CHAPTER III
RESULTS

3.1 Twenty four hour concentration ($\mu g/m^3$) of particulate matters, PM 2.5 and PM 10, in the air around Chiang Mai city

Monthly averaged 24 hour samples of PM 2.5 and PM 10 taken from May to October 1999 at various sampling sites (site 1 to site 4) were shown in Table 3.1. It revealed that from May – October 1999, PM 2.5 levels were varied from 21 - 69 $\mu g/m^3$ at site1 and 23 - 34 at site4 while PM 10 were varied from 22 - 45, 13 – 34, and 45 - 65 $\mu g/m^3$ at site 2, 3 and 4, respectively. Both of PM 2.5 and PM 10 levels were not exceed the 24 hour levels of USEPA standard which was 65 $\mu g/m^3$ for PM 2.5 and 150 $\mu g/m^3$ for PM 10. The levels of particulate matter collected from four different sites were compared in Figure 3.1, which showed the similar pattern of PM 10 levels. The levels of PM 2.5 and PM 10 collected from the same site (site 4) were compare in Figure 3.4 and PM 2.5 level was approximately 44.80% to PM 10 level as shown in Figure 3.5.

In addition, the daily levels of airborne particulate matters collected between outdoor (site 3) and indoor (site 2) sources from nearby place were compared in Figure 3.2. It showed that levels of particulate matters collected from outdoor and indoor sites were not different, however indoor fine particles were much higher than those from outdoor particles some days.

To perform the particulate matter concentration from different sources, 24 hour levels of PM 2.5 were studied from one outdoor source (site 4) and from one indoor source (site 1) which were distantly located approximately 4

km from each other. The result was showed in Figure 3.3 that the levels of PM 2.5 collected from the two sites were not different, and the airborne particulate matter levels collected from indoor and outdoor sites were not different, whether they were measured from nearby or from distant site. The results performed that the level of out door fine particles might affect the level of particulate matter inside a building.

Table 3.1. Monthly averaged 24 hour levels of airborne particulate matters, PM 2.5 and PM 10, concentration in ambient air of Chiang Mai city from four different stations during May – October 1999.

Month (1999)	24 – hour averaged of particulate matters, PM 2.5 and PM 10, concentration ($\mu g/m^3$)				
	PM 10 (mean ± SD)			PM 2.5 (mean ± SD)	
	Site 2	Site 3	Site 4	Site 4	Site 1
May	45 ± 33	34 ± 14	ND	ND	69 ± 38
June	27 ± 10	30 ± 13	47 ± 4	29 ± 13	30 ± 33
July	26 ± 14	18 ± 14	45 ± 14	23 ± 8	23 ± 7
August	28 ± 26	28 ± 14	52 ± 22	28 ± 14	25 ± 11
September	33 ± 21	17 ± 14	47 ± 29	34 ± 11	26 ± 7
October	22 ± 10	13 ± 14	65 ± 41	32 ± 11	21 ± 9

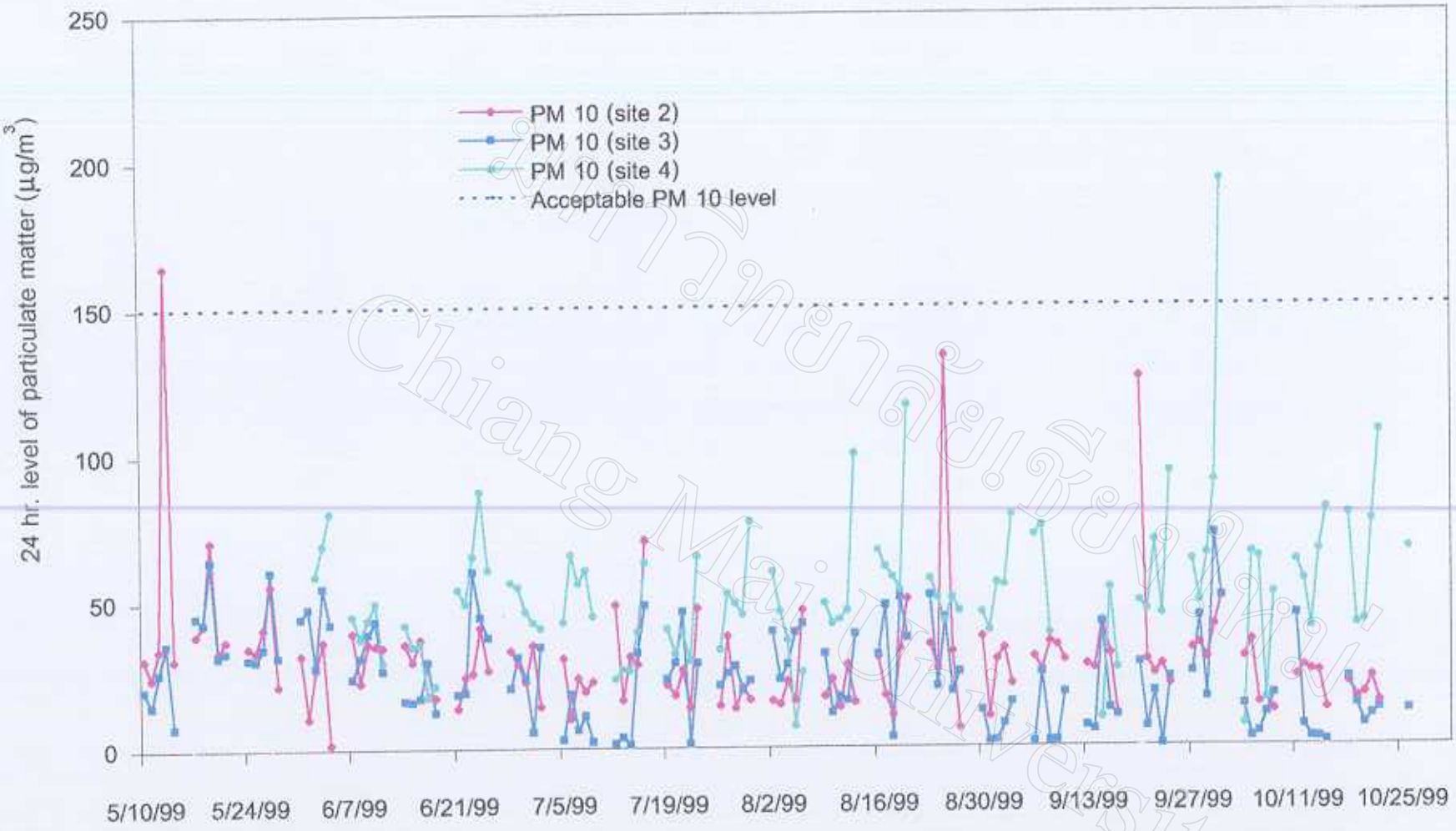

Figure 3.1 Daily 24 - hour levels of airborne particulate matters, PM 10, from various sites in Chiang Mai city, the samples were collected from Monday to Friday every week.

