4. DISCUSSION

4.1 Occurrence of PAHs in soil

By employing HPLC with UV detection, together with specific sample preparation, 16 EPA-PAHs were isolated and detected from soil samples (Tables 3.7-9). Acenaphthylene was not found in all of the samples analyzed, but all the other fifteen PAHs could be detected. Acenaphthylene has a relatively low melting point and occurs in the environment at very low concentrations. The method detection limit (MDC) of acenaphthylene is 2 ppb. In one of the three samples conducted by GC/MS, acenaphythalene could be determined with the concentration less 1 ng/g dry soil (Table 3.11).

Fluoranthene, pyrene, benzo(g,h,i)perylene, and phenanthrene were present at higher concentrations when compared with other PAHs both in Muang District and Sarapee District (Tables 3.7-8, Fig. 3.5). These PAHs tend to be predominant in soil samples. There are several environmental factors which can influence the concentration of PAHs, such as temperature, soil texture, sun light, and soil microorganisms. Chiang Mai is located in the tropical area, and its altitude is about 350 m above sea level. Photodegradation is one of the main limiting factors. Some PAHs, such as dibenz(a)anthracene, anthracene, are easy decomposed by photochemical reactions under a strong UV irradiation. Therefore, their concentrations are very low in soil. Because naphthalene, acenaphthene, acenaphthylene, and fluorene are easy evaporated during sample preparation for HPLC analysis, their concentrations analyzed may not reflect their actual conditions. Therefore, discussions and conclusions concerning these compounds are not included.

Two samples from Doi Suthep were not found to contain any PAH except anthracene, which was present at 1 ppb in the sample from the Wat Pahlaht Temple area. As mentioned in the site description, this area is far from the city and the residential areas. Traffic pollution, burning of waste, cooking, and other anthropogenic activities have not happened in this area although there has been an increasing number of tourists in recent years. This area is therefore considered as control, and the concentrations of PAHs reflect the background of Chiang Mai Province. It is possible in Doi Suthep area that forest fires happen. Wildfires may be caused by the accidental acts of man. And also, some burning of forest lands takes place according to planed decisions made by forest managers and hilltribes. However, the results clearly indicates that nearly all the PAHs contents were below the detection limits (Table 3.9) in this natural environment. Muang District and Sarapee District are subject to anthropogenic pollution. Soil samples from these two districts were found to contain total PAHs in the ranges of 34-127 and 10-117 ppb, respectively. The exposure types in these two districts are different. Muang District is the center of Chiang Mai Province where the traffic density is much higher than that in Sarapee District. Motor exhausts are the main sources of PAH pollution. In Sarapee District, one of main factors could be the burning of the home refuse. As no proper garbage disposal is available, villagers generally burn their accumulated garbage in the gardens. The vehicle density in this district is not comparable to Muang District. This is a suburban area and the samples collected from this area were in fact from villages. These villagers live on agriculture and most of them do not own cars. Comparison of total PAHs from these two districts showed significant difference ($p<0.05$). The concentration of individual PAHs is higher in Muang Distract that that in Sarapee District. Therefore, Muang District could be regarded as more polluted by PAHs (Table 3.10).

The samples collected from roadsides were presumably affected by exposure to motor exhausts, while the samples from garden areas, where the main source of PAHs

could be from burning wastes and cooking, were compare to roadside soil samples. As shown in Fig. 3.3, the concentration of total PAHs is higher in samples from the roadside in Muang District. The PAHs in soil were therefore contributed mainly from motor vehicles, including motor cars, diesel engines, buses, and motorbikes.

4.2 Comparison of PAHs in soil in Chiang Mai with other studies

Up to present there have been no reports on the amounts of PAHs in soil in Chiang Mai Province. In this study, soil samples from Doi Suthep were analyzed and only one PAH, anthracene, could be detected in trace amounts (1 ng/g dry soil). This suggests that the background concentration of PAHs in Chiang Mai is probably very low. The PAHs concentrations in Muang and Sarapee Districts at present are much higher than those Doi Suthep. However, as shown in Table 1.3, the soil PAHs in Chiang Mai are much lower than those in Saarland, Germany, even lower than those in rural areas. There is thus a big difference in soil PAHs in different countries. Germany is a developed country, and the Industrial Revolution which happened at first in Europe started several hundred years ago. PAHs are mainly from the industrial processes, such as burning of coal and oil fuels from coke plants. The accumulation of PAHs in soil makes the background level very high in such countries. Therefore, even in rural areas, soil PAHs may occur at high level. In contrast, Chiang Mai used to be a small town and the economic boom has happened only in recent years. As no big industry in this province, the sources from industrial processes are negligible.

