CHAPTER 2
LITERATURE REVIEW

2.1 Lichens

Lichens are one of the most sensitive living organisms to air pollution, which are found in nature. Lichens are a symbiotic association of a fungus and algae, resulting in a stable structure called a Thallus. The fungal partner of lichens (mycobiont) mainly belongs to a group known as Ascomycete. A few of Basidiomycetes, Coelomycete, Hyphomycete and Phycomycete also form lichens. For the alga (photobiont) is either a Chlorophyceae or a Cyanophyceae. In this association, the fungus protects its partner from desiccation and high light intensity and supplies the algae with water and minerals, while the algae is the part that carries out photosynthesis (Conti and Cecchetti, 2001).

According to the association of fugal and algal symbionts, the thallus of lichens can assume a variety of forms. The main types of growth forms of lichens are crustose, foliose and fruticose (Hale, 1979). Crutose lichens grow in flat patches firmly attached by their entire undersurface to soil or rock, with the algae lying below a distinct layer of fungal tissue termed cortex. Foliose lichens are leaf-like, with a distinct upper and lower cortex, often attached to substrate by rhizines and can be easily peeled off. Fruticose lichens are erect shrubby or beard-like pendent species attached to the substrate only at their base, circular or flattened in section.

Lichens occupy a wide range of habitats such as tidal zones, mountains, hot deserts, the artic and the antarctic. A few species occur in a very wide range of habitats over several continents, but most tend to be restricted to particular ecological niches and to have characteristic distributions. Both the chemical nature and the texture of the substrate and microclimatic variables are important limiting factors (Hawksworth and Rose, 1976).

2.2 Lichens as bioindicators for air pollution monitoring

Lichens are one of the organisms that have been used to assess air quality, due to their sensitivity to atmospheric pollutants such as sulfur dioxides, ozone and nitrogen compounds, fluorides, aromatic hydrocarbon and heavy metals. Lichens are generally absent in the cities with a polluted atmosphere or in the area with high number of industries. Nylander is cited as the first who realized their sensitivity to air pollution. He noticed that decrease in the number of lichens in Paris, in 1866 due to dark smoke and gaseous emissions (Galun *et al.*, 1984).

Lichens have special characteristics, which make them unique for air pollution monitoring and as bioindicators. For example, they lack a protective layer called the cuticle, which allows the nutrients and pollutants from the atmosphere to penetrate directly through their surface to lichen cells. Lichens can continue to metabolize at low temperatures and therefore can be used during the wintertime. Lichens grow slowly and injuries cannot be quickly restored. Unlike many vascular plants, lichens have no deciduous parts; therefore, the pollutants can accumulate in their thallus. Furthermore, lichens are ubiquitous, long-lived and perennial organisms available for monitoring throughout the year (Nimis and Purvis, 2002). Epiphytic lichens, which grow on trees, are best suited for the study of air pollution because they do not have direct access to soil nutrient pools and usually receive greater exposure to air pollutants.

Due to their sensitivity to air pollution, lichens may be used as bioindicators in two different ways. The first way is by mapping all species present in a specific area. Secondly, by transplanting lichens from an uncontaminated area to a contaminated one. The morphological changes in transplanted lichens are observed including physiological parameters and the bioaccumulation of the pollutants (Conti and Cecchetti, 2001). Although various techniques are used in determining air quality using lichens, the most widely used method is the mapping of lichens since it provides a valid, quick and economic way for assessing and mapping the long-range effect of pollution in a given area (LeBlanc *et al.*, 1972). Moreover, it provides results, on which predictions for human health can be based, as shown by the high correlation between lichen biodiversity and lung cancer in young male residents with $r = 0.95$, $p < 0.01$ in Veneto region, Northeastern Italy (Cislaghi and Nimis, 1997).