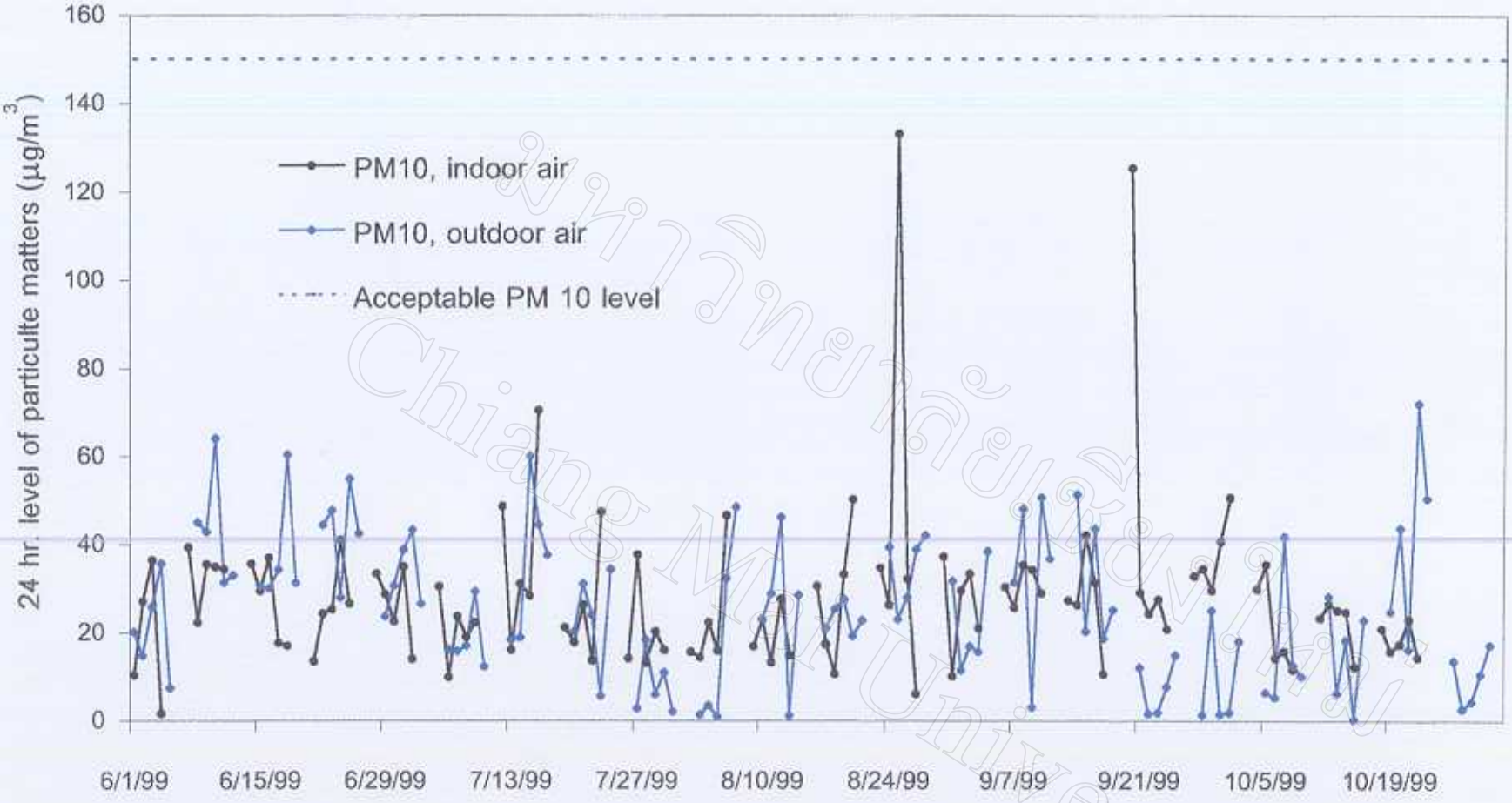

Figure 3.2 Daily 24- hour levels of airborne particulate matter, PM 10, from indoor site (site 2), and from outdoor site (site 3), the samples were collected from Monday to Friday every week.

