

Epicuticular wax concentration on *Syzygium myrtifolium* leaves

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Abstract : The pollution issues are a pervasive and increasing threat to human and forest ecosystem. Plants have been recognised as a method to reduce pollution. However, leaf is the most sensitive organ to pollution where changes in leaf morphological, physiological, anatomical and biochemical can occur. This study was conducted to determine the quantity of epicuticular wax on *Syzygium myrtifolium* leaves in urban, suburban and industrial areas. Three trees were selected in each of the study sites and new leaves on the branch was marked. 5 g of samples were collected for analysis. Leaf surface particles were extracted by using 20 ml of chloroform in conical flask and was left overnight for hardening where weight were recorded. Data analysis showed that the mean epicuticular wax ranged from 267.2-680.7 $\mu\text{g}/\text{m}^2$ with a mean increment of 309.6-950.7 $\mu\text{g}/\text{m}^2/\text{yr}$. The epicuticular wax concentration on *S. myrtifolium* leaves was significantly higher in industrial areas compared to those in the urban and suburban areas. *S. myrtifolium* at industrial areas are in the open and subjected to direct sunlight and also responding to stress caused by the air pollutions. The low concentration of epicuticular wax was recorded on *S. myrtifolium* leaves in urban area. These trees are subjected to high concentration of pollutants especially from exhaust emissions and possible is due to the aerosol deposition which have been carried by the wind. This initial study reveal that the level of physiological stress on the trees in industrial, suburban and urban areas are at a different scale where the levels of air pollution is different. Understanding the tree traits are important to capitalize their role as bio filters. This study provide the baseline data for future study where the relationship between the particulate matters deposited on the tree canopy and the tree trait could be explained.

Keywords: *Epicuticular wax, industrial area, suburban area, Syzygium myrtifolium, urban area.*

I. Introduction

Industrialization and urbanization is a global phenomenon and has transformed urban socially, economically and environmentally. Positive development also resulted in a degradation of the environment quality. Developing countries, in the case of India, has over 26.5 million vehicles which resulted in several environmental pollution problems [1] while in a review, the forests of East Asian region having the same fate [2]. The pollution issues are a pervasive and increasing threat to human and the forest ecosystem. This is because pollutants can travel long distances from their sources [3].

Plants have been recognised as a method to reduce pollution [4]. This is due to the fact that urban forest ecosystem plays an important role as interface between the atmosphere and forest ecosystems [5]. Hence, trees can improve quality urban life where they act as biological absorbers of pollutants [4], especially removing particulate matter and their associated semi-volatile organic compounds from the atmosphere [6]. The ability of plant species to mitigate these pollutants varies significantly due to differences in their leaf surface characteristics such as epicuticular wax, cuticle, epidermis, stomata and trichomes [7]. Epicuticular wax in plants is a wax coat covering the outer surface of the plant cuticle. It functions as a UV light reflector, decrease surface wetting and moisture loss, self-cleaning surface and anti-climb surface as in *Macaranga* spp. [8; 9]. Therefore, epicuticular wax is an important structural element of the leaf surface that facilitates in the plants and environment interaction [10].

In polluted areas, leaf is the most sensitive organ to pollution [11] as pollutants are deposited on the leaves surface. Many studies showed that pollutants cause alteration to the leaf morphological, physiological, anatomical and biochemical. Species that react to presence of air pollutants showing visible symptoms can be used as bio indicators. Studies found that epicuticular wax on the conifer needles are degraded due to the effects of air pollutions [12; 13], and changes in its structure [14]. The rate of wax erosion correlates with the level of air pollution. This causes changes needle or leaf wettability and rain retention. In addition, delay development

and rapid erosion of epicuticular wax were recorded in needles which are subjected to simulated acid-rain test and aerosol particles [15; 13]. These changes increases the cuticular transpiration rate and affect plant growth [16]. However, larger quantity of epicuticular wax occurs when trees are under drought condition [17].

