CHAPTER 4

Results and Discussions

The results of the present study were consisted of 2 parts.

Part 1: To develop a simple and portable EBC collecting device and evaluate the use of developed device with a group of volunteers

Part 2: To apply the developed EBC collecting device to collect EBC samples of school children who exposed to PM_{10}. After that, to evaluate the effects of PM_{10} exposure to respiratory health as well as pulmonary function, H_2O_2 and MDA concentrations in EBC of children in an urban and a highland school in Chiang Mai province during different seasons.

4.1 Results of part 1: To develop a simple and portable EBC collecting device and evaluate the use of developed device with a group of volunteers

4.1.1 Description of the developed EBC collecting device

The developed EBC collecting device in this work consisted of mouthpiece with one-way valve in which inspiratory and expiratory air were separated. The subjects were asked to breath at a normal frequency and tidal volume through a device while wearing a nose-clip. The mouth piece connected to the flexible plastic tubing for letting subjects move freely and having a comfortable position. This flexible plastic tubing was connected to the 50 mL polypropylene collecting tube that was acting as sampling container and placed inside a stainless steel chamber. The polypropylene collecting tube was designed to connect with the second one-way valve to flow the excess air of expired breath towards the top and to prevent the flowing of environmental air into the polypropylene collecting tube and contamination of the sample. A rubber ring was placed between the flexible plastic tubing and the hole in the stainless steel chamber to achieve an airtight connection. The polypropylene collecting tube was immerged in the stainless steel chamber that contained liquid nitrogen to cool down. The procedure allowed vapors, aerosols, and moisture in exhaled breath to condense along the wall of the polypropylene tube. The condenser maintains a temperature of less than -20ºC throughout a collection period. This device is quite simple to use, operate and affordable as shown in Figure 4.1.

