CHAPTER II

LITERATURE REVIEW

2.1 Polycyclic Aromatic Hydrocarbons (PAH)

PAH are ubiquitous pollutants in our environment. They consist in three or more fused benzene rings in linear or angular or cluster arrangement. PAH are member of a group of organic pollutants of critical public health and environment concern due to the following characteristics: Chronic health effects and carcinogenicity, microbial recalcitrance and high bioaccumulation potential. (Ruchaya Boonyatumanond, 1999)

As pure chemicals, PAH generally exist as colourless or white or pale yellow-green solids. Most PAH do not occur alone in the environment (including those found at hazardous waste sites), rather they are found as mixture of two or more PAH (ATSDR, 1990). They can occur in the air either attached on dust particles or in soil or sediment (WHO, 1998). Moreover they can be broken down by reacting with sunlight and other chemicals in the air, over a period of days to weeks (Somporn Chantara, 2000). PAH enter water through discharges from industrial and wastewater treatment plants. However, most of them do not dissolve easily in water, therefore, they stick to solid particles and settle to the bottoms of lakes or rivers. In soil, PAH are most likely to stick tightly to particles certain PAH move through soil to contaminate underground water. After a period of weeks or months, microorganisms can brake down PAH in soil or water. (ATSDR, 1990)

In the environment, human are exposed to gaseous PAH and PAH attached on dust and other particles in the air. PAH have been found in the USA. The background levels of PAH in the air were reported to be 0.02-1.2 mg/m^3 in rural areas and 0.15-19.3 mg/m^3 in urban areas (ATSDR, 1990).

US.EPA has identified 16 unsubstituted PAH as priority pollutants as shown in Figure 2-1.
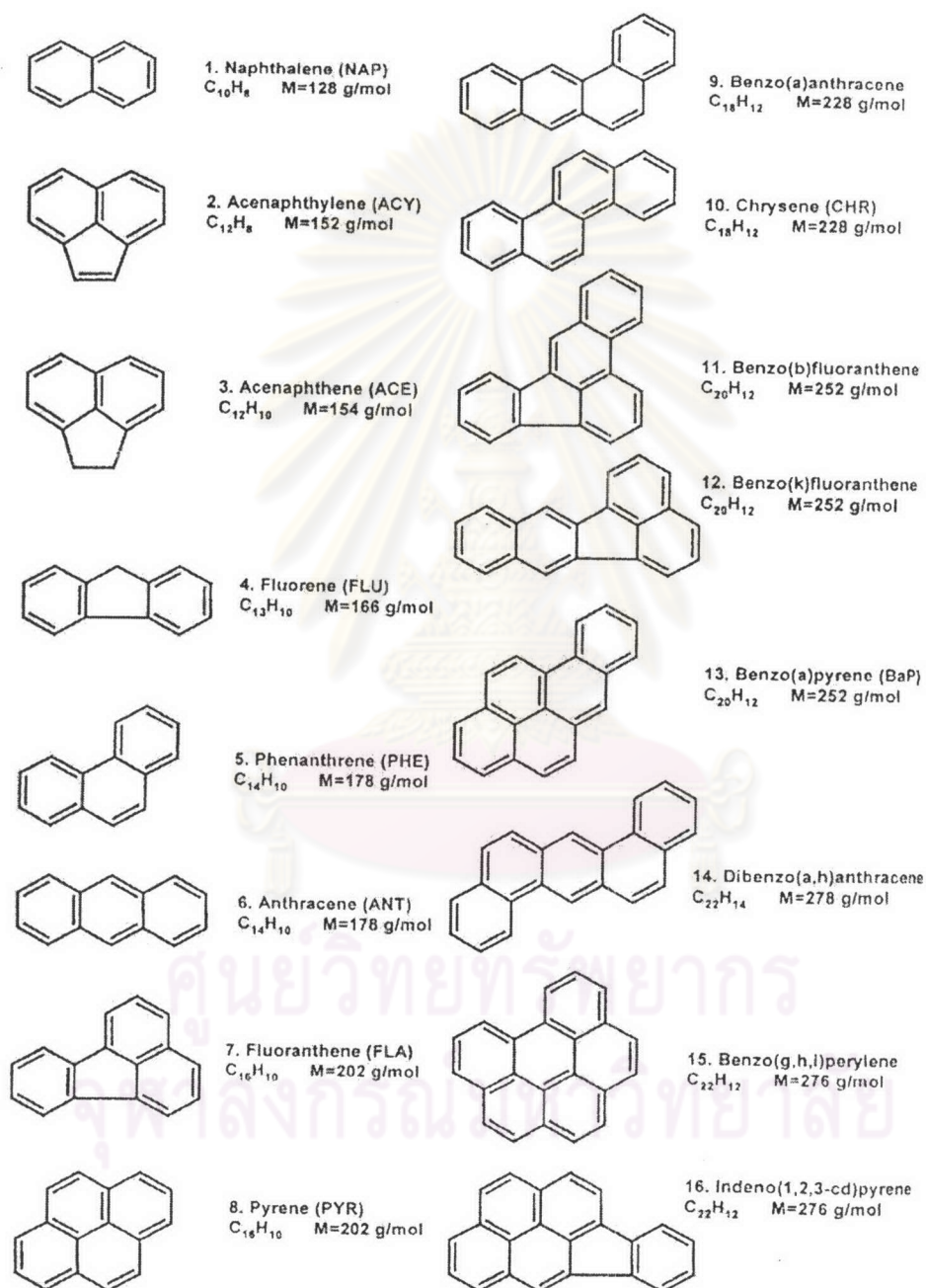

