CHAPTER 2

LITTERATURE REVIEW

2.1 Lichens

Lichens are the one of the most sensitive living organisms to air pollution, which are found in nature. Lichens are by definition symbiotic organisms (Nash, 1996). Lichen is a stable self-supporting association of a fungus (mycobiont) and an algae or cyanobacterium (photobiont) (Gilbert, 2000). More precisely the term "alga" indicates either a Cyanobacteria or a Chlorophyceae; the fungus is usually an Ascomycetes, although on rare occasions it may be either a Basidiomycetes or a Phycomycetes. In this association, the alga is the part that is occupied with the formation of nutrients, since it contains chlorophyll, while the fungus supplies the alga with water and minerals. These organisms are perennial and maintain a uniform morphology over time (Conti and Cecchetti, 2001). Furthermore, their lack of cuticle or stoma means that the different contaminants are absorbed over the entire surface of the organisms and pass to their tissues directly (Hale, 1970; Conti and Cecchetti, 2001; Hale, 1983 cited by Loppi *et al*., 2002).

Both of fungi and algae are living together in the special forming which called "thallus" that has no root, trunk, and true leaf like the vascular plants. The general structure of lichen is composed of layers of fungus and alga. It involves an upper cortex of densely packed cells that has a protective function and may incorporate pores to facilitate gas exchange. Below this, an algal layer is found, conveniently situated to receive plenty of light. Next there is a wide medulla formed of loosely packed fungal hyphae. This acts as a reservoir for water. In crustose lichens, the medulla also serves to attach the lichen to the substratum while foliose species have an additional lower cortex the underside of which bears swards of root-like structures (rhizinae) that act as attachment organs (Gilbert, 2000) (Figure 2.1).

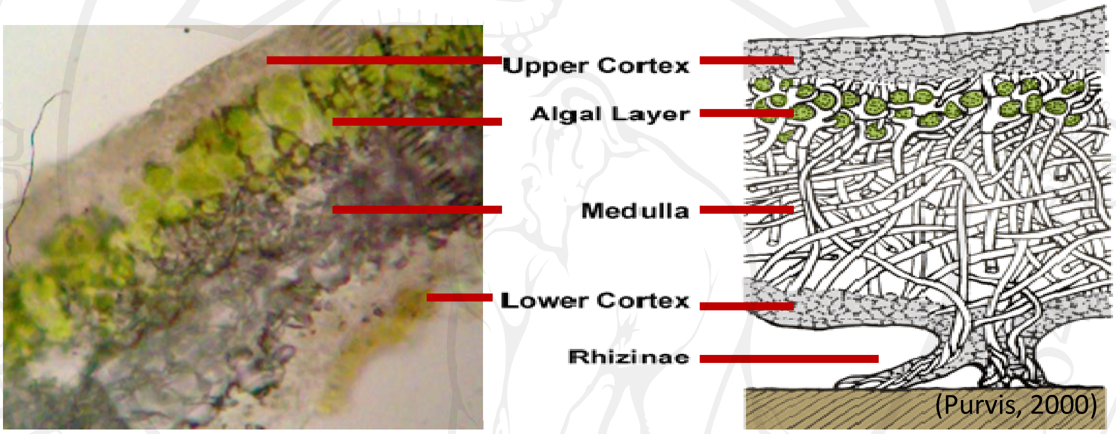
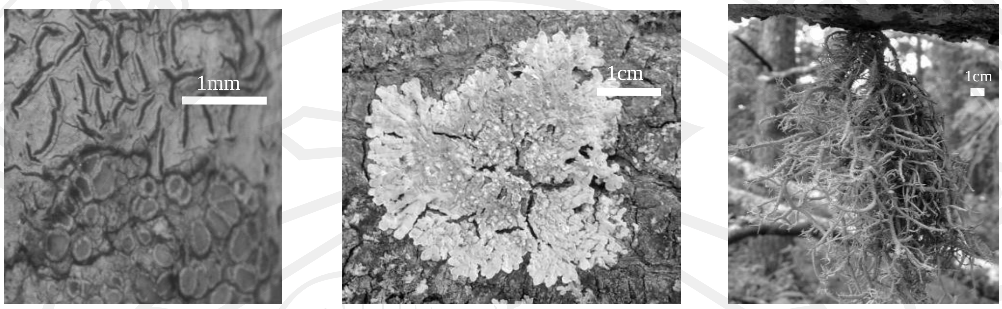


Figure 2.1 Thallus section of foliose lichen.