2.2.1 Mapping of lichens

The earlier study for estimating air pollution with lichens, the index of atmospheric purity (IAP), was carried out by De Sloover and LeBlanc in 1968 and later, the qualitative scale for estimating SO_2 pollution by Hawksworth and Rose in 1970 (Van Haluwyn and Van Herk, 2002). Since then, these two basis methods were modified by researchers and taking into account regional knowledge on lichens and on patterns of air pollution. All methods currently in use can still be classified as quantitative and qualitative methods. In the quantitative approach, the complete lichen composition of sample plots is reduced to a single value expressing air quality through a formula, for example, IAP values. In the original definition of the IAP, numerical values were assigned to species, expressing their sensitivity to air pollution, the mean number of companion species was often considered as a measure of sensitivity. In other methods, such as the German and the Italian guidelines, species sensitivities are no longer used, and only the sum of frequencies of selected species in a sampling grid of 10 units are taken into account. On the contrary, the qualitative approach, ecological information on species, species groups or communities is used to estimated air quality. The basis element is the species, each one having a range of tolerance to pollution which, if know can be expressed by ecological indicator values. Theses can consist of verbal expressions or can be expressed as numbers on ordinal scales. Such information can be derived from field observations, from circumstantial evidence, from correlations with measured pollution data and from fumigation experiments in which species are exposed to known level of pollution. Information on the conditions of species, such as vitality and damage, may be also used (Van Haluwyn and Van Herk, 2002).

Hawksworth and Rose (1970) performed the mapping studies. The qualitative scale of relating SO_2 concentrations ($\mu g\ m^{-3}$) for estimation of air pollution in England and Wales with epiphytic lichens was provided. Ten zones were devised, with zone 1 includes species indicating SO_2 levels more than 170 $\mu g\ m^{-3}$ whereas zone 10 representing purity atmosphere. The selected indicator species that indicate the polluted zones are *Pleurococcus viridis* s.l. and *Lecanora conizaeoides*. While the present of *Lobraria* sp., *Sticta* sp., *Pannaria* sp. and *Usnea* sp. indicate the clean air zones.

Johnsen and Søchting (1976) mapped the distribution of lichens and bryophytes in the Aalborg- Nørresondby area, Denmark, and compared lichen data with air pollution data and bark properties. They obtained a strong correlation (r = 0.958) between SO_2 emissions and the distribution of *Xanthoria parietina*. It was concluded that SO_2 emission and the pH of the bark are important ecological factors for cryptogammic epiphytes.

Thrower (1980) used the sensitive and tolerance species to assess air pollution in Hong Kong. The results showed that lichens are absent in the worst pollution zone. This zone includes the areas where power station exists and where there is dense industrial development. The estimated concentration of SO_2 in the air is over 150 $\mu g/m^3$. The clean air zones are the areas with the present of *Parmotrema tinctorum* and *Usnea* sp.

Poikolainen *et al.* (1998) carried out a systematic mapping of thirteen epiphytic lichen species on conifers in Finland. The mapping work was conducted to describe the regional distribution of the most common epiphytic lichens and to assess air quality based on the abundance of epiphytic lichens. All lichen species demonstrated an increase in their abundance between the year 1985 and 1995. The sensitive lichen species had become more common particularly in the central part of country, which is in agreement with decreasing sulfur deposition.

Loppi *et al.* (2002b) used biodiversity of epiphytic lichens as indicators of air pollution in the town of Siena in central Italy. Most of the study area was in the categories semi-natural or natural, according to a calibrated scale of environmental alteration. Compared with the situation in 1995, the results showed an improvement in air quality over time.

Gombert *et al.* (2004) constructed an air-quality map of the Grenoble area in southeast France using the Index of Atmospheric Purity (IAP). Each sampling station was characterized by a subjective Index of Human Impact (IHI), calculated according to four local environmental parameters influencing lichens. Lichen species and two algae were recorded and grouped into three ecological categories defined according to bark types and nutrient needs. It was shown that IAP varied in relation to the relative proportion of ecological groups of lichen.

Sommerfeldt and John (2001) investigated air pollution and the occurrence of lichens in the city of Izmir, Turkey using the VDI method. The lichen air quality map showed that five air quality classes were determined and a predominant part of the city area is heavily polluted. The best air quality values were determined in the southern and western parts of the city zone. They suggested that a range of 7.3 for the width of the air quality classes provides a method that is suitable for similar studies in Turkey.