Figure 2-1 Molecular structure of 16 PAH considered by US.EPA as priority pollutants

Source: ATSDR, 1990

2.1.1 Sources of Atmospheric PAH

PAH are recognized as carcinogens more than 50 years ago, such as BaP and DbA (ATSDR, 1990; WHO, 1998). The acivity and the level of activity is likely to depend upon molecular structure (Ruchaya Boonyatumanond, 1999). The presence of PAH in the environment is obviously concerned. Apart from specific occupational environment, human exposure to these compounds derives mainly from combustion products released into the atmosphere (Ruchaya Boonyatumanond, 1999).

The release of PAH into the environment has been detemined by identification of PAH characteristics. PAH, especially these of high molecular mass, entering the environment via the atmosphere are adsorbed onto particulate matter (WHO, 1998). The hydrosphere and geosphere are affected secondarily by wet and dry deposition. Creosote preserved wood is another source of release of PAH into the hydrosphere, and deposition of contaminated refuse, like sewage sludge and fly ash, contributes to emissions of PAH into the geosphere (WHO, 1998). Little information is available on the passage of PAH into the biosphere. PAH occur naturally in peat, lignite, coal and crude oil. Most of the PAH in hard coals are tightly bound within the coal structure and cannot be leached out (WHO, 1998).

Part of the PAH in the atmosphere arises from natural combustion such as forest fires, volcanic eruption, sediment diagnosis and biological conversion of biogenic precursors (Hathairatana Garivait, 1999). The largest emissions of PAH result from incomplete combustion of organic materials during industrial processes and other human activities (WHO, 1998). But emissions from human activities such as vehicle exhausts, agricultural burning are the predominant sources in urban areas. The anthropogenic sources of PAH can be divided into immoblie and mobile categories (Hathairatana Garivait, 1999). The major contributors of immobile category encompass a wide variety of combustion processes including residential heating, industrial activities, incineration and power generation. The amount of PAH produced and released to the atmosphere from all

those sources varies greatly and is dependent on factors such as fuel type, combustion conditions and emission control measures. (Ruchaya Boonyatumanond, 1999)

Coal and crude oils contain PAH in considerable concentrations owing to diagenetic formation in fossil fuels. The main PAH produced commercially are NAP, ACE, ANT, PHE, FLA, and PYR. The release of PAH during production and processing, predominantly of plasticizers, dyes, and pigments, is of only minor importance. Most PAH enter the environment via the atmosphere from incomplete combustion processes, such as processing of coal and crude oil, heating (wood, coal, and mineral oil), fires (forest and agriculture), vehicle traffic, and tobacco smoking. In some geographical areas, forest fires and volcanoes are the main natural sources of PAH in the environment. (WHO, 1998)

2.1.2 Physical and chemical properties of PAH

According to their SOC properties, PAH are found in the atmospheric in two forms: sorbed on suspended particles and in gas phase (Hathairatana Garivait, 1999). Individual PAH differ substantially in their physical and chemical properties. In overall, the low molecular weight PAH, such as NAP and its alkylated derivatives, are more volatile, water soluble and less lipophilic than their high molecular weight relatives. These physical and chemical properties largely determine the environmental behaviour of PAH.