According to the association of fungal and algal symbionts, the thallus of lichens can assume a variety of form. Lichens are traditionally divided into three main morphological groups: these are crustose, foliose and fruticose types (Hale, 1970 and Nash, 2008) (Figure 2.2). Crustose lichens are tightly attached to the substrate with their lower surface and may not be removed from it without destruction. Foliose lichens are leaf- like flat, with a distinct upper and lower cortex, often attached to substrate by rhizines and can be easily peeled off. The thallus lobes of fruticose lichens are hair- like, strap- shaped or shrubby and the lobes may flat or cylindrical. They always stand out from the surface of the substrate.



(a) (b) (c)

Figure 2.2 (a) Crustose lichen, (b) Foliose lichen and (c) Fruticose lichen.

Lichens are widely distributed, occurring in all terrestrial ecosystems, and collectively covering (in many cases dominanting) about 8% of the Earth's land surface (Purvis, 2007). A few species occur in a very wide range of habitats over several continents, but most tend to be restricted to particular ecological niches of the substrate and microclimatic variables are important limiting factors (Hawksworth and Rose, 1976). These features of lichens, combined with their extraordinary capability to grow in a large geographical range and to accumulate mineral elements far above their needs, rank them among the best bioindicators of air pollution (Garty *et al.*, 2003).

2.2 Lichens as bioindicators for air pollution monitoring

Lichens have been recognized as being very sensitive to air pollution for many years (Hawksworth 1971; Nimis *et al.*, 2002 cited by Nash, 2008). It is well established that lichens are sensitive to a wide range of habitat changes, most of them man- driven. This sensitivity is due to particular physiological characteristics of lichens, and allows them to be used as indicators and monitors of habitat changes, providing an integrated measure of all disturbances occurring in their environment (Pinho *et al*., 2004).

Understanding and predicting the response of species to climate change is essential to long-term conservation strategy. Many lichen species are heavily dependent on climate, often influenced by minor fluctuations. Climate change has a profound influence on the distributions of these sensitive organisms (Watsonetal, 2004 cited by Aptroot and Van Herk, 2007). A recent study in the Netherlands, based on monitoring at five-year intervals since 1979, has identified recent major changes in epiphyte distribution independent of pollution. Warm-temperate species have significantly increased, and species characteristic of cold environments have either decreased or disappeared (van Herk *et al.*, 2002). Aptroot and Van Herk (2007) studied the effect of global warming on lichen. They found the increasing evidence suggests that lichens are responding to climate change in Western Europe. More epiphytic species appear to be increasing, rather than declining, as a result of global warming. Many terricolous species (lichens which growth on the ground), in contrast, are declining. As same as Sancho *et al.* (2007) studied about extreme range in lichen growth rates supports their use as an indicator of climate change in Antarctica. From the result, they proposed that extreme rang of the cline in lichen growth in Antarctica means that lichen could be a sensitive detector for climate change throughout the continent. The expected changes in local climate driven by global change are increased temperature and/or increased precipitation. Both of these factors would drive faster growth rates of lichens.

Pollution in major cities tends to increase during the built up phase. Urban areas are exposed to numerous airborne contaminants emitted from anthropogenic sources in the form of solid particles or gases. The main sources of these pollutants are industrial plants, power stations, domestic heating systems and motor vehicles (Nriagu and Pacyna, 1998 cited by Alfani *et al*., 2000). The last two usually being prominent in urban areas. In recent decades, the increasing traffic has switched the attention to nitrogen oxides (NO_2), organic compounds and small particles. NO_2 are formed by oxidation of atmospheric nitrogen during combustion. The main part, especially from cars, is emitted in the form of the nontoxic nitric oxide (NO), which is subsequently oxidized in the atmosphere to the secondary "real" pollutant: NO_2. The emissions can be reduced by optimization of the combustion process (low NO_2 burners in power plants and lean bum motors in motor vehicles) or by means of catalytic converters in the exhaust.