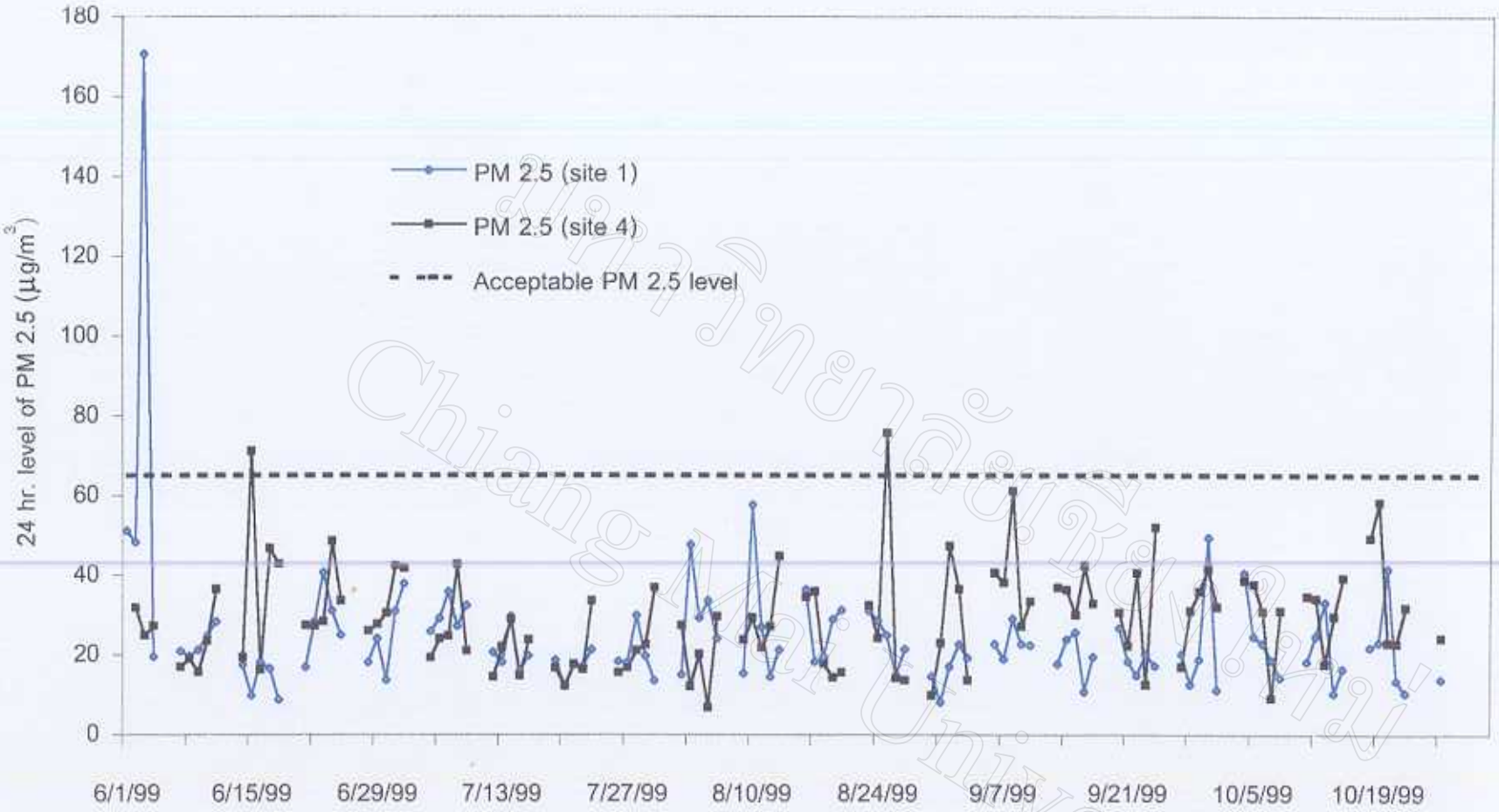

Figure 3.3 Daily 24-hour levels of airborne particulate matter, PM 2.5 collected from one indoor site (site 1) and from one outdoor site (site 4) during June-october 1999, the samples were collected from Monday to Friday every week.

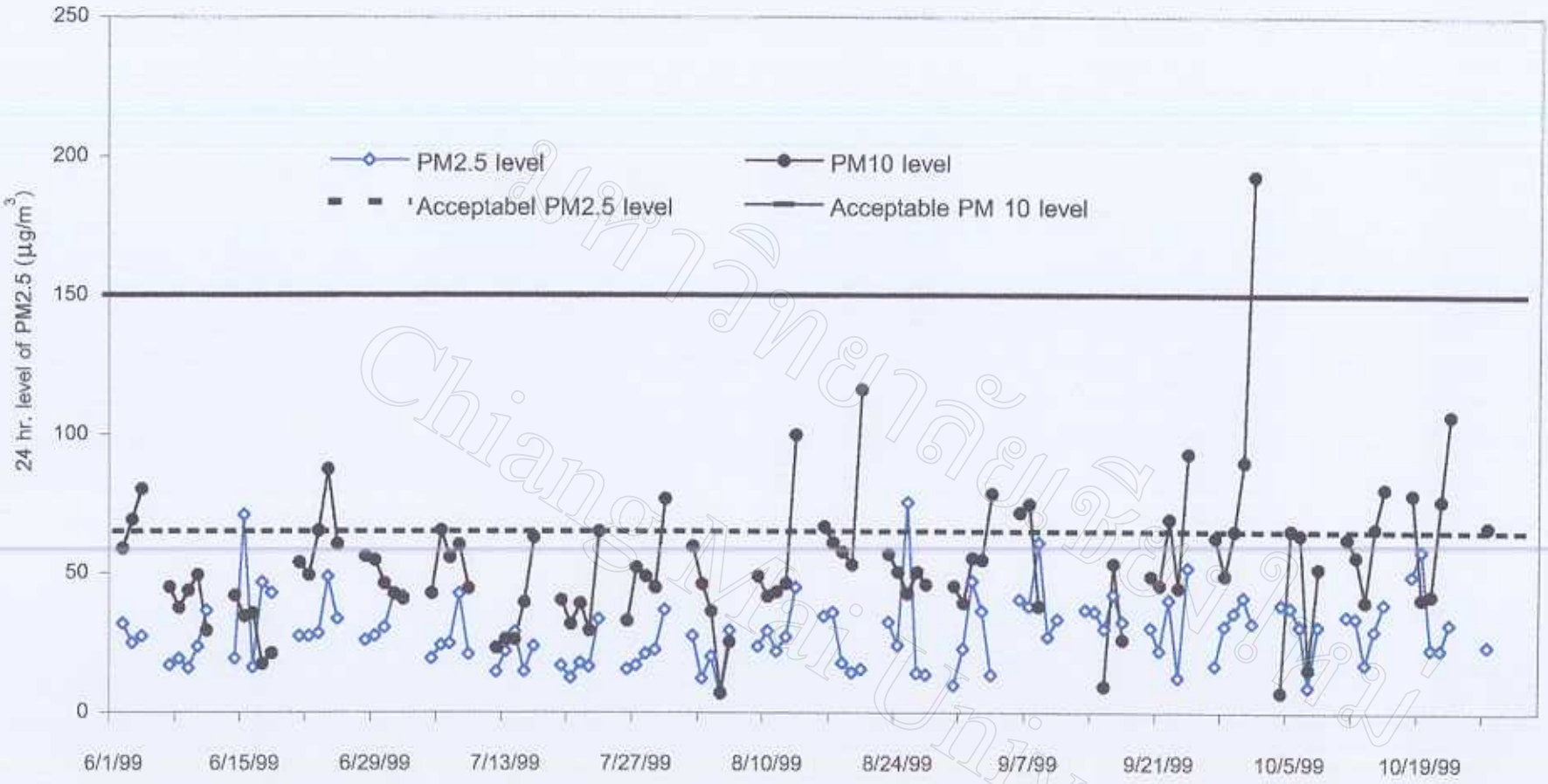

Figure 3.4 Daily 24-hour levels of airborne particulate matters, PM 2.5 and PM 10, collected at site 4 during June - October 1999, the samples were collected from Monday to Friday every week.