Saipunkaew (1994) carried out the first air quality study in Chiang Mai city using lichens as bioindicators. The air pollution map was developed based on the VDI method. The results are shown in a map indicating zones with different distributions of lichens. High lichen frequencies indicate better air quality while lower frequencies indicate worse air quality. Four air quality classes are distinguished. The air quality indices (AQI) vary from 2.3 to 31.5. The three zones of air pollution are determined by the drawing of isolines, with characteristic of very high pollution, very high-to-high pollution and high pollution, respectively. The air quality of Chiang Mai city is assessed again in 2001 by Subsri (2002). The same method is used and the results from the lichen mapping are compared. The author found that in 2001 the border of a high air pollution zone extends out the suburban area and is larger than in 1994. The results indicate that air pollution in Chiang Mai city has increased since 1994.

Hamada *et al.* (1995) examined the distribution of *Phaeophyscia limbata* and *Lecanora pulverulenta* and their relation to the distribution of SO_2 and NO_2 in the Osaka Plain, Japan. They found that the frequency of L. *pulverulenta* was lowest in the central area, where the concentrations of SO_2 and NO_2 were high, and increased gradually toward the periphery. While, the frequency of P. *limbata* was in contrast. They concluded that L. *pulverulenta* was more useful than P. *limbata* as a bioindicator of air pollutants such as SO_2 and NO_2.

In 2000, the standardized method to assess lichen diversity on tree barks was proposed (Asta *et al.*, 2002). The method in the guideline is largely based on the

German VDI lichen mapping guideline and the Italian guideline. The main modifications concern several elements of subjectivity in the sampling process, which were present both in the VDI and in the Italian guidelines. The other modifications, which are clearly difference from the VDI method, include the positioning and the size of the sampling grid on the tree trunks. In this method, the sampling grid of 10 × 50 cm quadrate with 5 sampling units of 10 × 10 cm is attached to the tree trunk corresponding with the 4 aspects (north, east, south and west), instead of using a 20 × 50 cm quadrate with 10 sampling units of 10 × 10 cm and attach to the one aspect of tree trunk where the lichen cover is highest, as describe in the VDI method. However, the data analysis for both VDI and standardized method is based on the sum of frequency of lichen species on a defined portion of tree bark. For this standardized method, the frequency of lichens is used to calculate the lichen diversity values (LDVs) and the LDVs map can be constructed in the similar way of the VDI method. Moreover, the LDV results can be used to assess magnitude of alteration as the deviation from natural conditions when the natural area is available (Loppi *et al.*, 2002a). Therefore, this procedure provides a rapid, low cost method to define zones of different environmental quality. It can be used to detect hot spots of environmental stress over a large-scale area as well as applied in the vicinity of an emission source to prove the existence of air pollution to identify its impact.

Pinho *et al.* (2004) used a standard protocol to determine lichen diversity value (LDVs) as an indicator of environmental quality. The study was carried out in a region in southwest Portugal, where foliose and fruticose lichens diversity and frequency were sampled in an area with large industrial facilities. They conclude from the study that the obtained LDVs map can identify the areas with either great or little disturbance, which later allows us to limit and concentrate the chemical sampling. They suggested that further pollution measurement and chemical analysis could be useful to improve the interpretation of data and accurately relate each pollutant level to the observed LDVs.

Castello and Skert (2005) compared the standard protocol of a study of epiphytic lichen diversity proposed by Asta *et al.* (2002) with the standard methodology, which previously used in Italy. Biodiversity values obtained from the two sampling methods are highly statistically correlated ($r = 0.9759$, $p < 0.001$) and their consistency is shown by very similar spatial diversity patterns obtained from the survey area. The regional scale of environmental alteration based on lichen diversity in the North Adriatic submediterranean bioclimatic area was provided with a seven-class scale of naturality/alteration. The LDVs vary from 1-15 for the high alteration, while the LDVs for high naturality are greater than 75.