The physical and chemical properties are largely determined by the conjugated electron systems, which vary fairly regularly with the number of rings and molecular mass, giving rise to a more or less wide range of values for each parameter within the whole class. At room temperature, all PAH are solids (WHO, 1998). The general characteristics common to the class have high melting and boiling point, low vapor pressure, and very low solubility in water. PAH are soluble in many organic solvents and are highly lipophilic. (WHO, 1998)

Vapor pressure of PAH tend to decrease with increasing molecular mass, varying by more than 10 orders of magnitude. This characteristic affects the adsorption of individual PAH onto particulate matter in the atmosphere and their retention on particulate matter during sampling on filters. Vapor pressure increases markedly with ambient temperature, which additionally affects the distribution coefficients between gaseous and particulate phases. Solubility in water tends to decreases with increasing molecular mass. (WHO, 1998)

PAH are chemically inert compounds. When they react, they undergo two types of reaction; electrophilic substitution and addition (WHO, 1998). As the latter destroys the aromatic character of the benzene ring that is affected, PAH tend to form derivatives by the former reaction; addition is often followed by elimination, resulting in net substitution (WHO, 1998). The physical and chemical properties of PAH are shown in Table 2-1.

Table 2-1 Physical and chemical properties of the typical atmospheric PAH

PAH	MW	Mp. (^{o}C)	Bp. (^{o}C)	S (at 25°C) (μg/l)	H (at 25°C) (Pam^{3}mol^{-1})	log K_{ow}	log K_{OA} at 25°C (Beyer et al., 2000)	VP (at 25°C) (Pa)
NAP	128.2	81	218	3.17×10^4	44.6	3.40	5.13	10.4
ACY	152.2	92	265	$3.93 \times 10^{3*}$	8.4	4.07	6.47	8.9×10^{-1}
ACE	154.2	95	279	3.93×10^3	12.7	3.92	6.23	2.9×10^{-1}
FLU	166.2	116	295	1.98×10^3	7.91	4.18	6.68	8.0×10^{-2}
PHE	178.2	101	340	1.29×10^3	7.47	4.60	7.45	6.9×10^{-4}
ANT	178.2	217	342	73	7.34	4.50	7.34	8.0×10^{-4}
FLA	202.3	109	375	260	8.6	5.22	8.60	1.2×10^{-3}
PYR	202.3	150	393	135	8.61	5.18	8.61	6.0×10^{-4}
BaA	228.3	161	400	14	9.52	5.61	9.54	2.8×10^{-5}
CHR	228.3	254	448	2.0	10.42	5.91	10.44	8.4×10^{-5}
BbF	252.3	168	481	1.2^*	10.17	6.12	-	6.7×10^{-5}
BkF	252.3	216	480	0.76	11.18	6.84	11.19	1.3×10^{-8}
BaP	252.3	178	496	3.8	10.77	6.50	10.77	7.3×10^{-7}
IP	276.3	164	536	62	-	6.58	-	1.3×10^{-8}
DbA	278.4	267	524	0.5	13.91	6.50	-	1.3×10^{-8}
BPER	276.3	278	545	0.26	11.01	7.10	11.02	1.4×10^{-8}