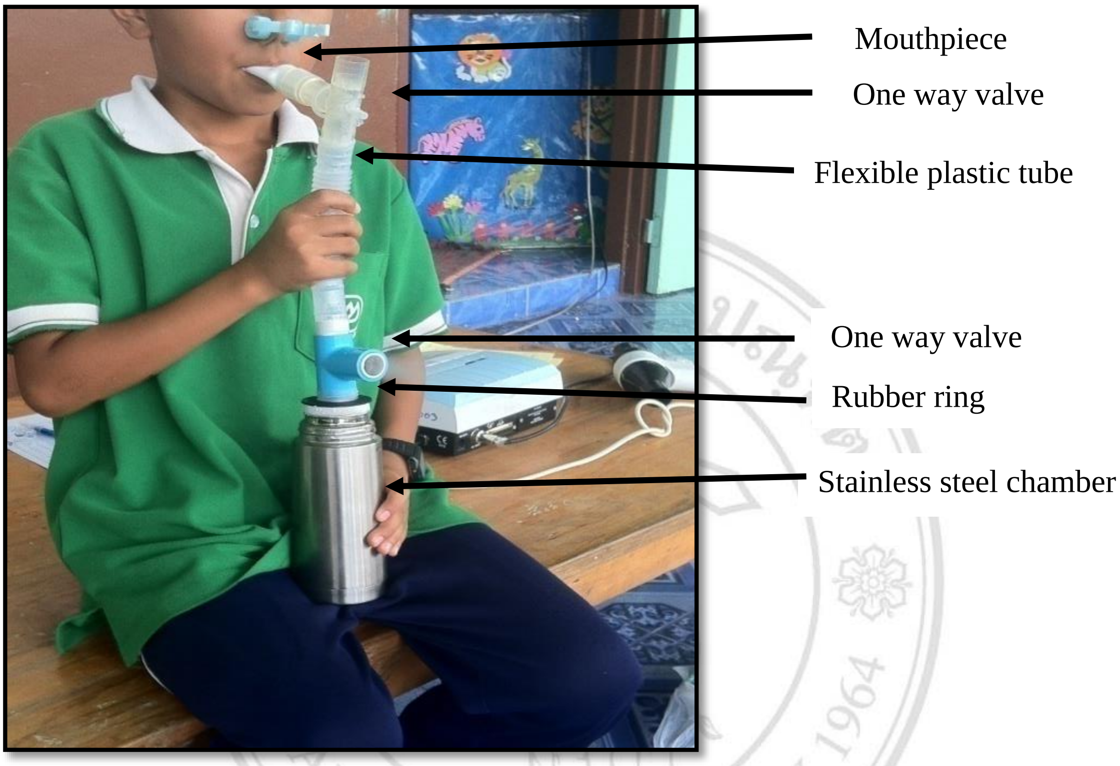

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Figure 4.1 Photograph of developed EBC collecting device used to collect EBC

4.1.2 Determination of suitable collection periods and breath patterns of EBC sample collection from healthy subjects.

After the development was completed, this device was first used with 5 healthy subjects. All of subjects were healthy as assessed by a history of any chronic diseases or airway infections in the previous one month. The subjects consisted of 1 male and 4 female (age range 12-44 years). Their range heights were 155-172 cm and weights were 45-95 kg. The basic characteristics of the 5 healthy subjects are shown in Table 4.1.

Table 4.1 Basic characteristic of the healthy subjects

Variables	No. of subject				
	1st	2nd	3rd	4th	5th
Sex*	F	F	F	F	M
Age (years)	12	16	25	39	44
Height (cm)	162	172	155	170	168
Weight (kg)	45	58	48	60	95

* F = Female, M = Male

EBC samples were collected at 2 consecutive days. On day one, subjects were asked to perform the same EBC collection procedures twice of each subject. They breathed tidally at normal frequency into developed EBC collecting device for 10 minutes to collect EBC samples. After that, they had 30 minutes for technical break and then performed a second EBC collection in the same manner for 20 minutes. On day two, two sampling periods were performed the same as day one but the subjects were asked to breathe with increased tidal breathing by breathing with deep inhalations and forcing exhalations. For each of sampling periods, EBC samples were collected separately. In total 4 different EBC samples were therefore collected from each subject.

All of the subjects were able to successfully complete the EBC procedure without difficulty. In subjects with 10 minutes of normal frequency breathing, range of EBC volumes was 1.15-1.31 mL and 20 minutes of normal frequency breathing, range of EBC volumes were 1.25-1.37 mL. Otherwise, when the subjects asked to breathe at a forced frequency breathing in 10 minutes of collection period, range of EBC volumes were 1.24-1.39 mL and 20 minutes of forced frequency breathing, range of EBC volumes were 1.34-1.45 mL. Mean of EBC volume by different duration period and breathing pattern are summarized in Figure 4.2, and individual data are shown in Figure 4.3 and 4.4. The EBC volume was highest in 20 minutes of collection period by forced frequency breathing. But the subjects were complained of feeling tired and weary. Although the study in this stage was studied in adults, they still felt tired and weary. If using the same procedure among the childrenaged10 to 14years old in part 2, it is likely to have more complications. In part 2 studying, the subjects were required 10 minutes of normal tidal breathing to provide sufficient EBC volume for further analysis. Recently, Mutlu and coworkers (2001) indicated that the subjects usually takes 5-10 minutes in adults and up to 15-20 minutes in children to obtained 1-3 mL of EBC.