2.3 Effect of air pollution on lichens

Sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) are common pollutants that have adverse effects on lichens (Richardson, 1992). The main anthropogenic source of SO_2 has been stationary source combustion, particularly from coal burning power plants, while NO_2 is mainly emitted by fossil fuel combustion in urban area (Spiro and Stigliani, 1996). Both pollutants can be transported over long distances following the pattern of prevailing winds.

SO_2 is a soluble gas and can dissolve in rainwater or in the moisture within the cell wall of wet thalli. The sensitivity of lichen lies in the acidic characteristic of SO_2. Since the cell membrane of lichen provides little barrier, the emitted SO_2 can readily pass inside the cell both in the gaseous form and in the dissolved form, as sulfate, sulfite, bisulfate ions or sulfurous acid (Hawksworth and Rose, 1976).

Fields (1988) reviewed the physiological responses of lichens when exposed to laboratory fumigations with pollutants such as SO_2, hydrogen fluoride, ozone and NO_2 and peroxyacetyl nitrate. The order of sensitivity appeared to be headed by N_2 fixation as the most sensitive process to pollution, followed by K^+ efflux/total electrolyte leakage, photosynthesis and respiration and pigment status. He concluded that even though, the various pollutants have different physiochemical properties; they affected the physiological system of lichens in similar way. Another study carried out by Kong *et al.* (1999) showed that lichens fumigated with 0.5 ppm SO_2 lead to a 10% decrease in phaeophytinization of chlorophyll in lichen *Xanthoparmelia mexicana*. Also, chlorophyll *a*, *b* and protein content decreased with the increase of SO_2.

Vokou *et al.* (1999) surveyed epiphytic lichen vegetation of 20 sites around Thessaloniki (Macedonia, Northern Greece) to monitor any changes in lichen communities and consequently, in air quality. They found impoverishment of lichen community, which was concluded to be the result of air pollution, chiefly SO_2 and NO_2. Showman (1997) used lichen to study air pollution in two forests, Fernwood and Yellow Creek at Ohio State, which served as recording data for future comparison. The reduction in lichen communities due to the impact of air pollution was observed. van Dobben *et al.* (2001) demonstrated the relation between the abundance of epiphytic lichen species and pollutant concentrations in the Netherlands. They reported that nearly all species decrease with increasing concentration of atmospheric SO_2 and NO_2, which appeared to be the most important factors determining lichen diversity.

2.4 Passive Sampling

Passive sampling is defined as any sampling technique based on free flow of analyte molecules from the sampled medium to a collecting medium, as a result of a difference in chemical potentials of the analyte between the two media (Górecki and Namieśnik, 2002). 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 a 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 objects and on persons, 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). Hence, it is ideally suited for developing a wide spatial network for atmospheric pollutant monitoring. This method can be used in a large-scale project for the measurement of atmospheric pollutants at an extremely low cost (Krochmal and Kalina, 1997). Carmichael *et al.* (2003) used the passive sampler to measure level of gaseous SO_2, NH_3 and O_3 at 50 stations in Asia, Africa, South America and Europe. They concluded from the study that diffusive samplers are ideal for measurements at remote sites, for checking transport model, screening studies, mapping concentration in cities, personal monitoring, etc. Bower *et al.* (1991) carried out a nationwide survey of ambient NO_2 concentrations in urban areas of the U.K. by utilizing passive diffusion tube samplers. They found that this survey provides, for the first time, a nationwide picture of urban levels of this pollutant. They examined the correlations between measurements of SO_2, smoke and NO_2. The closest correlation is observed between NO_2 and smoke, indicating traffic to be a dominant source of these pollutants. Stevenson *et al.* (2001) established an 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 can be utilized in large numbers to determine the spatial distribution of NO_2 and high light areas of high concentration

2.4.1 Operating Principle

The passive sampler is based on the principle of air diffusion. The atmospheric NO_2 diffuses into the tube where it gets absorbed on the absorber triethanolamine (TEA) coated. TEA absorbs NO_2 from the air in the form of nitrite ion. The reaction product of TEA and NO_2 has been studied and is still a subject of controversy. Glasius *et al.* (1999) proposed the reaction product as triethanolamine N-oxide on the basis of the following reaction

$$2NO_2 + N(CH_2CH_2OH)_3 + 2OH^- \rightarrow 2NO_2^- + {}^-O\text{–}N^+(CH_2CH_2OH)_3 + H_2O \qquad (2.1)$$

This reaction is in accordance with the observed 1:1 conversion of NO_2 to nitrite ions. Hydroxyl ions in the reaction probably stem from dissociation of TEA in water, and the reaction will therefore not take place in completely dry air.