The urban forest ecosystems in Bintulu, Sarawak showed the potentially problems in relation to pollutions because of the increase use of oil products and the physical development due to high investment on heavy industries. Among the common trees in the urban ecosystem in Malaysia are such as Kelat paya, merawan, kayu manis, angkana and rain tree [18]. *Syzygium myrtifolium* or locally known as Kelat Paya is from the family Myrtaceae. It has a dense canopy with a mix of green and red-maroon leaves. However, information in relation to the epicuticular wax of tropical trees in this country is rather limited. Some efforts were recorded where [19] conducted chemical constituent of wax lipids from leaf surfaces of 10 common roadside trees in Kuala Lumpur, Malaysia while [20] did similar analysis for *Litsea* spp in Sarawak, Malaysia.

This study was conducted to determine the quantity of epicuticular wax on *S. myrtifolium* leaves. The leave samples were collected from trees in various locations in Bintulu District where some are near to the oil-based industrial estates (Kidurong), wood-based industrial estates (Kemena), town centre and suburban area (Stadium Muhibbah and UPMKB). Information will help to provide understanding on the effects of anthropogenic factors on the concentration of wax on the leaves.

II. Materials and Methods

2.1 Study sites

The study was conducted in various locations in the Bintulu District, Sarawak, Malaysia. Bintulu has been designated to be an industrial city by the State Government of Sarawak. Major physical development is occurring which may elevate certain environmental issues. In this study six sites were selected where 3 sites are located near the industrial estates, 1 in urban area and 2 in suburban areas. Site 1 (Sekolah Kebangsaan Asykirin), Site 2 (Vocational Bintulu) and Site 6 (Sekolah Menengah Kai Dee) are near to the industrial estates. Sites 1 and 2 are on the northern site (Fig 1) which is in the Kidurong Industrial Area (KINDA) and Kidurong Light Industrial Estate (KLIE). The main industries are the oil and gas processing industries, palm oil refinery, wood based, glue and cement factories including a power plant. As for Site 6 (southern side) is near to timber-based industries in the Kemena Industrial Estate (KIE). Site 3 (Stadium Muhibbah), Site 4 (Universiti Putra Malaysia Bintulu Sarawak Campus) is considered as suburban area while Site 5 (St Anthony Church) in located in the town centre (Fig. 1) is considered as urban area.