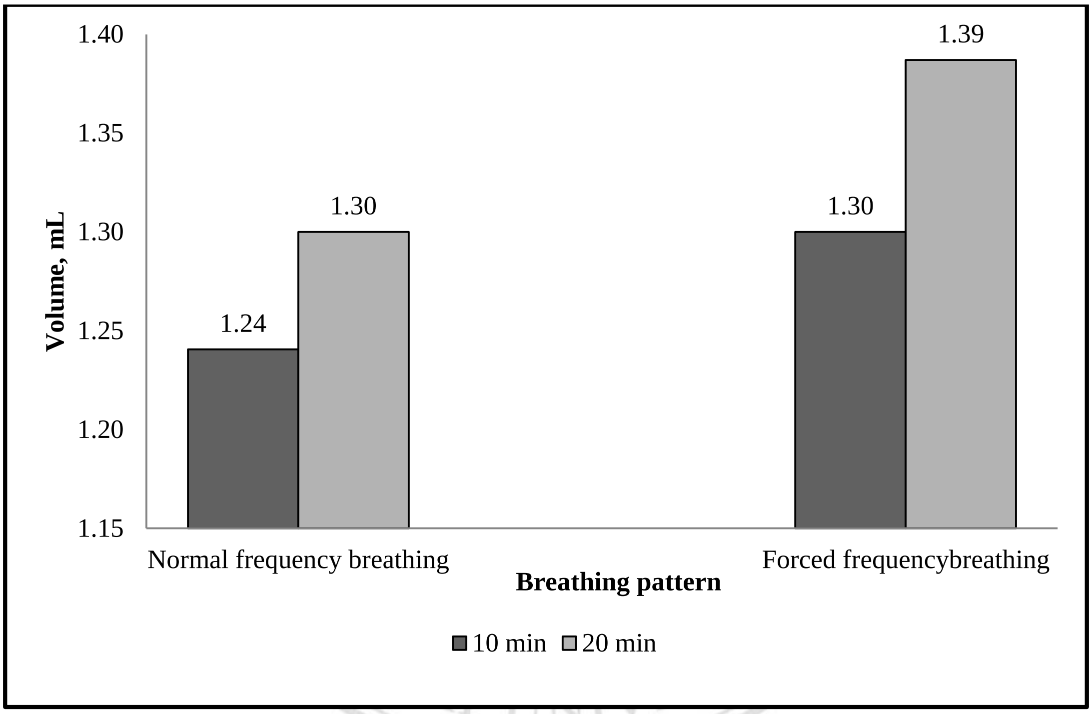

Figure 4.2 The mean of EBC volume by duration period and breathing pattern

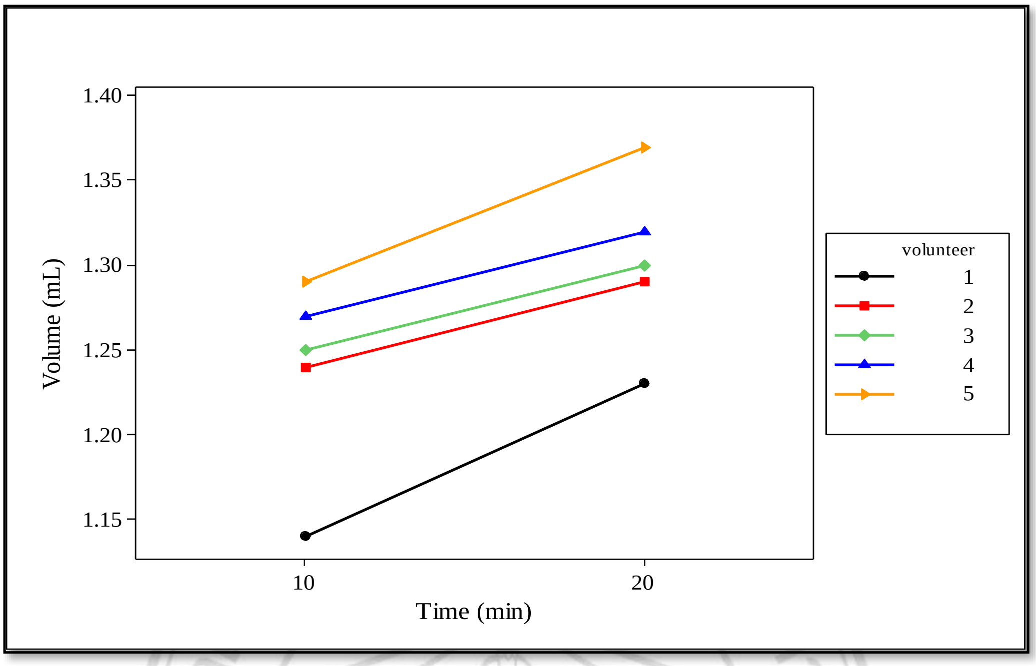

Figure 4.3 The EBC volume of each subject asked to breath at a normal frequency in 10 minutes and 20 minutes of duration period

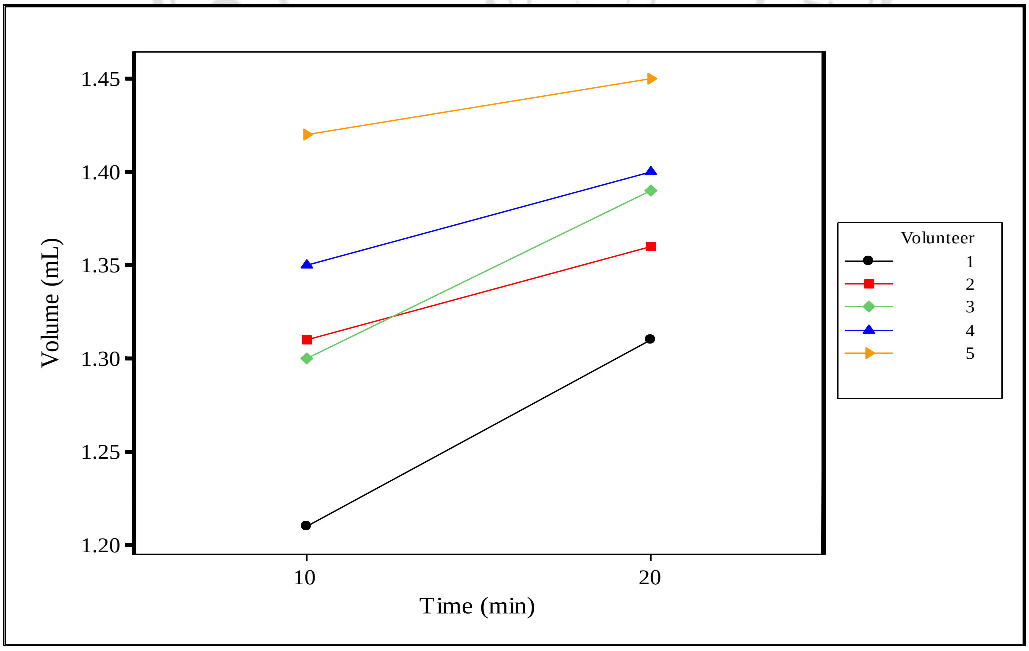

Figure 4.4 The EBC volume of each subjects asked to breath at a forced frequency in 10 minutes and 20 minutes of duration period

4.2 Discussion of part 1

The results of the present study, all subjects were found completing the collecting procedure. A total of 20 samples from 5 healthy subjects were collected throughout the study. The present study shows that the procedure of EBC collection is well accepted, safe, and feasible in all subjects. The success rate was 100%. None of the subjects had to stop the procedure because of side effects. Therefore this procedure was suggested safe, rapid and simple to use and operate.