Due to their sensitivity to air pollution, lichens may be used as bioindicators in many ways as Branquinho (2001) and Conti and Cecchetti (2001) were studied. But both studies were noted the different ways of using lichens as bioindicators. Branquinho (2001) found that it can be done in three ways: using variations in diversity and/ or abundance, using variations in physiological parameters, or using lichens as accumulators of pollutants. While Conti and Cecchetti (2001) noted that there are two different ways to used lichens as bioindicators. The first way is by mapping all species present in a specific area and the second is by transplanting lichens from an uncontaminated area to a contaminated one. The morphological changes in transplanted lichens are observed including physiological parameters and bioaccumulation of the pollutants. Although various techniques using lichens are used in determining air quality, the most widely used method is the mapping of lichens since it provides a valid, quick and economic way for assessing and mapping long-range effect of pollution in a given area (LeBlanc *et al*., 1972).

2.2.1 Mapping of lichens and the effect of air pollution on lichens

Epiphytic lichens are known to be sensitive to phytotoxic gases (mainly NO_x and SO_x) and lichen are long since known to be damaged by sulphur dioxide (SO_2) and its derivatives formed in aqueous solution (Hawksworth and rose, 1976; Richardson, 1993 cited by Conti and Cecchetti, 2001; Nash and Gries, 2002, Ferretti *et al*., 2004). Other oxidizing compounds, such as nitrogen oxides, are also toxic to lichens (Sigal and Nash, 1983). Lichen biodiversity can be taken as estimates of environmental quality which high values correspond to good situations and low values indicate poor quality (Loppi *et al.*, 2002). Such measures are chiefly depending from the two main reactions of epiphytic lichen communities to air pollution by phytotoxic gases, especially SO_2 and NO_x : a decrease in the number of species and in their cover frequency (Nimis, 1999 cited by Loppi *et al.*, 2002). Several methods have been proposed for estimating and mapping the effects of atmospheric pollution on lichens. Sommerfeldt and John (2001) investigated air pollution and the occurrence of lichens in the city of Izmir, Turkey using VDI method. The lichen air quality map showed that five air quality classes were determined and a predominant part of the city area was heavily polluted. The best air quality values were determined in the southern and western parts of the city zone. They suggested that the width of the air quality classes of 7.3 provided a method that was suitable for similar studies in Turkey, which correspond with the study of Bačkor *et al.* (2003). The author also found difference in number of species and lichen abundance at sites close to a steel factory south of the city, the city center, and peripheral parts north of the city (Košice city). In the city center and sites close to the steel factory, lichens which were more tolerant to pollution were typical. They found lichen *Ramalina fastigiata*, which very sensitive to air pollution on the north periphery of the city. Samsudin *et al*. (2012) used the lichen frequency counts calculate the frequency points and air quality score of each area (Universiti Kebangsaan Malaysia Campus). This study found lichen *Arthonia*, *Caloplaca*, *Chrysothrix*, *Dirinaria*, *Hyperphyscia adglutinata*, *Laurera*, *Lecanographa*, *Lecanora*, *Parmotrema tinctorum*, *Parmotrema praesorediosum*, *Physcia*, *Pyxine cocoes*, *Rinodina*, *Trypethelium* and *Graphidaceae*. These epiphytic lichen gave values for air quality at the study site because each lichen will give a specific score according to its tolerance toward air pollution. The air quality in this study can be categorized to 4 levels; clean air, moderate, slightly polluted and highly polluted. The lowest score of quality is -7.83 at Aman Building which near to the main road.

There are many researches which demonstrate the relationship and the effect between lichen and NO_2 that released from traffic. Van Dobben and Ter Braak (1998) and Gombert *et al.* (2003) found the effect of NO_2 on lichen distribution. Van Dobben and Ter Braak (1998) found that NO_2 predominantly released by traffic, was the second-most important factor explaining the distribution of lichen species apart from SO_2. As same as Gombert *et al.* (2003) who studied the influence of urban roads on the nitrogen concentration of a nitrophytic lichen, *Physcia adscendens* and an acidiphytic one, *Hypogymnia physodes* in the Grenoble area (France). They analyzed lichen and nitrogen concentrations and mapped them for the whole urban area. It was indicated that roads influenced the nitrogen concentrations in *P. adscendens*, but not those in *H. physodes.* Significant positive correlations were found between the traffic index and the total nitrogen concentration in *P. adscendens.*