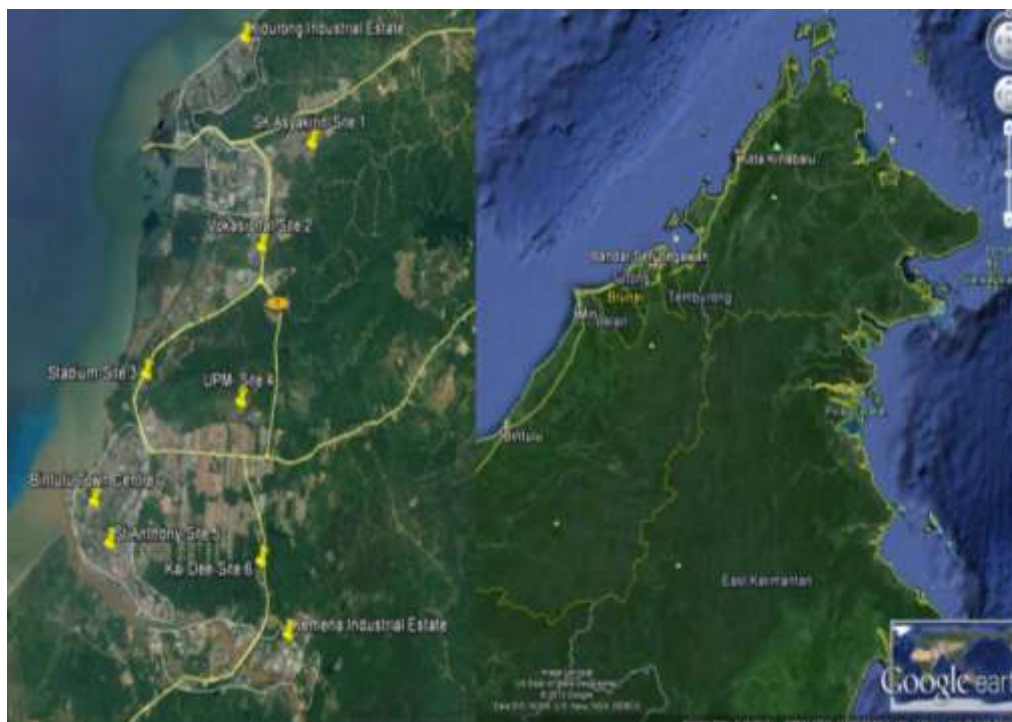


Figure. 1. The location of study sites in this study

2.2 Leaf samples.

The common tree species found in the six study sites was *Syzygium myrtifolium*. On May 2015, three trees were selected in each of the study sites and new leaves on the branch was marked. The new leaves are

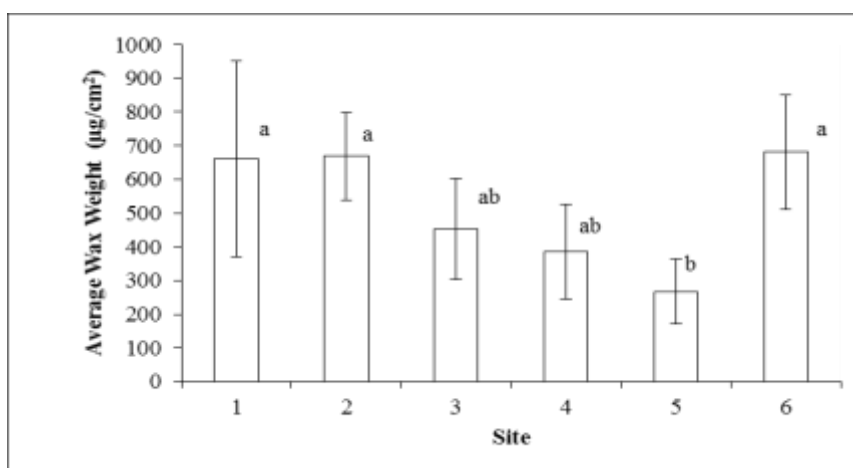
indicated by the maroon-coloured leaves. Leaves were harvested in January and March 2016. Leaves from the 3 branches are composited and 5 g of samples were collected for analysis. At the laboratory, the leaf samples were washed with de-ionized water and gently dried under room temperature on the filter paper. Leaf-surface particles were extracted by using 20 ml of chloroform in conical flask for 30 seconds to dissolve epicuticular wax on the leaf surface. The epicuticular wax was left overnight for hardening and the weight were recorded.

2.3 Data analysis

Analysis of variance was used using the SAS for window version 9.13 software. This was used to determine the significant differences epicuticular wax concentration among the study sites. Results which showed significant differences in means were differentiated using Tukey's Range Test.

III. Results and Discussion

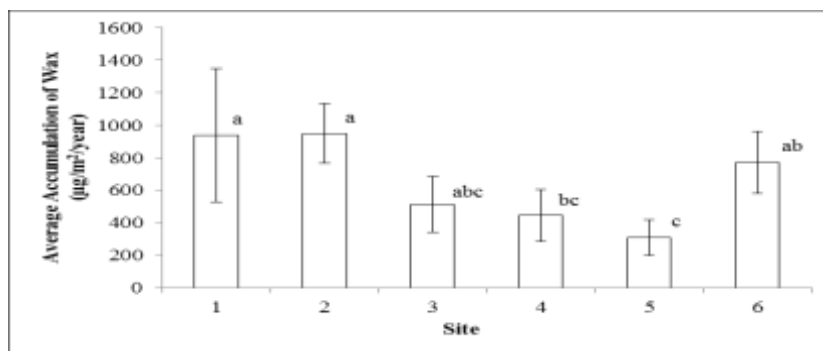
The mean epicuticular wax ranges from 267.2-680.7 $\mu\text{g}/\text{m}^2$ (Fig. 2) with a mean increment of 309.6-950.7 $\mu\text{g}/\text{m}^2/\text{yr}$ (Fig. 3). There is relative little information data regarding to the epicuticular wax concentration on the urban trees in Malaysia to compare to this study. As a guide, studies conducted by various researchers suggested that trees that are found in low relative humidity with a range of 20-30% has higher epicuticular wax than those found in high relative humidity condition (98%). It is also found that the wax thickness is higher during warm, dry site than on cool, moist site. Environmental factors such as light intensity, relative humidity, temperatures are important factor in inducing wax development [21].