Sources: WHO, 1998

* Ruchaya Boonyatumanond, 1999

2.2 Characteristic of plant leaves

2.2.1 Structure of leaves

The plant leaves usually compose with 3 layers, which are epidermis, mesophyll, and veins (as show in Figure2-2). (Moore et al., 1995; Raven, Evert and Eichhorn, 1986)
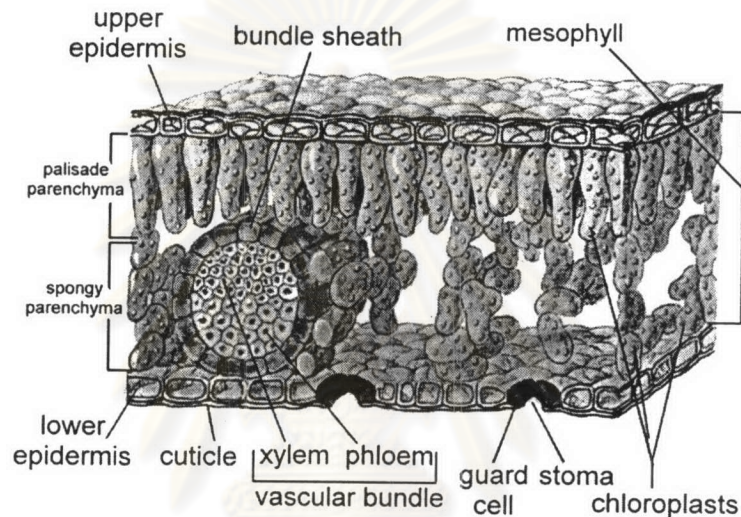
Figure 2-2 The structure of plant leaf
Source: Moore et al., 1995

The epidermis protects inner tissues from desiccation, pathogens, herbivores, etc. It usually has one layer thick over upper and lower leaf surfaces. It mostly produces waxy cuticle, which may be thicker in hotter and drier environments. The cuticular membrane is described as an extra-cellular physiological compartment that mainly consists of soluble waxes and insoluble polymers referred to as cutin (Muller, Hawker and Connell, 1994). Therefore it is convenient to treat the cuticular membrane separate to the cellular lipids. The cells in this layer are lacked of chloroplasts and may have hairs (trichomes) for protection. The stomata regulated by guard cells may be found in either (or both) upper

and lower epidermis. Guard cells regulate water loss and gas exchange by regulating opening and closing of the stomata. (Moore et al., 1995)

The mesophyll (middle leaf) includes the major photosynthetic tissues that are palisade parenchyma and spongy parenchyma. Palisade parenchyma is found beneath the upper epidermis in dicots packed with chloroplasts. It may have one or more cell layers thick, depending on plant. Spongy parenchyma is found in both monocots and dicots, in dicots. It will be found below the palisade layer. It forms large air spaces to provides the storage for carbon dioxide, oxygen and also provides large surface area for absorption of carbon dioxide into cell. (Moore et al., 1995)

The veins contain the vascular tissues. The xylem and phloem in leaves is continuous with xylem, phloem in stems. In some dicots (never in monocots), xylem and phloem may be surrounded by specialized layer of cells, called bundle sheath. The bundle sheath cells are usually found in plants living in hotter or drier environments, they sequester xylem and phloem away from air spaces, which caused the reducing of water loss and they also play a role in C-4 photosynthesis. (Moore et al., 1995)
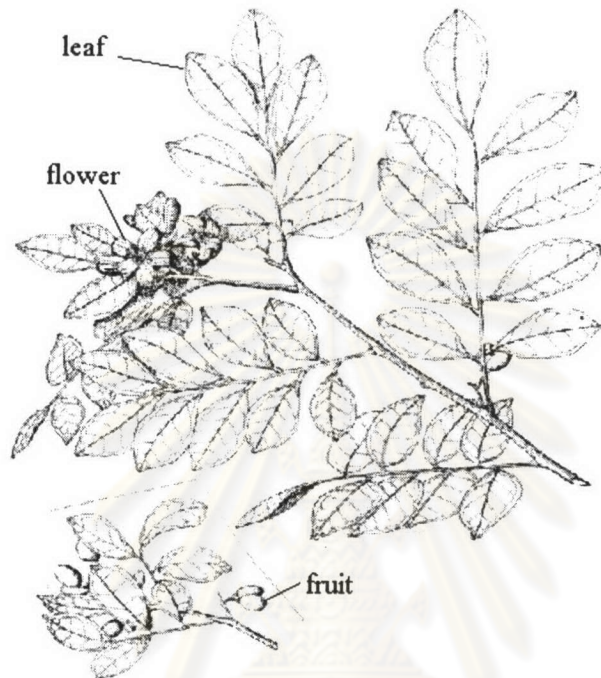
2.2.2 *Murraya paniculata* (L.) Jack