Frati *et al.* (2006) studied the effect of NO_2 and ammonia (NH_3) from road traffic on epiphytic lichens in central Italy, while, Davies *et al.* (2007) investigated the distribution and diversity of epiphytes in London and their relationship in relation to NO_x using fine-scale atmospheric dispersion modeling. Both of them found that there were corresponded with the result of each other. NO_2 and NH_3 emitted by road traffic can influence on lichen diversity, lichen vitality and the accumulation of nitrogen in lichen thallus. There was a significant inverse relationship between lichen diversity and NO_x. Diversity declined where NO_x exceeded 70 μgm^{-3} and NO_2 exceeded 40 μgm^{-3}, suggesting a phytotoxic effect. NO_2 concentrations, although rather low, were negatively correlated with distance from the highway according to a typical logarithmic function. No association between NO_2 concentration and the diversity of epiphytic lichens was found, probably because of the low NO_2 values measured. However, Davies *et al.* (2007) found a significant positive relationship between NO_x and lichen abundance due to the ubiquitous distribution of pollution tolerant species, mainly associated with eutrophication.

Van Dobben and Ter Braak (1998) found that monitoring it was very difficult to separate the effects of many intercorrelated variables in a field and this was especially true for pollution studies, as pollutant concentration trend to be correlated to the general level of human activity and were therefore correlated among each other. Which, correspond to work of Munzi *et al.* (2007) who found that besides air pollution, the most important variable affecting the epiphytic lichen flora of Rome, currently updated to 102 taxa, was the influence of the Tyrrhenian Sea.

Van Dobben *et al.* (2001) determined the joint effect of gaseous atmospheric pollutants and trace elements on epiphytic lichens in The Netherlands. It was found that atmospheric SO_2 and NO_2 appeared to be the most important factors determining lichen biodiversity. Nearly all species were sensitive to these compounds. All species decreased with increasing concentrations of these compounds, which therefore strongly affect species diversity. It is well-documented that epiphytic lichens respond to atmospheric pollution, a negative relationship of most species was observed only for SO_2, or the combination of SO_2 and NO_x which generally are strongly correlated, and thus biodiversity can only be used as a monitor for SO_2 (Van Dobben and Ter Braak, 1999).

Besides the effect of NO_2 from traffic, there were several pollutants sources effected on lichens which had been studied. Pinho *et al.* (2004) sampled foliose and fruticose lichen diversity and frequency in large industrial facilities, in a region in southwest Portugal (Sines). A long-term study has been underway in the same area since 1970s using lichens as bioindicators to evaluate air quality. It was found in the areas close to the industrial areas, less lichen species were found less than in other areas. A relationship between the specific lichen community existing at a site and degrees of air pollution had been detected. A rich variety of lichen species exists more frequently in areas with clean air.

The next study which using epiphytic lichens as indicators of environmental quality was from Munzi *et al.* (2007). The authors studied in Rome and used the applied Lichen Diversity (LD) method. The epiphytic lichen flora of Rome was mostly adapted to an anthropogenic environment. Significant changes in the lichen flora have been noted over the past 20 years, with the lowest diversity now being found in the urban centre and in the eastern and southern sectors, while the ‘‘lichen desert’’ area has decreased in parallel with decreasing concentrations of CO, NO_x and SO_2.

In Thailand, some lichen studies had been conducted. Saipunkeaw (1994) and Subsri (2001) used lichens as bioindicators for air pollution monitoring in urban and suburban in Chiang Mai city by VDI method (VDI, 1995). Subsri (2001) found that high polluted areas were extended to suburban more than in 1994. Moreover, lichen *Parmotrema tinctorum* (Nyl.) Hale was transplanted in the city. Chlorophyll and phaeophytin content of the lichen in urban area with higher traffic and human activities were lower than lichen in suburban area where lower traffic presented. As same as Pomphueak (2005) used lichens as bioindicators for air quality monitoring in Amphoe Mueang

Lampang by using lichen diversity values; LDVs method. A total of 21 lichens species were recorded, 15 species belonging to the crustose group and six species were the foliose group. Eight air quality classes were identified and the mean NO_2 concentrations of each air quality class were significantly different. This result indicated that NO_2 deposition was one of the major factor influencing the LDVs of lichen in this study area. Pruksakorn (2007) also used lichen as biomonitoring for air pollution monitoring in Lamphun province by VDI method. The result showed that Air Quality Index (AQI) in the Lamphun municipal was categorized into 3 Air Quality Class (AQC). Lamphun municipal area had a high air pollution level while result from the study of lichen diversity in the Northern Region Industrial Estate which divided into two sites, East and West. *Lecanora* cf. *leprosa* was found mostly in the area from both of study site (55% and 70% of total lichen number).