Note: Different alphabets denotes significant differences $p \leq 0.05$ using Tukey Range Test

Figure 2. The distribution of average wax concentration at different study sites

The epicuticular wax concentration on *Syzygium myrtifolium* leaves was significantly higher in industrial areas compared to those in the urban areas. *S. myrtifolium* at industrial areas are in the open and subjected to direct sunlight. The annual accumulation of epicuticular wax was higher on *S. myrtifolium* leaves located near the industrial zones compared to the urban areas. It is difficult to find enough data to compare to this study. This is because there is relatively lack of studies in the aspect of changes in accumulation of epicuticular wax that can occur during the year. However, there are studies conducted on Douglas-fir's needles in Washington and Oregon, USA, found that there are significant increment in wax thickness during the year from late June/early July to early November [22].



Note: Different alphabets denotes significant differences $p \leq 0.05$ using Tukey Range Test

Figure 3. The mean annual accumulation of wax on *S. myrtifolium* at different sites

The possibilities of heat related stress are experienced by *S. myrtifolium* in that location. This is because there is an increasing trend in the average max-min temperature recorded in Bintulu for 2006 where the mean air temperature ranged from 23.7-31.5°C compared to 23.8-32.2°C in 2013 [23; 24]. Generally, epicuticular wax production increases in stress condition or drought-stresses plant [25]. This will enhance the plant tolerant to high temperature stress, disease, insects and moisture stress. Roadside trees experiencing water deficiency due to heat related stress, therefore a well-developed epicuticular wax layer is crucial. This is to increase the efficiency of stomatal functions, reduce water loss, and any change in the original morphological structure will make these plants more sensitive to water stress [1; 26].

However, there is another possibility that the *S. myrtifolium* trees is responding to stress caused by the air pollutions. Sekolah Kebangsaan Asykirin and Vocational Bintulu is near to the petrochemical and refinery plants at the northern region whereas Sekolah Menengah Kai Dee is near to the wood factories. Similar pattern has been recorded on *Cryptomeria japonica* tree in Japan which is near to an electrochemical plant. Epicuticular wax concentration showed an increase in 10% where it is a physiological response to stress caused by the air pollutions [17]. Studies suggested that particulate matter (PM) deposited on the epicuticular wax can cause physiological and morphological impacts including increased cell alkalinity, photosynthetic inhibition, leaf senescence, stomatal damage, and reduced growth and yield [27; 28].

The low concentration of epicuticular wax was recorded on *S. myrtifolium* leaves in urban area. These trees are subjected to high concentration of pollutants especially from exhaust emissions. Similar pattern has been recorded in India, where studies found that in *Asparagus racemosus*, *Azadirachta indica*, *Bougainvillea spectabilis*, *Cassia fistula*, *Ficus religiosa*, *Nerium indicum*, *Polyalthia longifolia* and *Thevetia neriifolia*, the epicuticular wax lost its original shape, got disorganized and subsequently eroded. This is probably due to the continuous fumigation of the plants with exhaust emissions under high temperatures (30–50°C approx.), owing to the close proximity of the plants to the sources of pollution [1]. Studies using scanning electron microscopical (SEM) found that Norway spruce needles, Finland showed deleterious effects of exposure on the epistomatal wax of needles where the epicuticular wax around the stomata was injured for trees along the highway [14]. These fuel combustion created sulphur dioxide and nitrogen oxides which are responsible for the formation of acid rain. The acid rain has been reported to be able to erode epicuticular wax [29].