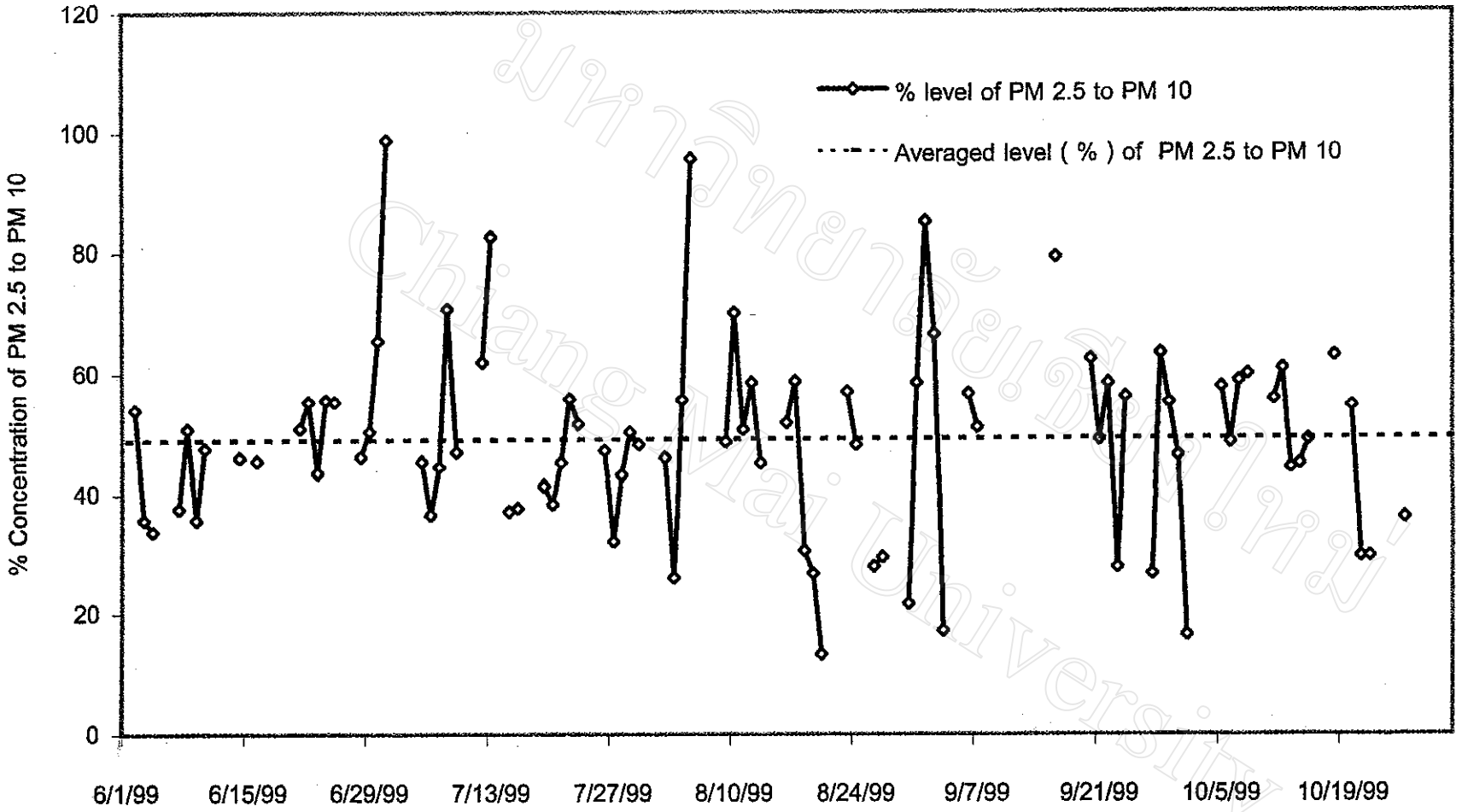

Figure 3.5 Concentration of PM 2.5 subseted (%) to PM 10, the samples were collected from Monday to Friday every week.

3.2 Day-time and Night-time concentration ($\mu g/m^3$) of airborne particulate matters, PM 2.5 and PM 10, in the air around Chiang Mai city

The comparison with airborne particulate matter levels during day-time and night-time, PM 2.5 and PM 10, from various sites in Chiang Mai was shown in Table 3.2. It revealed that particulate matter concentrations collected during night-time were much higher than those collected during day-time, and the particulate levels were gradually increased from November 2000 to February 2001 which was winter time and slightly decreased in March 2001. The monthly 8 hour averaged of PM 2.5 was varied from 20 – 57 $\mu g/m^3$ in the day-time and 26 – 76 $\mu g/m^3$ in the night-time, respectively.

As shown in Figure 3.6 that the levels of fine particles during day-time and night-time at all sites express a similar pattern which were gradually increased from November 2000 to February 2001.

Day-time and night-time levels of particulate matters collected from nearby site between site 3 (out door) and site 1 (indoor) were shown in Figure 3.7. The result showed that day-time and night-time levels of PM 2.5 collected from the two sites were not different during November, but the levels of PM 2.5 both day-time and night-time at site 3 were much higher than those at site 1 since December 2000. Figure 3.8 also showed the same pattern as Figure 3.5, but between site1 (indoor) and site 4 (outdoor) which was distantly located from each other.

Table 3.2. Day-time and Night-time averaged of airborne particulate matters, PM 2.5 and PM 10, concentration in ambient air of Chiang Mai city collected at various stations from November 2000 – March 2001

Month	8 - hour averaged of particulate matters, PM 2.5 and PM 10, concentration ($\mu g/m^3$)									
	PM 2.5 (mean ±SD)								PM 10 (mean ±SD)	
	Site 1		Site 2		Site 3		Site 4		Site 3	
	Day-time	Night-time	Day-time	Night-time	Day-time	Night-time	Day-time	Night-time	Day-time	Night-time
November 2000	20 ± 4	26 ± 5	20 ± 5	32 ± 5	23 ± 6	37 ± 10	33 ± 5	56 ± 12	35 ± 8	63 ± 11
December 2000	24 ± 5	33 ± 8	25 ± 5	44 ± 8	35 ± 15	76 ± 17	38 ± 9	69 ± 14	57± 15	95 ± 19
January 2001	29 ± 6	41 ± 8	32 ± 9	50 ± 14	47 ± 5	76 ± 7	46 ± 4	71 ± 10	70 ± 5	109 ± 12
February 2001	-	-	-	-	44 ± 2	76 ± 1	45 ± 4	69 ± 17	72 ± 2	111 ± 11
March 2001	-	-	-	-	40 ± 11	75 ± 9	44 ± 8	70 ± 12	71 ± 4	110 ± 9