Pimwong (2002) studied distribution and frequency of lichen *Pyxine cocoes* Swartz. and *Dirinaria picata* Swartz. in Chiang Mai City. *P. cocoes* was widely distributed in the centre of the city and suburban area with similar frequency while *D. picta* was found in some area. It showed narrower distribution and lower frequency than *P. cocoes*. Especially, *D. picta* was not found in high traffic density; In area with lower traffic, *P. cocoes* trened to be more tolerant to air pollution than *D. picta*, therefore it was widely distributed in the study area. *D. picta* might be able to be used as a bioindicator which was sensitive to air pollution. Chaithaswad (2002) also studied distribution and frequency of lichen in Chiang Mai City but different lichen species. *Hyperphyscia adglutinata* Flörke and *L.* cf. *leprosa* were selected for this study. *H. adglutinata* showed highly distribution with high frequency in the center of the city where air pollution was rather high. It also found in suburban areas but in low frequency. *L.* cf. *leprosa* was rarely found and showed low frequency in the center of the city. This species was found in higher frequency in some area outside the center of the city. Therefore, *H. adglutinata* was more tolerant to pollution than *L.* cf. *leprosa*.

The result of Kulapirak (2006) was corresponded with Pimwong (2002) and Chaithaswad (2002). *P. cocoes* was widely distributed in all study area in different frequency. *P. cocoes* was found in higher frequency at the centre of the city while *Lecanora* cf. *leprosa* was found in higher frequency in suburban area. *P. cocoes* and *L.* cf. *leprosa* might be served as good bioindicators for anthropogenic impacts. *L.* cf. *leprosa* trened to be more sensitive to air pollution, whereas, *P. cocoes* trened to be more tolerant to air pollution.

Saipunkeaw *et al.* (2005) and Saipunkeaw *et al.* (2007) used epiphytic lichens as indicators of environmental health in the vicinity of Chiang Mai, Thailand. Epiphytic macrolichens on mango trees in urban and suburban in northern Thailand were surveyed. Theses paper also used the VDI method to obtain frequency of lichens. Selected sites included highly polluted sites in urban and adjacent industrial areas of Chiang Mai city, disturbed rural sites and undisturbed forest in Doi Suthep Mountain. The study found that macrolichen species of epiphytic communities in the lowlands could be used to distinguish urban and industrial sites from agricultural and rural sites. Lichen diversity was correlated with the human population, and the lichen diversity was lowest in the cities with the highest population. Moreover, lichen diversity were correlated with rainfall and population density. Comparison with records of pollution data and local pollution model, greater corresponded of lichen data with the effects of PM10 than with anthropogenic sulphur was found.

Muangsuwun (2006) monitored the growth rate of the lichen *P. cocoes* Swartz on the mango tree (*Mangifera indica* L.) in Chiang Mai City. It was found that growth rate of *P. cocoes* in Chiang Mai city was slower than in Chiang Mai University because the air pollution in city was higher than Chiang Mai University. Less lichen species was found in the urban area while more lichens were found in Chiang Mai University. This study was corresponded with previous studies of Saipunkeaw (1994) and Subsri (2001). It indicated that air pollution had been increasing in the city.

Runnawut (2008) did the surveying of lichen diversity around Mae Mo Power Plant areas Mae Mo District, Lampang province. A total 25 species were found in this study and the lowest of lichen diversity index was found in Ban Sop Mo located near to Mae Mo coal- fired power plant. Lichen *D. picta*, *Chrysothrix xanthina*, lichen in family Graphidaceae and lichen crustose which had no fruiting body were the common lichen in this study. In every study area lichen crustose was found more than foliose. Moreover, lichen was found in the direction which avoid from Mae Mo coal- fired power plant. Kanjoem (2010) studied lichen diversity around Mae Moh power plant area, Mae Moh district, Lampang province. Most lichen genera found in study sites were such as

Dirinaria, *Pyxine*, *Chrysothrix*, *Cryptothecia*, *Arthonia*, *Lecanographa*, *Laurera* and *Hyperphyscia*, while few lichen genera found in study sites were such as *Buellia*, *Ocellularia* and *Chapsa*. *D. picta* and *P.* cocoes were found in all study areas. Crustose lichens species was more abundant than foliose lichens species in every study sites. Lichens grow on the investigated trees were found mostly in the direction which avoided from the power plant.