The other possibility is due to the aerosol deposition which have been carried by the wind. The wind rose chart for Bintulu showed several wind directions (Fig. 4). Aerosol and other pollutants from the industrial area in the northern region could be blown by the wind to the south-west region when the centre town is located. It has been reported that excessive aerosol deposition causes wax degradation [13]. Aerosol has shown to have reduced the plant drought tolerance especially in pines trees [30]. The concern would be that the level of aerosol concentrations have increased globally due to industrialization and wind erosion by land use changes [31; 32]. This will create another issues relating to trees health and survivability. Therefore, there is a possibility that the wax degradation level can be used as an indicator for massive aerosol deposition.

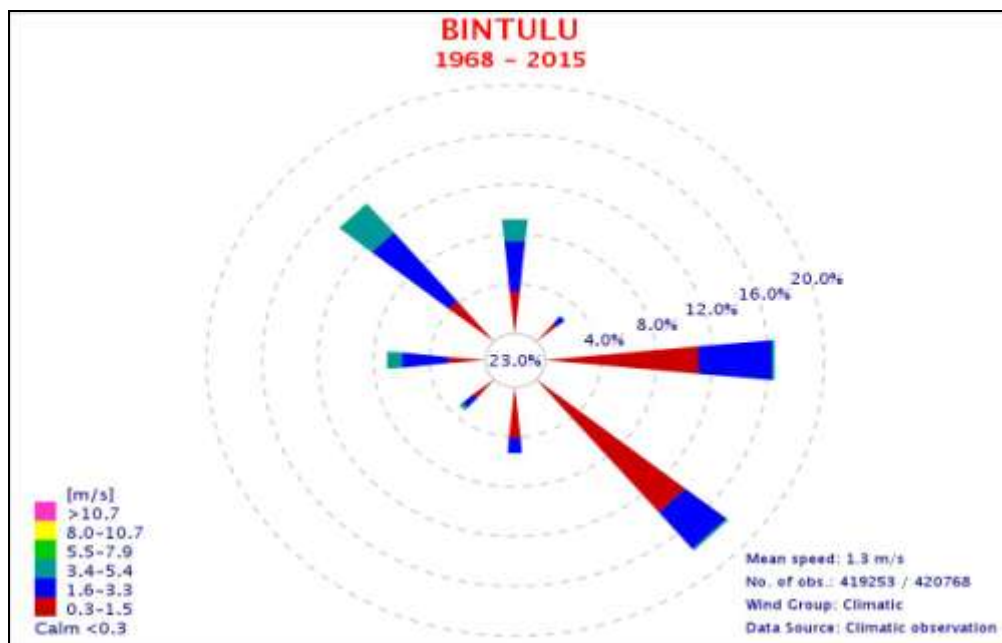


Figure 4. The wind rose chart for Bintulu

This initial study reveal that the level of physiological stress on the trees in industrial, suburban and urban areas are at a different scale. This could be due to the different levels of air pollution in these areas. Understanding the changes occurring at the epicuticular wax can help understand the types and level of pollutants in a locality. Some studies suggested that the wax structure can be used as an early indicator of traffic exposure effects [14; 12]. Apart from that, the epicuticular wax act as a receptor for the particulate matter. The capability of the trees depends on the microstructural like leaf rough surface, pubescence, thickness of epicuticular wax while macrostructural such as plant height, leaf arrangement and leaf areas [33; 34; 35; 36]. Therefore, to maximize the capabilities of the trees as bio filters, the relationship between these tree traits and environment should be articulated, yet at the moment this information is rather limited.

IV. Conclusion

The setting created by industrialization and urbanization where vehicles and industries plays a significant role in decreasing the air quality. Urban plants are constantly exposure to the increasing level and types of pollutants. These pollutants will cause physiological stress to the trees which will appear in their plant's physical structure or physiology. Understanding, the tree traits are important to capitalize their role as bio filters. This study provide the baseline data for future study where the relationship with the particulate matters deposited on the tree canopy and the tree trait could be explained.

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