The selected plants, *Murraya paniculata* (L.) Jack (orange jasmine) is classified as Rutaceae family, which is in the same family of lime (*Citrus aurantifolia*). It is a local plant in South East Asia, and mostly found in every part of Thailand. It is the hardy evergreen shrub with glossy green leaves which have glandular punctate to produce wax. It has flowers all year with highly perfumed creamy white color. Prefers a sunny position to flower but will tolerate part shade. Enjoys fertile soil for rapid growth. It is mostly planted along the roadside, and can grow in a wide range of dry to wet sites.

It has alternated obovate leaf arrangement with odd-pinnately compound type. The leaf blade length is less than 2 inches. The small red berries appear throughout much of the year and attractive to birds. The shrub is well-suited to shearing into a formal hedge

or screen and can take on a boxwood-like effect in a formal garden. However, it had rapid growth rate while plants are young assures that numerous shearings will be needed throughout the growing season.

Figure 2-3 *Murraya paniculata* **(L.) Jack (orange jasmine)**
Source: The Royal Institute, 1995

2.2.3 Composition of leaves

Leaves are the most active and conspicuous organs of plants. The most importance of their functions is absorbing sunlight for photosynthesis. To do this, they expose large amounts of surface area to the environment. (Moore, 1995)

Leaves consist of various phases including intercellular air, water, lipids, structural carbohydrates, and proteins (Muller et al., 1994). Each of these phases has characteristic physicochemical properties. The cuticular membrane mainly consists of soluble waxes and insoluble polymers referred to as cutin. Therefore it is convenient to treat the cuticular membrane separate to the cellular lipids.
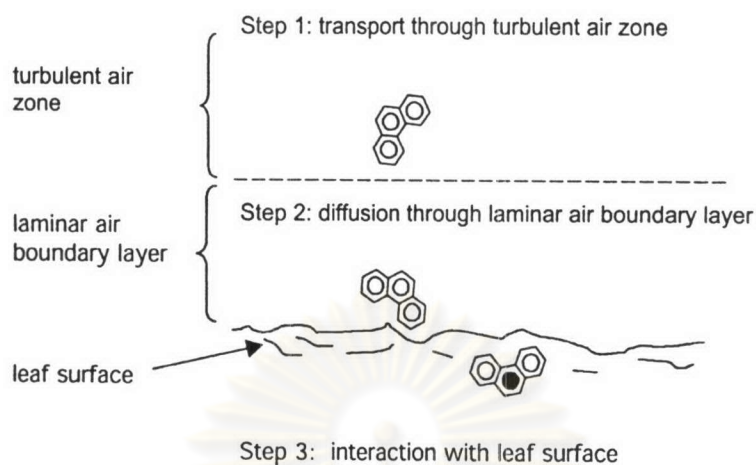
The outermost layer (cuticle) of leaves that comprise the waxes, called cuticular wax. Waxes are complex mixtures of fatty acids linked to long – chain alcohols. This mixture also contains free fatty acids, fatty acids with hydroxylgroups, and long-chain hydrocarbons. Waxes are usually harder and more water-repellent than other fats. Cuticular wax is also assosiated with cutin, another waxy substance, which makes up most of the cuticle. Cutin consists of a variety of hydroxylated fatty acids link together.(Moore, 1995)

2.3 Partitioning of SOC in the biota system

2.3.1   Pathways of PAH deposition

Deposition of atmospheric PAH to plants occurs via several pathways. Because PAH partition between the gas phase and atmospheric particles, a major division can be made between gaseous and particle-bound deposition. Since the solubility of the hydrophobic PAH is very low, the rain droplets or other precipitation, wet deposition of gases is of minor importance. Compounds bound to atmospheric particles can reach the plant surface by both dry and wet deposition. Uptake of compounds that are volatilized from highly contaminated soil is another pathway, particularly in more basal aerial plant parts. Also, contaminated soil particles can be transported directly to the plant surface by wind or splash. (Bakker et al., 2000)

The deposition of airborne compounds, whether they are gaseous or particle-bound, to plant leaves involves three steps as shown in Figure 2-4.