Kheawsalab (2010) studied lichen diversity on some tree species in Chiang Mai. Lichens in the city were found mostly on a royal palm. Dominant lichen species in city was *P. cocoes*. Dominant species in urban area; Chaechang subdistrict, San Kampang was *Lecanora* sp. which was less tolerant than *P.* cocoes.

2.3 Passive sampling

A passive sampler operates by diffusing the sample gas from the atmosphere across the sampler volume, usually an inverted tube, to a skin or chemical absorbent according to Fick's law. Rate of pollutant gas absorption for a simple diffusion tube sampler is controlled by the diffusion path length and the internal cross-sectional area of the sampler (Cox, 2003). It can be used for the determination of both inorganic and organic compound in a variety of matrices, including air, water and soil.

In recent year, passive sampling has been gaining increased attention since it has the advantages of being cheap, lightweight, robust and simple technique, which is easy to operate and handle. It does not require any power source, calibration or maintenance. It can be fixed to any object and on person, depending on the objective of the measurement. Passive samplers remain stable over several months after sampling and can be conveniently transported before and after exposure. Also all sampler parts are reusable (Varshney and Singh, 2003). The flexibility of placement for passive sampling systems also make them attractive alternatives for assessing exposures at locations that are difficult to access, such as within the forest canopy. Passive samplers may also be used to identify areas receiving air pollution events, that were previously unknown, and where additional infrastructure for instrumental monitoring may be required (Cox, 2003).

Stevenson *et al.*(2001) established a NO_2 coordinated monitoring network, involving more than 1000 monitoring sites in urban areas throughout the UK, using diffusion tube samplers. The results showed that diffusion tubes could be utilized in large numbers to determine the spatial distribution of NO_2 and high light areas of high concentration. Cox (2003) found that passive samplers were useful tools for monitoring exposure of ecosystem components to gaseous pollution on different spatial scales, and to verify atmospheric transport and chemistry models and their extension over remote areas. However, there are still many uncertainties that need consideration in their application. Choice of passive sampling techniques for O_3, NO_2 and SO_4 can be depended on local conditions and on the frequency of sampling. Short periods (1–2weeks) favour the badge-type samplers, whereas longer periods (a month or more) or high concentrations (pollution source regions) may favour the tube-type samplers, except with O_3 and NO_2 where low residence time with in the diffusion path of the sampler, reduces their chance of interaction.

Borisuttichun (2008) constructed the test kit for determination of SO_2 in ambient air by passive sampling. The polypropylene diffusion tube provided high precision and at the same time provided less percent error (high accuracy) in comparison with fluorescence technique. Moreover, 3 days exposure was found to be appropriate period for measurement of SO_2 in ambient air by the designed passive diffusion tube.

Stranger *et al.* (2008) monitored of NO_2 in the ambient air with passive samplers before and after a road reconstruction event. They found NO_2 concentrations correlated well with the traffic density. Sampling sites located further from the road works, enclosed to the group 'less polluted' (low traffic density), showed the lowest NO_2 concentrations. The highest NO_2 level was found for the locations close to reconstruction works, which belonged to the group 'heavily polluted'. The contribution of NO_2 was at the same level before and after the road works. This result similar to Parra *et al.* (2009) measured volatile organic compounds (VOC) and NO_2 in a medium size city in Northern Spain. They found that The lowest levels of VOC and NO_2 occurred during summer, owing to the increase in solar radiation and to lower traffic densities. Mean concentrations of benzene and NO_2 exceeded the European limits at some of the monitored points.

Bootdee (2009) monitoring of NO_2 levels in Chiang Mai province. The result showed that NO_2 concentrations in rural area were significantly less than those of urban area and sub-urban area ($\alpha < 0.05$), while those of urban area were significantly higher than those of sub-urban area. The highest NO_2 concentrations (28.1- 45.1 ppbv) of each sampling month was found at site U3 (Waroros market), which was located in the urban area of Chiang Mai with high traffic density. That was corresponded with Thammapanya (2012) who tested an efficiency of the lab made passive samplers for long term NO_2 measurement and to monitor spatial and temporal NO_2 levels in ambient air of Chiang Mai City. The result showed that NO_2 levels were found to be correlated with traffic volume and site characteristic. The first 4 sites are located in Chiang Mai downtown, where the traffic volume is high, while the MF (Mae Faek Municipality) is the background site surrounded by agriculture area and has low traffic volume. Ambient NO2 concentration was low in rainy season (June – September 2011) and started to increase at the end of October and reached its highest concentrations in February 2012 (middle of the dry season). After that it was slowly decreased and remained low in wet season.