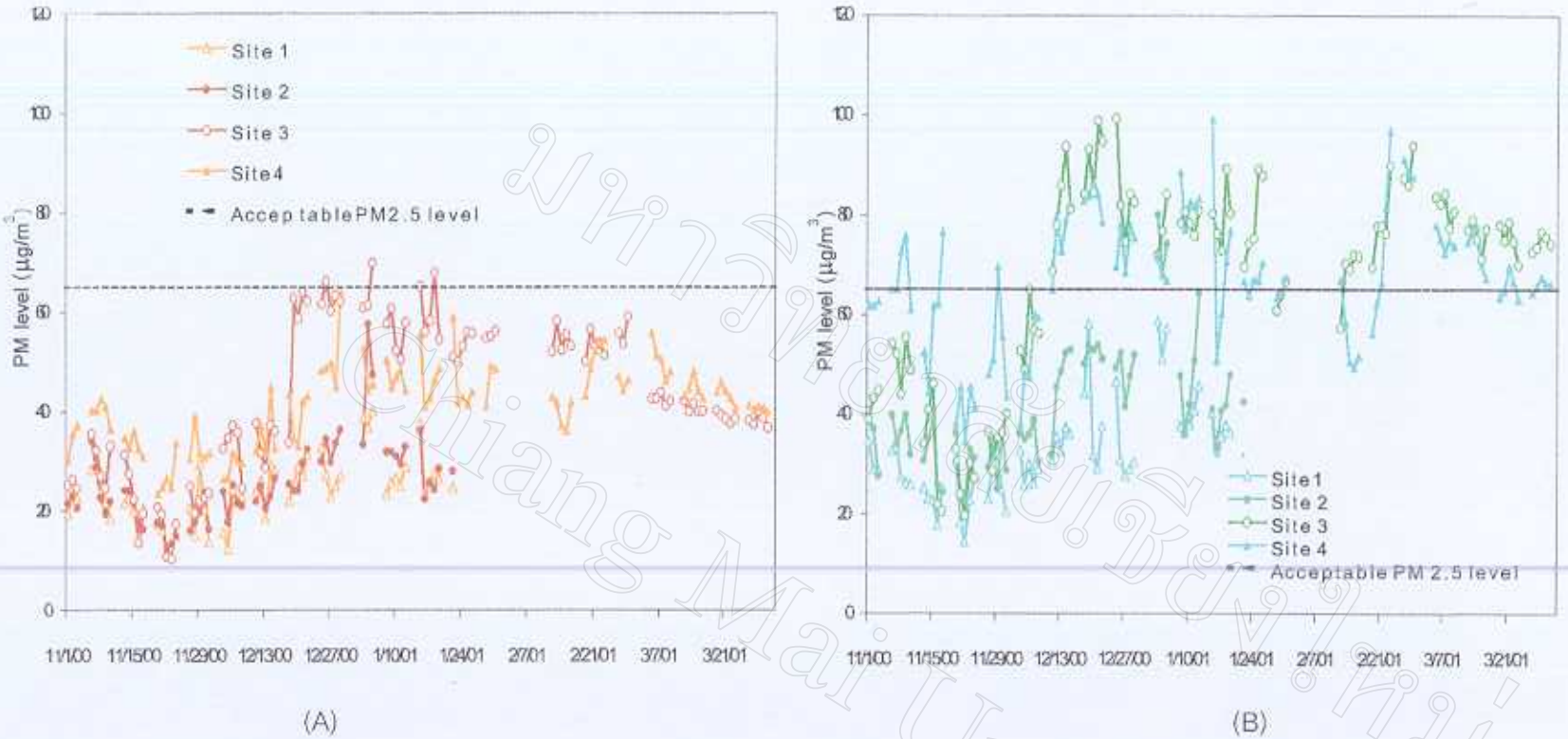

Figure 3.6 Day-time (A) and Night-time (B) levels of airborne particulate matter, PM 2.5, collected at four sites during November 2001 – March 2002, the samples were collected from Monday to Friday every week

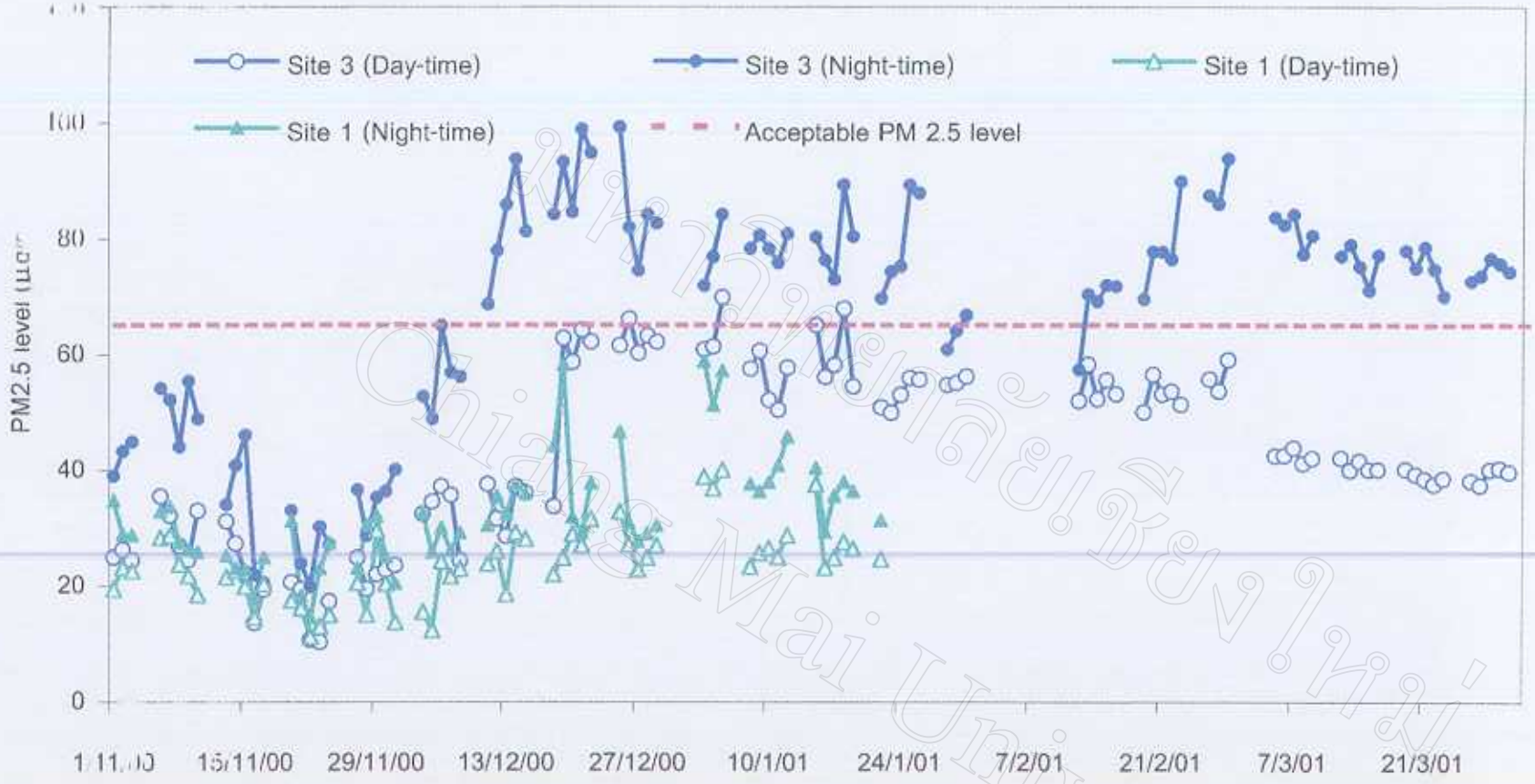

Figure 3.7 Day-time and Night-time levels of particulate matter, PM 2.5, collected from outdoor site (site 3) and from indoor site (site 1) during November 2001 - March 2002, the samples were collected from Monday to Friday every week.