The PAH concentrations in the environmental are also largely influenced by topographic and meterographic factors [1,13,14]. Chiang Mai is located in tropical areas and in higher altitude. Therefore, the degree of evaporation of PAHs in environmental is possibly greater in Chiang Mai because of higher temperature and

stronger UV irradiation. The degradation of PAHs in the environment should be relatively fast.

Because no official standards and guidelines concerning PAHs in soil could be found, the data from this study are compared to reference values of the Netherlands guidelines for soil restoration [59]. As showed in Table 4.1, PAH concentrations are much lower in Chiang Mai except pyrene. The results demonstrate the environment in Chiang Mai is not serious polluted by PAHs. However, as discussed in next part, most soil samples tested showed mutagenic activity. The pollution could not be measured only with PAHs.

Table 4.1 Comparison of reference values of the Netherlands guidelines and data from this study on PAHs in soil (ppb).

	NAP	ANT	PHE	FLU	PYR	BaP	Total PAHs
Netherlands	100	100	100	100	10	100	1,000
Muang District	4.3	0.9	9.0	0.4*	15	4.0	85.9
Sarapee District	2.9	1.2	4.6	0.4*	4.2	2.0	40.6

* including ACE.

4.3 Mutagenic activity of soil in *Salmonella typhimurium* strains

Fifteen soil samples were conducted with *Salmonella typhimurium* TA98 for examining their mutagenic potential. The other 3 samples, D1, S3, and S4, were not tested because of the difficulty in soil extraction. The amounts of extractable organic matter of these three samples are 0.07, 0.08, and 0.08 mg/g soil, respectively.

In case of a sample induced a doubling of revertant colonies at two consecutive dose levels, it was considered positive results [8]. The specific activity and weighted

activity could be calculated. Otherwise, the specific activity and weighted activity were considered below detection. With this criteria, twelve of fifteen soil samples tested showed the positive response in *Salmonella typhimurium* TA98 with metabolic activation. Among of them, samples M1, M2, M3, S1, S2, S5, and D2 could induce revertant colonies both with and without metabolic activation. Mutagenicity of soil extracts increased in the presence of S9 mixture. The results suggested that both direct acting and indirect acting mutagens existed in these soils. While some other samples, such as M5, M7, S6, S7, and S8, contained only indirect acting mutagens because they could induce revertant colonies only after having been activated by rat liver microsomal enzymes. Only two samples, M2 and S8, showed mutagenic activity in TA100. TA98 detects specifically frame-shift type mutation, and TA100 for base-pair change type mutation. Therefore, the soil contains mainly frame-shift type mutagens.

At a dose lever of 1 mg/plate in TA98 with metabolic activation, samples M1, S1 and S8 yield higher his^+ revertants, 125, 113, and 125 net his^+ revertant colonies, respectively. The comparison of specific activity at 1 mg/plate between Muang District and Sarapee District gave no significant difference. However, soil samples from Sarapee District weas likely to induce more his^+ revertants without metabolic activation. Samples S1 and S5 induced 112 and 145 net revertants at 1 mg extract per plate. The results indicated the extracts from Sarapee soil contained relative stronger direct acting mutagens. The comparison of weighted activity of soil between Muang District and Sarapee District did not show the significant difference (Table 3.17 and Fig. 3.10), although soils from Muang District had the tendency of higher weighted activity with metabolic activation.

Soil is a complex mixture containing a variety of chemical classes. Three important classes of organic compounds, PAHs, nitropolycyclic aromatic hydrocarbons (nitro-PAH), and polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are frequently detected in contaminated soil [24,60,61]. The

mutagenicity of soil is the result of the interaction between the compounds containing in soil. From this study, the correlation between PAH contents in soil and the mutagenic activity is difficult to be drawn. Although some samples (M1 and M7) with higher PAH level demonstrated higher mutagenic potential, still there were several samples which showed the opposite result. For example, both M5 and S7 yield a high PAH content, with the total PAH about 127 and 117 ppb, respectively, but both of them did not exhibit higher weighted activities. Especially, one sample from Doi Suthep area (D2) showed a positive response in TA98 although no PAHs could be detected in soil. Such data could not emphasize the importance of PAHs in the mutagenic potential of soil.