Step 1: transport through turbulent air zone

turbulent air zone

Step 2: diffusion through laminar air boundary layer

laminar air boundary layer

leaf surface

Step 3: interaction with leaf surface

Figure 2-4 Schematic representation of the three steps in the deposition process of hydrophobic organic compounds from the atmosphere to a leaf

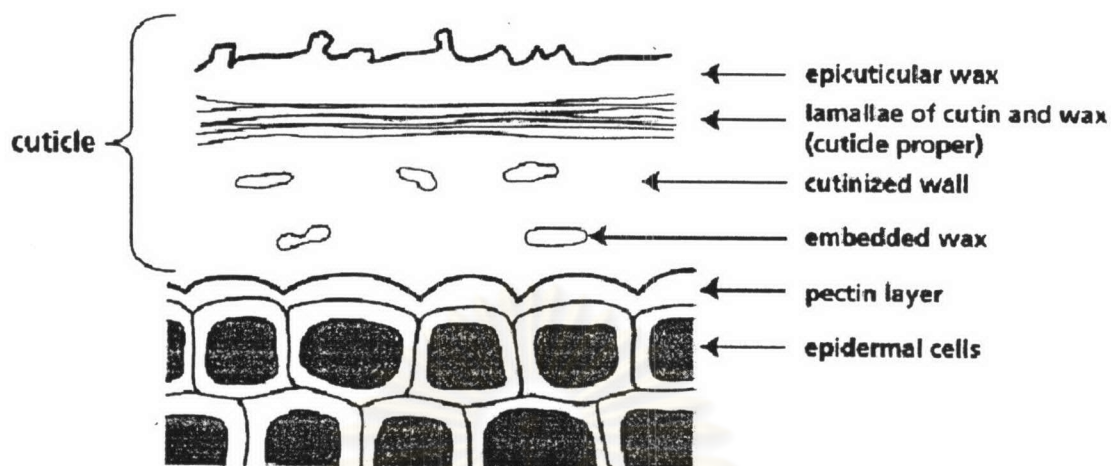**Source:** Bakker et al., 2000

First, the contaminant is transport from the (turbulent) atmosphere to the laminar air boundary layer surrounding the leaf. Subsequently, the contaminant has to be carried across the boundary layer. In this layer the airflow is parallel to the leaf surface. The final step in the uptake process is the interaction of the compound with the leaf surface. Deposited particles may simply adhere to the leaf surface, react with it chemically, or bounce off if the surface is smooth. For gases, the third step comprises the adsorption to the surface or diffusion into the cuticle (Schreiber and Schonherr, 1992).

The different pathways are all function of (1) the physicochemical properties of the compound (such as vapor pressure, hydrophobicity, molecular weight), (2) environmental characteristics (temperature, wind) and (3) plant characteristics (surface area, lipid content and composition, architecture of the plant). (Paterson et al., 1990)

The particle-bound fraction of PAH in the atmosphere depends on the ambient temperature, the available particle surface and the compound's volatility. The compound's K_{OA} to govern the gas-particle distribution, with high K_{OA} values favoring high particle-bound fractions. (Bakker et al., 2000)

PAH may be transferred from the deposited particles to the cuticle, which has been studied with wash-off experiments. Washing of lettuce with water removed a considerable amount of the high molecular weight PAH, but little of the small PAH phenanthrene, indicating that only the latter, gaseous PAH was sorbed in the cuticle (Bakker et al., 2000). In contrast, rinsing maize leaves with aqueous solutions could only extract a minor part of the high MW PAH, suggesting that the compounds were desorbed from the particles or that the particles were encapsulated in the cuticle (Kaupp, 1996 cited in Bakker et al., 2000).