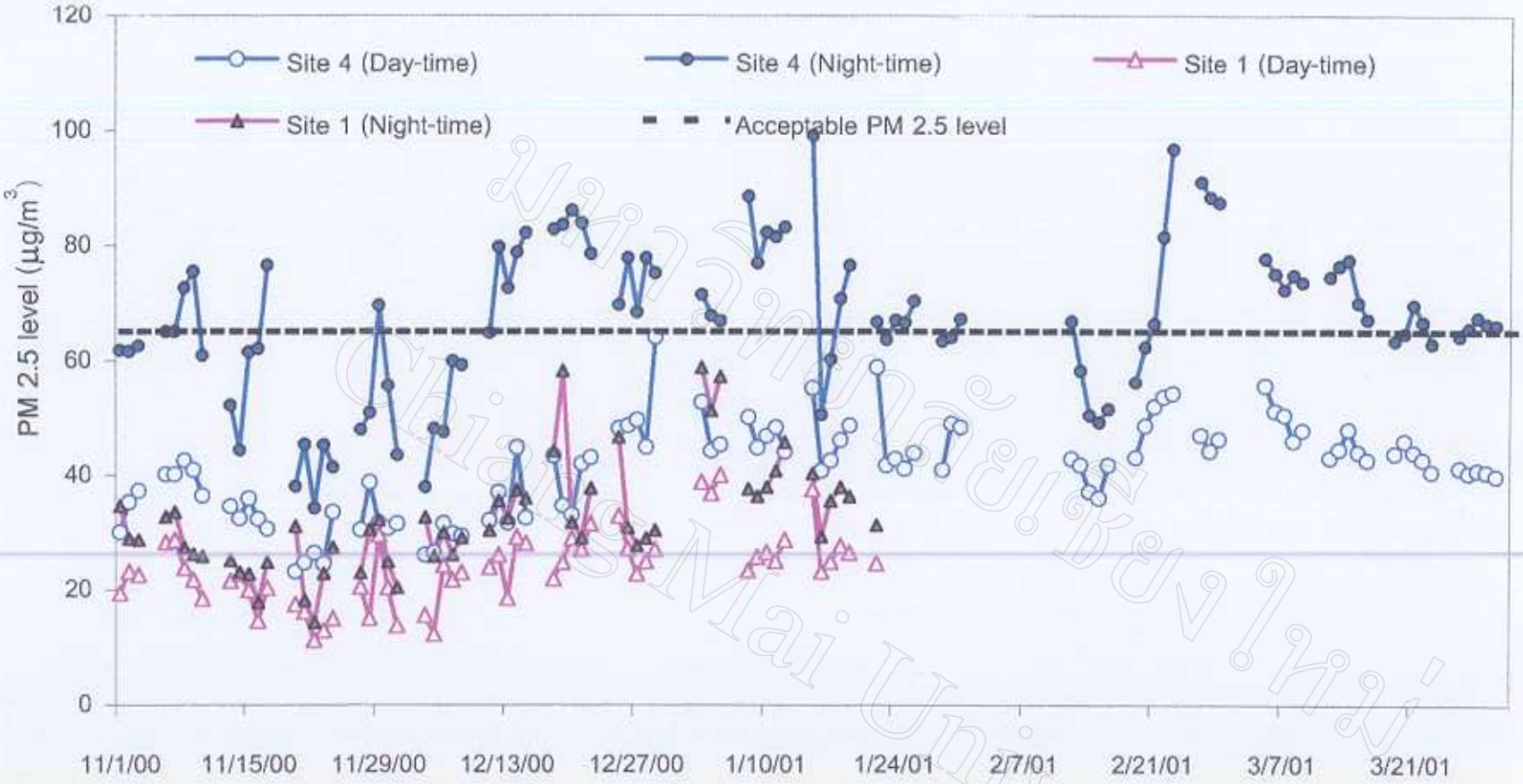

Figure 3.8 Day-time and Night-time levels of particulate matter, PM 2.5, collected from outdoor site (site 4) and from indoor site (site 1) during November 2001 - March 2002, the samples were collected from Monday to Friday every week.

In addition, Figure 3.9 and Figure 3.10 revealed the correlation between particulate matter concentration and unclear air.

Figure 3.9 expressed the visibility of Doi Suthep with clear air, and the PM 2.5 and PM 10 concentrations in that day time were 40 $\mu g/m^3$ and 70 $\mu g/m^3$, respectively. Besides, it was shown to be covered with smog in Figure 3.10 and Doi Suthep was hardly visible with 58 $\mu g/m^3$ of PM 2.5 and 70 $\mu g/m^3$ of PM 10. These results performed that the particulate matter concentration would getting high when the air was covered with smog and seem to be unclear.

Figure 3.9 The visibility of Doi Suthep in the clear air day

Figure 3.10 The hardly visible Doi Suthep in the unclear air day

3.3 Mutagenicity of extractable organic matter from particulate matters, PM 2.5 and PM 10 collected in Chiang Mai city

Dichloromethane extracts of airborne particulate matters either PM 2.5 or PM 10, collected in Chiang Mai city were mutagenic to *Salmonella typhimurim* stain TA100 with and without mutabolic (S9mix) activation when metabolic activation is the biotransformation of relatively inert chemicals to highly reactive metabolites with numerous chemically induced toxicities (Particia, 1994). The results showed that mutagenicity in 24 hour sample of airborne particulate matter extracts from PM 2.5 or PM 10 was detectable during the winter month (October 1999). As shown in Table 3.3 and Table 3.4 that direct- acting mutagenicity was detected and the mutagenic activity was higher in the presence of metabolic activation (S9 mix) especially from PM 10 only in the sample collected at site 4 which was located in Chiang Mai downtown area.

In addition, the mutagenicity in day-time and night-time airborne particulate matter extract from PM 2.5 and PM 10 was also detectable during November 2000 to March 2001 as shown in Table 3.5 and 3.6. Direct-acting mutagenicity was detected as well as the mutagenic activity was higher in the presence of S9 mix especially from night-time particulate samples.

Table 3.3 Mutagenicity of particulate matters, PM 2.5 and PM 10, (revertant colonies / plate) to *S. typhimurium* TA 100 with (+) and without (-) metabolic (S9 mix) activation of sample extract at site 4. Spontaneous revertant colonies of TA 100 have been subtracted already.

Month	Mutagenicity of PM 2.5 and PM 10 (revertant colonies / plate) at site 4			
	PM 2.5 (mean ± SD)		PM 10 (mean ± SD)	
	+S9	-S9	+S9	-S9
June 1999	23 ± 8	18 ± 8	32 ± 13	7 ± 5
July 1999	7 ± 13	17 ± 9	97 ± 19	81 ± 6
August 1999	86 ± 15	68 ± 8	108 ± 18	99 ± 10
September 1999	89 ± 7	70 ± 9	118 ± 5	111 ± 4
October 1999	*135 ± 13	*91 ± 11	*227 ± 10	*135 ± 9

Number of spontaneous revertant colonies ; with S9 mix = 120 ± 6, without S9 mix = 91 ± 4

Number of 2.5 μg/plate of B(a)P revertant colonies = 1200 ± 32

* ; Positive mutagenicity

Amount of extractable organic matter in each plate = 350 μg

Table 3.4 Mutagenicity of particulate matters, PM 2.5 and PM 10, (revertant colonies / m^3) to *S. typhimurium* TA 100 with (+) and without (-)metabolic (S9 mix) activation of sample extract at site 4. Spontaneous revertant colonies of TA 100 have been subtracted already.