From the results obtained in this study, the correlation between total PAHs and the mutagenic potential in soil is weak (correlation efficient 0.51, $p = 0.05$) in TA98 with metabolic activation. There is no correlation in TA 98 without metabolic activation. As PAHs are a group of indirect acting mutagens; they need to be metabolized by microsomal enzymes to demonstrate the mutagenicity and carcinogenicity [55]. Therefore, risk assessment for mutagenic and carcinogenic potential of environmental complex based on chemical analysis of PAHs is limited because synergistic, antagonistic, and additive interaction between the compounds in the complex and the direct acting mutagens could not be predicted by PAHs. Previous studies showed that PAH levels are consistent with the mutagenicity of environmental complex [62,63]. but, some studies obtained a different result. The nitro-PAHs were responsible for a large portion of the mutagenic activity of air samples, and it is estimated that less than 2% of the mutagenic activities of air sample could be contributed for by benzo(a)pyrene content [64]. There are several reasons that could explain the lack of correlation observed in this study. One of the facts is that not all measured PAHs are mutagenic in this test system. Fluorene, naphthalene, acenaphthylene, and acenaphthylene are reported to be negative [22,65], and even

contradictory regarding mutagenicity. Synergistic interactions can also explain why PAH contents do not correlate with the mutagenicity. Inhibition of metabolism of mutagenic PAHs to the mutagenic metabolites may also partially account for the observed lack of correlation between mutagenicity and the individual PAHs in these samples. Soil may contain both PAHs and heterocyclic aromatic components. Many of these compounds are possibly competitive, or otherwise, for the cytochrome P450 metabolic systems [48]. Hermann [65] showed that at a low concentration, benzo(e) pyrene enhanced the mutagenic activity of benzo(a)pyrene, but inhibited at higher concentration. Schmaltz *et al.* [66] reported naphthalene inhibited the induction of skin tumors by benzo(a)pyrene. Donnelly *et al.* [67] found that nonmutagenic PAHs inhibited the mutagenic potential of benzo(a)pyrene in the extract of soil from Superfund sites. The above studies demonstrate the difficulties to predict the mutagenic potential by a chemical analysis alone.

An interesting topic arose from the result that soil D2 showed a positive response in bioassay although this area would be considered as a clean site. The chemical analysis supported this expectation as no PAHs could be found, but the Ames test provided a controversial result. It is likely that some compounds in soil contributed the mutagenic potential of this soil sample. Further investigation suggested that the mutagenic activity may come from fertilizers and herbicides as well as their interactions. The soil was collected from Wat Prathat area, which actually is in a National Park. The park was decorated with some ornamented vegetation in order to attract tourists. Therefore, fertilizers, insecticides and herbicides, which probably contain nitro-compounds, a group of mutagens, were used. The soil texture identification also indicated the agricultural activities, with higher nutrients than normal soils from the mountain. Although the presence of PAHs has not been reported in this type of soil, the application of herbicides, insecticides, and fertilizer are also able to yield mutagenic activities.

The sources of mutagenic activities in the garden sites are more complex because the biocides may be used in the gardens. Cooking and burning of wastes can produce both PAHs and nitro-PAHs. Open fire places burn the organic wastes or coal most inefficiently. The temperature of a typical open fire can not burning volatile matter completely to carbon dioxide and water vapor because the supply of secondary air is not controlled. One of important sources may be from the heterocyclic amine as the result of cooking. The fact that PAHs in garden areas generally were very low although the mutagenic potential was apparent indicates other related compounds might exist. A number of close compounds have been identified as arising from the heat treatment of proteins and aminiacids. They result from a complex interaction between meat components, cooking surface characteristics, time and temperature conditions of cooking [68,69]. Home heating and cooking sources have been shown to account for a great degree of the mutagenic activity.

4.4 Risk assessment to humans and ecosystems

Human beings are exposed to various mutagens and carcinogens, which are main causes of cancer. Studies on cancer etiology demonstrate over eight percent of cancer incidence are due to the exposure to environmental factors [10]. PAHs are a group of compounds whose carcinogenicity has been revealed by a number of animal experiments. The lack of human epidemiological data fails to clarify the carcinogenicity to humans. Several compounds are listed by IARC [9,10] as probable and possible carcinogens to humans. Humans can be exposed to soil mutagens and carcinogens through skin contact, inhalation, or ingestion. Exposure of person within community may occur through inhalation of dust derived from soil at a contaminated soil. Dermal absorption of contaminated soil that has fallen out of the air onto the skin. Accidental uptake by directly ingestion of contaminated soil by adults is generally not a

concern since most adults do not intentionally eat dirt. The ingestion of soil by children, aged 2-6 years, who have mouthing tendencies needs to be considered. Particularly, most soil samples were collected from the garden areas or along the roadside in this study, the ingestion by children is almost certainly the primary hazard [70].