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;

$$F_1 = -D_{12}\, dc_1/dz \qquad (2.2)$$

Where

F_1 the flux of gas (mol cm^{-2} s^{-1})

D_{12} 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 seconds for a cylinder of radius r is given by the following equations;

$$Q_1 = F_1(\pi r^2)\, t \text{ mol} \tag{2.3}$$

Therefore

$$Q_1 = -D_{12}\,(c_1\text{-}c_0)(\pi r^2)\, t/z \text{ mol} \tag{2.4}$$

Where

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

Then the concentration of NO_2 and SO_2 in µg m^{-3} are calculated by applying the equation (Plaisance *et al.*, 2002);

$$C = \frac{Q \times z}{(\pi r^2) \times t \times D} \tag{2.5}$$

Where

C the concentration measured by passive sampling tube (µg m^{-3})

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

r the radius of diffusion tube (m)

t the sampling time (s)

z the diffusion length (m)

D the diffusion coefficient (m^2s^{-1}), 0.154×10^{-4} m^2s^{-1} for NO_2 and 0.127×10^{-4} m^2s^{-1} for SO_2

The corresponding quantities of NO_2 and SO_2 are calculated by the following equations;

$$QNO_2 = m\ NO_2^- \tag{2.6}$$

$$QSO_2 = \frac{64}{96} \times m\ SO_4^{2-} \tag{2.7}$$

Heal and Cape (1997) measured NO_2 concentrations in urban and rural ambient air by using passive diffusion samplers with TEA as adsorbent. The interferences from peroxyacetyl nitrate (PAN) and others were observed to be low for British conditions. The systematic error of within-tube chemistry was also known to be responsible for overestimation of NO_2 by the diffusion sampler, which previously was thought to be due to wind effects. They found that passive sampling is more efficient in rural ambient air compared to urban ambient air. The combined error due to the effect of wind on path length and chemical effect with cities caused up to 70% overestimation of NO_2.

Perkauskas and Mikelinskiene (1998) used passive diffusion samplers for the evaluation of SO_2 and NO_2 concentration levels in the Lithuanian capital Vilnius. The results show that the SO_2 concentrations levels depend mainly on heating and exhibit average values of 7-13 $\mu g/m^3$ for warm seasons and 17-23 $\mu g/m^3$ for cold seasons. The NO_2 average rates depend strongly on traffic (sampling place) and are highest in crossroads (52-82$\mu g/m^3$) and lowest at the background-suburban level (9-16 $\mu g/m^3$).

Kasper-Giebl and Puxbaum (1999) used polyethylene diffusion tubes and TEA as an absorbent for the determination of ambient air concentrations of sulfur dioxide and nitrogen dioxide. They found the concentrations of NO_2 were 50% lower than the results given by nearby chemiluminescence monitors. The underestimation could be corrected by placing two grids into the diffusion tube. The determination of sulfur dioxide was strongly biased by the collection of particulate sulfate at the entrance part of the tube and along the tube walls.

Cruz *et al.* (2004) constructed a passive sampler, which was designed to minimize particle interference and turbulent diffusion. They tested the SO_2 diffusive passive sampler using Na_2CO_3 filter impregnation under ambient conditions, during periods of exposure ranging from 1–4 weeks. Its precision varied between 2.4% and 10% for a SO_2 concentration range of 1.9–13 mg m^{-3}, when applied to two different types of tropical environments. The field measurement results showed good agreement between passive and active methods during the same exposure period. The authors concluded that, considering the growing demands for environmental monitoring, passive samplers represent a cost-effective tool for SO_2 monitoring.