Month	Mutagenicity of PM 2.5 and PM 10 (revertant colonies / m^3) at site 4			
	PM 2.5		PM 10	
	+S9	-S9	+S9	-S9
June 1999	2	2	3	1
July 1999	1	1	8	7
August 1999	7	6	9	8
September 1999	8	6	10	9
October 1999	*12	*11	*19	*12

*; Positive mutagenicity

Table 3.5 Day-time and night-time mutagenicity of particulate matters, PM 2.5 and PM 10, (revertant colonies / plate) to *S. typhimurium* TA 100 with (+) and without (-)metabolic (S9 mix) activation of sample extract. Spontaneous revertant colonies of TA 100 have been subtracted already.

Month	Mutagenicity of PM 2.5 and PM 10 (revertant colonies / plate)											
	PM2.5 (mean ± SD) at site3				PM 10 (mean ± SD) at site3				PM 2.5 (mean ± SD) at site4			
	Day-time		Night-time		Day-time		Night-time		Day-time		Night-time	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
November 2000	89±9	52±8	90±11	48±6	96±8	36±6	97±11	85±10	85±7	79±11	*122±15	85±10
December 2000	*117±11	62±7	*121±10	82±9	*161±2	48±5	*180±13	90±13	110±6	55±16	*128±14	78±7
January 2000	*119±8	62±4	*138±16	*124±10	*176±16	61±5	*210±21	*124±9	*124±7	88±15	*140±12	*128±13
February 2000	*123±9	81±6	*132±10	*120±9	*175±17	62±11	*202±19	*129±9	*129±16	*110±13	*130±10	*118±8
March 2000	*120±11	73±4	*136±11	*119±5	*169±13	64±7	*191±12	*133±11	*127±8	90±8	*136±17	*120±11

Number of spontaneous revertant colonies ; with S9 mix = 116 ± 5, without S9 mix = 89 ± 8

Number of 2.5 µg/plate of B(a)P revertant colonies = 1160 ± 35, * ; Positive mutagenicity

Table 3.6 Day-time and night-time mutagenicity of particulate matters, PM 2.5 and PM 10, (revertant colonies / m^3) to *S. typhimurium* TA 100 with (+) and without (-)metabolic (S9 mix) activation of sample extract. Spontaneous revertant colonies of TA 100 have been subtracted already.

Month	Mutagenicity of PM 2.5 and PM 10 (revertant colonies / m^3)											
	PM 2.5 (mean ± SD) at site3				PM 10 (mean ± SD) at site 3				PM 2.5 (mean ± SD) at site 4			
	Day-time		Night-time		Day-time		Night-time		Day-time		Night-time	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
November 2000	16.12	9.57	29.79	15.87	16.54	4.49	34.05	11.28	15.79	16.54	*30.45	28.13
December 2000	*21.75	11	*40.05	23.83	*27.79	8.29	*45.22	18.04	18.59	10.22	*42.37	25.82
January 2000	*22.12	11.52	*45.68	*37.73	*30.39	10.53	*49.78	*32.29	*23.05	16.36	*46.34	*42.37
February 2000	*22.86	17	*41.68	*32	*32	10.70	*52.03	*35.87	*23.98	*20.45	*43.03	*39.06
March 2000	*22.32	13.28	*33.3	*37.26	*30.86	9.87	*51.29	*33.28	*24.01	18.22	*44.25	*40.59

*; Positive mutagenicity

3.4 *In vitro* genotoxic effect of the airborne particulate extract on human peripheral blood leukocytes : DNA damage (COMET assay)

The average values (mean ± SD) of head length and tail length in human peripheral blood leukocytes following *in vitro* treatment with 24 hour sample of particulate matter extract are presented in Table 3.7. In the experiments performed no significantly different of head length (μm) between control and sample whether with or without exogenous metabolic activation system (S9 mix). Whereas, at site 4, the extent of DNA fragmentation expressed as tail length was significantly increased above the control values at the particulate matter extracts from both PM 2.5 and PM 10 during September to October 1999, especially in the presence of S9 mix as determined by one-way analysis of ANOVA test.

To obtain more detail information on DNA damage about airborne particulate matter, head length and tail length of day-time and night-time sample extracts were studied. The results showed in Table 3.8 that there was no significantly different between the head length of control and sample. However, DNA damage performed as the tail length of day-time and night-time extracts showed significantly higher than the control during winter time (November 2000 – March 2001) with and with out metabolic activation especially in the night-time.

Table 3.7 The Comet assay of 24-hour samples, PM 2.5 and PM 10, collected at site 3 and site 4 during June-October 1999

Sample	PM 10 (mean ± SD)				PM 2.5 (mean ± SD)			
	Head length (μm)		Tail length (μm)		Head length (μm)		Tail length (μm)	
	+S9	S9-	+S9	S9-	+S9	S9-	+S9	S9-
Negative Control	2.47 ± 0.90	2.44 ± 0.92	3.04 ± 2.10	3.13 ± 1.25	2.47 ± 0.90	2.44 ± 0.92	3.04 ± 2.10	3.13 ± 2.25
Positive Control	2.28 ± 0.61	2.15 ± 0.76	*13.65 ± 6.33	*8.41 ± 4.13	2.28 ± 0.61	2.15 ± 0.76	13.65 ± 6.33	11.41 ± 4.13
Extract in June	2.25 ± 0.91	2.36 ± 0.93	4.50 ± 3.01	4.26 ± 3.09	2.20 ± 0.86	2.23 ± 1.03	3.73 ± 2.01	4.01 ± 2.07
Extract in July	2.23 ± 0.76	2.32 ± 0.98	4.62 ± 3.02	4.20 ± 2.98	2.25 ± 0.98	2.31 ± 0.65	4.10 ± 2.72	4.06 ± 3.91
Extract in August	2.20 ± 0.68	2.30 ± 0.69	5.02 ± 3.12	4.34 ± 2.40	2.22 ± 0.12	2.30 ± 0.77	4.53 ± 2.49	4.09 ± 2.22
Extract in September	2.21 ± 0.73	2.26 ± 0.79	*5.25 ± 3.40	*5.14 ± 3.86	2.20 ± 0.69	2.20 ± 0.81	*5.33 ± 3.68	*5.15 ± 3.13
Extract in October	2.17 ± 0.30	2.22 ± 0.79	*7.55 ± 5.42	*6.58 ± 4.41	2.20 ± 0.48	2.19 ± 0.66	*6.27 ± 5.80	*5.60 ± 4.22

*; $p<0.01$ by ANOVA

Table 3.8 The head length of day-time and night-time sample, PM 2.5, collected at site 3 and site 4 during November 2000 – March 2001

