-Jan-07		6.57	58.22	12.21			19.25
15-Jan-07		7.67	20.62	13.43			19.66

sob	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06	8.31	0.98	8898.55	326.43	12.22	20.52	46.91
11-Oct-06	3.68	4.59	7237.69	160.79	22.97	11.48	40.43
17-Oct-06	0.95	1.91	6379.74	111.72	38.19	9.55	28.17
23-Oct-06	3.95	4.44	7295.31	150.62	5.43	11.36	37.04
29-Oct-06	5.24	7.62	7124.23	146.60	28.56	11.42	39.98
22-Dec-06	4.97	4.97	7954.88	168.44	20.87	11.92	44.72
28-Dec-06	3.79	3.32	7838.94	172.43	12.32	15.16	52.11
3-Jan-07	2.42	3.38	5931.19	129.02	4.35	10.15	32.86
9-Jan-07	3.45	3.94	6682.09	159.00	4.43	11.32	46.27
15-Jan-07	2.93	3.91	7021.21	157.88	4.40	11.24	40.57

PMB	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5-Oct-06							
11-Oct-06	12.73	840.03	773.21	25.46	305.46	143.19	614.11
17-Oct-06	19.40	633.64	678.90	25.86	200.44	45.26	384.71
23-Oct-06	9.68	587.18	512.98	12.91	432.32	29.04	390.38
29-Oct-06	38.79	465.51	1008.61	32.33	924.56	132.54	303.88
22-Dec-06	9.18	443.89	900.03	24.49	186.74	131.64	251.03
28-Dec-06	9.15	613.25	1382.09	39.66	61.02	54.92	207.47
3-Jan-07	6.00	558.24	690.30	21.01	24.01	27.01	108.05
9-Jan-07	5.99	446.59	884.19	53.95	29.97	89.92	221.80
15-Jan-07	12.18	554.21	1352.03	42.63	91.35	91.35	1120.61

BIOGRAPHY

Mr. Teerawet Titseesang was born on December 14, 1971 in Bangkok. He received his B.Sc (2^{nd} class Honors) in General Science (concentrated in Environmental Science) from Chulalongkorn University. He also received degrees B.Pol.Sc (International Relations and Comparative Government and Politics), B.A (Information Sciences), B.Ed (Educational Administration), B.P.H (Occupational Health and Safety), B.B.A (Marketing), and B.B.A (General Management) from Sukhothai Thammathirat Open University. He gained his M.Sc (Environmental Science) from Chulalongkorn University. He was a lecturer at Department of Health Science, Faculty of Science, Thammasat University before moving to industrial sector as Head of Occupational Health, Safety and Environment department. At present, he works as a lecturer in a private university in Bangkok.