The Center for Disease Control (CDC) of USA assumed that 10,000 mg/day of soil would be ingested by children aged 1.5-3.5 years, and during other age periods ingestion would be much less (Table 4.2). When all this published information on soil ingestion is considered the data indicate that a consensus estimates for soil ingestion by children (ages 1.5-3.5 or ages 2-4) is about 25-50 mg/day. The 100 mg/day figure was used by EPA in its risk assessment. Even having considered the contribution of poor hygiene and soil contaminated food, for example, fruits and vegetables, the 100 mg/day figure used by CDC to estimate soil uptake by adolescents and adults at residential sites containing contaminated soil seems unlikely, and a figure of 2-5 mg/day seems more reasonable and justifiable based on available revertant data [71].

Quantitative estimates of the dermal uptake of chemicals of the dermal uptake for humans contain more uncertainty than estimates for other routes of entry. Wester *et al.* [72] conclude the absorption in human skin of benzo(a)pyrene is 35-50% for 24 hours exposure. In CDC's assessment of contaminated soil, they assumed that dermal exposures would follow "an age-dependent pattern of deposition" similar to ingestion as showed in Table 4.3.

Table 4.2 Ingestion of dirt[a] (CDC Assumption) [71].

Age group	Soil ingested (mg/day)	Lifetime uptake (%)
0-9 months	0	0
9-18 months	1,000	2.6
1.5-3.5 years	10,000	70.0
3.5-5 years	1,000	5.2
5-70 years	100	22.6

[a] Adjusted for seasonal variation.

Table 4.3 Amount of soil deposited on skin (CDC Assumption) [71].

Age group	Soil on skin (day)
0-9 months	0 g
9-18 months	1 g
1.5-3.5 years	10 g
3.5-5 years	1 g
5-70 years	100 mg

The degree of inhalation hazards is generally dictated by the volatility of a chemical, its toxicity, and the amount of dust generated at the site. CDC assumed that the average air concentration of total suspended particulates (TSP) was 0.14 mg/m^3 and that 100% of this amount (by weight) was respirable.

According to the CDC assumptions, the exposure of total PAHs to humans is calculated (Table 4.4). Uptakes of PAHs through oral and dermal exposures are 0.186 and 0.083 ng/kg day for the residents in Muang and Sarapee Districts, respectively. The exposures of PAHs of residents in Chiang Mai are lower than

previous studies [71,73]. Because actual PAH concentrations in airborne particulates are not available, the exposure of total PAHs based on the soil PAHs is limited.

Table 4.4 Human exposure of total PAHs from soil.

Route	CDC Assumptions	Total PAHs uptake (ng/kg.day)[b]	
		Muang	Sarapee
Oral	0.41 g/day ingested (lifetime) 30% absorption	0.18	0.08
Dermal	0.41 g/day of soil on the skin Dose is weighted by age over life 1% absorption	0.006	0.003
Inhalation	Total suspended particulates is 0.14 mg/m^3 15 m^3 /day 10% in lung 100% bioavailable	NA[a]	NA

[a] not available

[b] average body weight = 60 kg

Contaminated soil can pose not only a direct hazard to humans but also indirect effects [73]. Ecological effect levels are used to protect the reproductive viability of wildlife populations. According to exposure modeling and necessary assumptions, the ecological effect levels for ingestion exposure, dermal exposure, and inhalation exposure could be estimated. Kappleman [74] calculated that the effect levels on individual animals for total PAHs in soils were 4.8 mg/kg soil, based on a dermal exposure of *Peromyscus* mice to benzo(a)pyrene. Total PAHs effect levels based on

reproductive population effects resulted in a soil level of 160.6 mg/kg soil, based upon an ingestion exposure of mice to benzo(a)pyrene. In this study, the total PAHs both in Muang District and Sarapee District were much lower than these figures. PAHs are unlikely to be a problem to wildlife.