Month	Head length of PM 2.5 (μm)							
	Site 3 (mean ± SD)				Site 4 (mean ± SD)			
	Day-time		Night-time		Day-time		Night-time	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
November 2000	2.39 ± 0.78	2.33 ± 0.66	2.32 ± 0.59	2.32 ± 0.73	2.37 ± 0,66	2.40 ± 0.77	2.33 ± 0.63	2.36 ± 0.58
December 2000	2.30 ± 0.90	2.29 ± 0.88	2.27 ± 0.56	2.30 ± 0.42	2.31 ± 0.55	2.29 ± 0.63	2.29 ± 0.78	2.32 ± 0.91
January 2001	2.27 ± 0.54	2.29 ± 0.65	2.29 ± 0.83	2.27 ± 0.36	2.30 ± 0.61	2.33 ± 0.53	2.28 ± 0.70	2.26 ± 0.88
February 2001	2.31 ± 0.64	2.28 ± 0.59	2.26 ± 0.63	2.30 ± 0.62	2.28 ± 0.51	2.31 ± 0.43	2.27 ± 0.62	2.29 ± 0.78
March 2001	2.26 ± 0.71	2.30 ± 0.62	2.30 ± 0.55	2.31 ± 0.58	2.32 ± 0.40	2.33 ± 0.84	2.28 ± 0.59	2.30 ± 0.94

Head length of positive control ; with S9 mix = 2.53 ± 043 μm, without S9 mix = 2.46 ± 0.39 μm

Head length of negative control ; with S9 mix = 2.40 ± 044 μm, without S9 mix = 2.44 ± 0.57 μm

*; $p<0.01$ by ANOVA

Table 3.9 The tail length of day-time and night-time sample, PM 2.5, collected at site 3 and site 4 during November 2000 – March 2001

Month	Tail length of PM 2.5 (μm)							
	Site 3 (mean ± SD)				Site 4 (mean ± SD)			
	Day-time		Night-time		Day-time		Night-time	
	+S9	S9-	+S9	S9-	+S9	S9-	+S9	-S9
November 2000	4.97 ± 3.24	4.92 ± 4.06	4.06 ± 3.26	4.01 ± 2.68	3.96 ± 3.01	3.81 ± 2.65	4.98 ± 3.50	4.71 ± 3.78
December 2000	*5.54 ± 4.23	4.68 ± 4.52	*5.75 ± 3.56	4.98 ± 4.22	*5.60 ± 5.12	*5.61 ± 4.65	*5.18 ± 4.52	*5.69 ± 4.63
January 2001	*5.98 ± 5.09	*5.66 ± 4.61	*6.69 ± 5.12	*6.74 ±3.97	*5.87 ± 4.12	*5.93 ± 3.45	*6.11 ± 3.33	*6.23 ± 3.78
February 2001	*5.35 ± 3.91	*6.01 ± 4.65	*7.23 ± 4.92	*6.55 ± 3.88	*5.46 ± 4.02	*5.50 ± 3.78	*6.47 ± 4.77	*6.46 ± 4.48
March 2001	*6.06 ± 5.01	*5.94 ± 5.62	*6.65 ± 5.44	*6.04 ± 4.56	*5.36 ± 4.03	*5.38 ± 3.89	*5.99 ± 3.67	*5.87 ± 4.01

Tail length of postive control ; with S9 mix = 12.67 ± 5.14 μm, without S9 mix = 9.01 ± 4.67 μm

Tail length of negative control ; with S9 mix = 3.11 ± 2.04 μm, without S9 mix = 3.06 ± 1.97 μm

*; $p<0.01$ by ANOVA

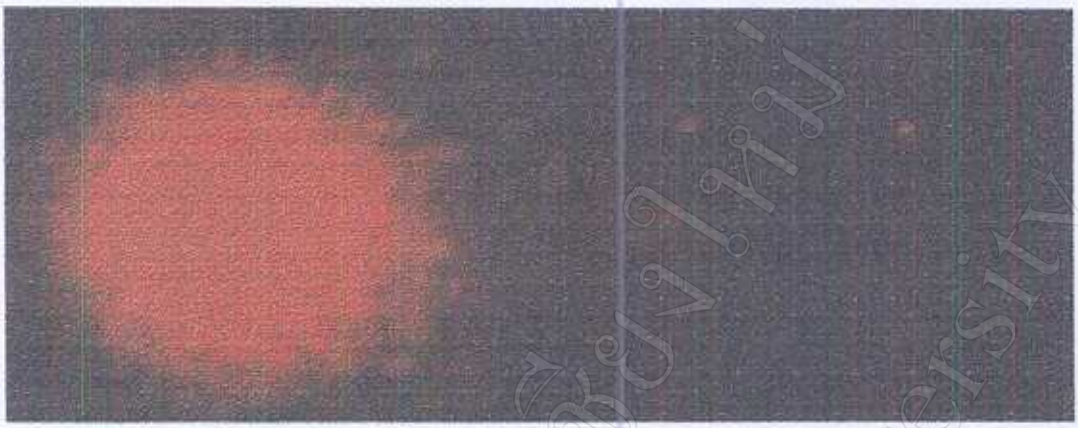

Figure 3.11 The Comet cell with short length of the tail stained with ethidium bromide under flulorescent microscope (6250x)

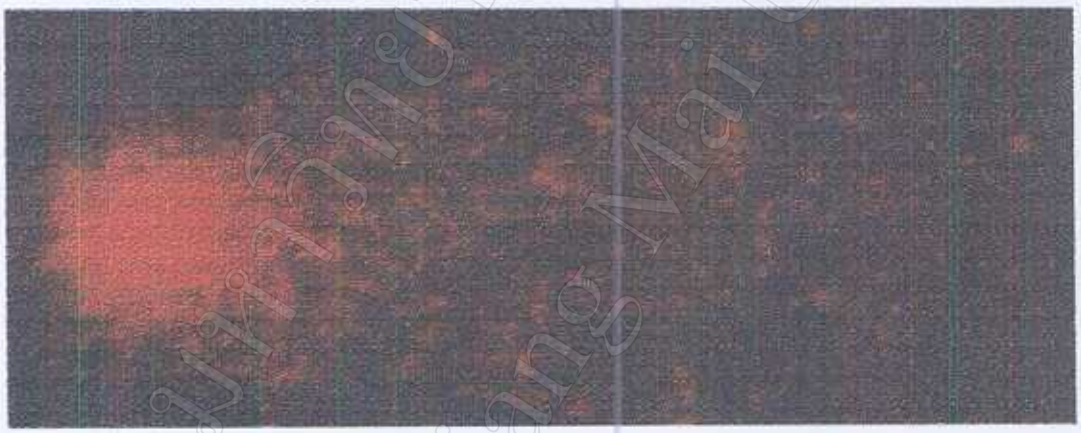

Figure 3.12 The Comet cell with long length of the tail stained with ethidium bromide under flulorescent microscope (6250x)