Huang *et al.* (2013) studied land use patterns and SO_2 and NO_2 pollution in Ulaanbaatar, Mongolia. The NO_2 concentrations at 19 traffic/road sites (12.85 ppb in the warm season and 20.48 ppb in the moderate season) were significantly higher than those at 19 urban sites (7.60 ppb and 14.39 ppb in the moderate season). The NO_2 concentration at the location 4.83 km from the city center was decreased by 18% and at the location 4.79 km from the power plants by 21%. NO_2 concentrations are very high in Ulaanbaatar, especially in the winter, and can be explained by several land use variables, including the distance to the *ger* areas, the city center, the main roads, and the power plants.

There were some studies of NO_2 and lichens had been carried on in Thailand. Pomphueak (2005) collected NO_2 and SO_2 by using passive sampling technique and used lichens as bioindicators for air quality monitoring in Amphoe Mueang Lampang and around Mueang Lampang. Sampling tubes were placed for two weeks period of exposure. The results from lichen data correlated well with concentration of NO_2 from passive sampling. The study indicated that NO_2 deposition was one of the major factors influencing the Lichen Diversity Values (LDVs) of lichen in this study area. Muangsuwun (2006) monitoring the levels of NO_2 and SO_2 in the air by using passive sampling method. Concentration of both gases were analyzed by a spectrophotometer. Chiang Mai city had higher level of NO_2 and SO_2 than in Chiang Mai University and corresponded with growth rate of lichens in Chiang Mai City which showed lower growth rate. Furthermore, Kanjoem (2010) measured SO_2 concentration around Mae Moh power plant area, Mae Moh district, Lampang province. The concentrations of SO_2, which were measured in dry and rainy seasons, were 0.84 – 8.65 ppbv and 0.51 – 1.72 ppbv, respectively. There was no significant correlation at 95 % confidence between lichen diversity and measured SO_2 concentrations in the study areas. Sulphur dioxide (SO_2) concentrations measured during the study period showed no effect on lichen diversity and bark pH.

2.3.1 Operating principle

Passive collection of a given air pollutant is achieved by chemical and physical sorption on a sorbent or filter impregnated with a sampling medium. In diffusion sampling the gas is passively transported to the sorbent by molecular diffusion. Passive sampling is sampling technique based on free flow of analyze molecules from the sampled medium to a collecting medium, as a result of a difference in chemical potentials of the analyze between the two media. (Gorecki and Namiesnik, 2002)

The principle of diffusion in passive sampling refer to Flick's First law as describe by Gair *et al.* (1991). The unidirectional flow of gas_1 through gas_2 is given as the following:

$$F_1 = -D_{1,2} dc_1/dz \qquad (2.1)$$

Where:

F_1 = the flux of gas (mol cm^{-2} s^{-1})
$D_{1,2}$ = the diffusion coefficient of gas_1 in gas_2 (cm^{-2} s^{-1})
c_1 = the concentration of gas_1 in gas_2 (mol cm^{-3})
z = the length of diffusion (cm)

The quantity of gas transferred (Q_1 mol) in *t* second for a cylinder of radius r is given by the following equations;

$$Q_1 = F_1 (\pi r^2)\ t \ \text{mol} \qquad (2.2)$$

Therefore

$$Q_1 = -D_{1,2} (c_1 - c_0)(\pi r^2)\ t/z \ \text{mol} \qquad (2.3)$$

Where:

c_0 is the concentration experienced at the absorber surface, therefore $(c_1-c_0)/z$ is the concentration gradient along the cylinder length (z). If an efficient absorber is used to remove gas_1, then c_0 efficiently become zero.

Then the concentration of NO_2 in $\mu g/m^3$ are calculated by applying the equation (Plaisance *et al., 2002*);

$$C\ (\mu g/m^3) = \frac{[Q \times z]}{[(\pi r^2) \times t \times D]} \qquad (2.4)$$

Where:

C = the concentration measured by passive sampling tube ($\mu g/m^3$)

Q = the quantity of absorption products present in the sampler (μg)

Z = the diffustion length (m)

r^2 = the radius of diffusion tube (m)

t = the sampling time (s)

D = the diffusion coefficient (m^2s^{-1}), 0.124 x 10^{-4} m^2s^{-1}

The calculation of gas quantity presented in diffusion tube is depended on the final product after reacting with sampling medium.