The uptake of gaseous PAH involves passive diffusion of the compounds between the atmosphere and the cuticle of the plant. The cuticle is an extracellular, non-living, lipid layer which forms the interface between the atmosphere and the plant and protects the plant from desiccation and from fungal and insect attack, as shown in Figure 2-5 (Bakker et al., 2000). It is usually characterized by the presence of two specific classes of lipids: soluble waxes and insoluble polyester cutins. Waxes occur embedded in the cuticular membrane (intracuticular waxes) and lying across the surface of the membrane (epicuticular waxes). Waxes consist of complex mixtures of long-chain aliphatic and cyclic components, including primary alcohols, alkanes, esters, fatty acids and triterpenoids. The cuticular is not homogeneous, but composed of a number of layer of which the characteristics vary according to species, age of the plant and environmental conditions. (Bakker et al., 2000)

Figure 2-5 Schematic representation of the cuticle of a plant
Source: Bakker et al., 2000

Uptake of SOC via stomata is probably not important. They will prefer to enter the cuticle, hence the contribution of the stomatal pathway will be negligible to that of the cuticle (McLachlan, 1999). Moreover, there is some evidence that the cuticle extends farther along the cell wall that from the epidermal boundary of the substomatal cavity. So, even after entering the stomata, SOC can still be sorbed to these internal cuticular waxes.

2.3.2 PAH and plant leaves relationship

Atmospheric SOC can be deposited to plants by dry gaseous and wet and dry particle-bound deposition. Once deposited to the leaves or stems of the plant, the compound can be transported through the cuticle, the lipid-like layer which forms the interface between the atmosphere and the plant. (Bakker et al., 2000)

Higher plant leaves, as well as lichens, mosses, and grasses, constitute an important environmental compartment, in which the bioconcentration of trace organic chemicals can occur. Among the three main pathways for the uptake of organics in plant leaves (1) Translocation from contaminated soil via roots. (2) Atmospheric deposition of

contaminated particulates and (3) vapor intake from the air. The air to leaves transfer of gaseous organics can be considered a key process, particularly for less water soluble compounds. (Bacci Eros et al., 1990)

Several studies have shown that partitioning from the soil into vegetation does not occur to a great extent for lipophilic SOC (Simonich and Hites, 1994; Nakajima et al., 1995). Same as the study of Granier and Chevreuil (1992), used tree leaves from the plane tree *Plantanus vulgaris* as bioindicators of aerial organochlorines. Plants absorb PCB mainly from the surrounding atmosphere and not from the roots. And root uptake followed by translocation within the plant is not significant for substances with log octanol/water partitioning coefficients larger than 3 (Kylin, Grimvall and Ostman, 1994).

The study concerning the PAH are ubiquitous and accumulation in the terrestrial environment. These compounds have been found to accumulate in moss, in tree bark and particularly in the leaves of trees and grasses (Toll and McLachlan, 1994).

Thomas (1984), studied the accumulation of PAH, chlorinated hydrocarbons and trace metals in moss (*Hypnum cupressiforme*) in Europe and found that mosses were suitable for monitoring the chlorinated hydrocarbon, BHC and PAH. The results showed a clear PAH concentration gradient in mosses, which was high in the industrial centers in middle Europe and low in northern Europe. The content of fluoranthene (1.0 ng/g) and benzoperylene (0.6 ng/g) in moss samples in Iceland were regarded as background levels. While in Thailand, Somporn (2000) studied the suitability of a bioindicator for air quality concerning PAH in Chiang Mai City. Epiphytic lichens, leaves, bark of mango trees (*Mangifera indica* L.) and soil were investigated to find out which one would be a suitable bioindicator. After a systematic investigation upon analyzing for PAH in all sample types, the bark of mango trees was found to be the suitable indicator for monitoring levels of PAH and construct an air quality map. The results obtained from the summation of the concentrations (dry weight) of 16 PAH found in bark samples were in the range 20.8-162.2 ng/g and 8.1-205.4 ng/g.

Kylin, Grimvall and Ostman (1994), have used Pine needles Scot pine (*Pinus sylvestris*) as passive samplers for monitoring polychlorinated biphenyls (PCB) to mapping the distribution of organochlorine compounds in Europe. They analyzed pine needle wax from the central and northern parts of Europe and found that high concentrations of PCB were found in Western Germany 47 ng/g of wax as compared to the other investigated geographical sites 4-7 ng/g of wax.

For the seasonal changes in the concentrations of PAH, Nakajima et al. (1995) studied perylene, BaP and PYR in azalea leaves and in the atmosphere (measured separately, in the vapor and particulate phases) were followed at two sites with different traffic conditions in Tokyo. The concentrations of the compound in leaves (PAH-lev) and in suspended particulate matter (PAH-pat) decreased in summer and increase in winter.

2.3.3 Factor influencing plant leaf-air partitioning system

2.3.3.1 Temperature and wind

Temperature and wind are important environmental parameters. Under equilibrium conditions, temperature has a large influence on air to plant partition coefficient (K_{PA}). Because of different wind speeds, the rate at which plant concentrations adjust to new situations can be different sites. Wind can increase the transfer of the chemical from the air to the plant surface, by increasing the turbulence and decreasing the thickness of the laminar boundary layer. This illustrates the important role of the wind, although the effect of wind on uptake rate has never been studied directly. (Bakker et al., 2000)

The partitioning process between air and leaf is expected to be dependent on the ambient temperature. This means, as the ambient temperature decreases, partitioning to leaf increases. (Bakker et al., 2000)

2.3.3.2 Lipid content

Difference in accumulation in many plants results from the lipid content of the species. The influence of different lipid content in different part of a plant (bark, needle, seed, leaf) has been observed (Simonich and Hites, 1994). The extractable lipid content may not represent the actual storage volume, as SOC may also accumulate in non-extractable lipid material, such as cutin. On the other hand, SOC may not have reached the internal lipids, but these are included in the total lipid content. In practice, however, this factor is difficult to estimate (Bakker et al., 2000). Under equilibrium conditions, the large differences between PAH concentrations in some plants may be explained by the amount and composition of the lipids. However, it is difficult to ralate the presence of certain wax components to the stroage capacity of leaves.

2.3.3.3 Growth dilution

The large difference found between SOC concentrations in pine needles and lichens in Norway, it was suggested that the difference in plant age (2 years for the pine needles and 25-50 years for the lichens) may play a role (Ockenden et al., 1998). SOC in air of a few decades ago were likely higher than those in contemporary air. Hence, the older lichens may have been approaching higher equilibrium concentrarions of PCB, and are now releasing the compounds very slowly (Ockenden et al., 1998).

In cases where equilibrium has not been approach, the age of the leaves and the plant architecture have been shown to be key factors in determining the PAH concentrations in plants.

2.3.3.4 Degradation

- Photodegradation

PAH are photodegraded in air by two processes: direct photolysis by light with a wavelength < 290 nm and indirect photolysisby least one oxidizing agent such as OH, O_3 and NO_3 in air. In general, indirect photolysis-photooxidation- is the more importantprocess. The reaction rates of PAH with airborne OH radicals measured under standard conditions, which shows that the calculated half-lives are one day or less. Under environment conditions, PAH of higher molecular mass, i.e. those with more aromatic rings, are almost completely adsorbed onto fine particles, this reduces the degradation rate markedly. (WHO, 1998)

The degradation was much slower in the absence of sunlight. PAH did not react significantly with SO_2. PAH in wood smoke and gasoline exhaust did not degrade significantly during winter in extreme northern and southern latitudes owing to low temperatures. In summer, however, at a temperature of 20 $^{\circ}$C, the half-lives of individual PAH were in the range of 30-60 min. the degradation rate increased further with increasing humidity. The rate of degradation of absorbed individual PAH seen: to be independent of their physicochemical characteristics but dependent on their molecular structure (WHO, 1998).

- Biodegradation

PAH can be biodegraded or biotransformed by microorganisms such as yeast, fungi and bacteria. The PAH with up to four aromatic rings are biodegradable under aerobic conditions but that the biodegradation rate of PAH with more aromatic rings is very low. Biog\degradation under anaerobic conditions is slow for all components (WHO, 1998). Algae have been reported to degrade benzo(a)pyrene to oxides, peroxides, and dihydroxydiols. Owing to the high biotransformation rate, the concentrations of PAH in organisms and water are usually not in steady state. Freely dissolved PAH may be rapidly degraded under natural conditions if sufficient biomass is available and the turnover rates are fairly high. For example biodegradation of acenaphthene with the concentration of 5 and 10 mg/l PAH at $25^{o}C$ and found the significant degradation with rapid adaptation of 100% degradation after 7 days.