The EBC collecting device presented was based on the similar principle as the system used previously with some modifications (Dean *et al.*, 2007). The collection device for EBC at its basic level is composed of mouthpiece, saliva trap, and a condenser surround by a cooling apparatus. The condensation chamber in this study was constructed of stainless steel, in agreement to the recommendation of EBC collection in the previous study (Horváth *et al.*, 2005). The present study used a polypropylene tube to obtain EBC sample by immerging in a condensation chamber that contained liquid nitrogen. Polypropylene tube is a hypo-reactive plastic which is very stable in different temperature and hard to break while as a glass or Pyrex tube is easy to break. The liquid nitrogen was contained into the condensation chamber to obtain the lowest temperature. At atmospheric pressure, liquid nitrogen boils at −196 °C. Because of its extremely low temperature, careless handling of liquid nitrogen may result in cold burns. However, liquid nitrogen is widely used, e.g. to store cell at low temperature for laboratory work, immersion freezing and transportation of food products. The danger related to the use of liquid nitrogen is a limitation of the practicality of EBC collection, but these low temperatures were chosen to maximize the volume of EBC collected over a period of 10 minutes. It has been demonstrated that lowering the temperature increase the volume of EBC collected (Goldoni *et al.*, 2005). In the preliminary studies, wet ice contained in the condensation chamber was used to obtain the lower temperature and it found the EBC volume less than 0.2 ml, which was insufficient for further analysis (data not shown), while the liquid nitrogen allowed collection of sufficient volume for laboratory analysis. To minimize the problem that may occur, the field staffs are closely monitored throughout the procedure while the EBC samples were collected from the subjects.

Successful collection EBC utilizing different devices has been reported (Montuschi *et al*., 1999; Mutlu *et al.*, 2001). Various collector systems have been described widely by different research groups; however, the principle of rapid cooling of the exhaled air with consequent condensation of water vapor is similar for all systems used. In general, the subject breathes tidally via a mouthpiece through a non-rebreathing valve by inhaling normal room air and exhaling into the condenser system. The most simple and originally widely used systems included Teflon-lined tubing immersed in an ice-filled bucket or passed through a section of pipe packed with ice (Dohlman *et al*., 1993). Others used a condensing device made of two glass chambers is cooled by ice (Montuschi *et al.*, 1999; Baraldi *et al*., 2003). However, no standardize methods have been established for EBC collection.

The methodology for collection of EBC is simple and single operator was sufficient to undertake EBC collection from the subjects. The volume of EBC samples from subjects with 10 minutes of normal frequency breathing were 1.15-1.31 mL and subjects with 20 minutes of normal frequency breathing were 1.25-1.37 mL. This result was agreed with previous study (Mutlu *et al*., 2001; Hoffmeyer *et al*., 2007). In each collection duration (10 minutes and 20 minutes), the effect of breathing pattern on EBC volume was tested. The mean volumes of EBC sample by normal tidal breathing over a period of 10 minutes and 20 minutes were similar. Otherwise, the EBC volume was significantly higher in 20 minutes of collection duration compared with 10 minutes when the subjects breathe with increased tidal breathing. But the subjects were complaining of feeling tired and weary when they instructed to breathe by increased tidal breathing over 20 minutes. Although this work was studied in adults, they still felt tired and weary. If using the same procedure among the childrenaged10 to 13years old, it is likely to have more complications. In order to reduce this problem, the subjects were required 10 minutes of normal tidal breathing to provide sufficient EBC volume for further analysis.

4.3 Results of part 2: To apply the developed EBC collecting device to collect EBC samples of school children who exposed to PM_{10}.

To study the effects of PM_{10} exposure to respiratory health of school children among urban area and highland areas in Chiang Mai province, the developed EBC collecting device was apply to collect EBC samples from the school children. Prior to analysis EBC samples, the analytical characteristics of H_2O_2 and MDA in EBC were investigated.

4.3.1 Analytical characteristics of H_2O_2 and MDA in EBC

1) Calibration curve

1.1) Calibration curve of H_2O_2 standard solution

The calibration of H_2O_2 standard solution was investigated by spectrophotometer. Absorbance of individual H_2O_2 standard solution was measured at 450 nm. Concentrations were plotted against their absorbance. Linear regression equation and regression coefficient (R^2) of individual H_2O_2 standard solution are present in Figure 4.5

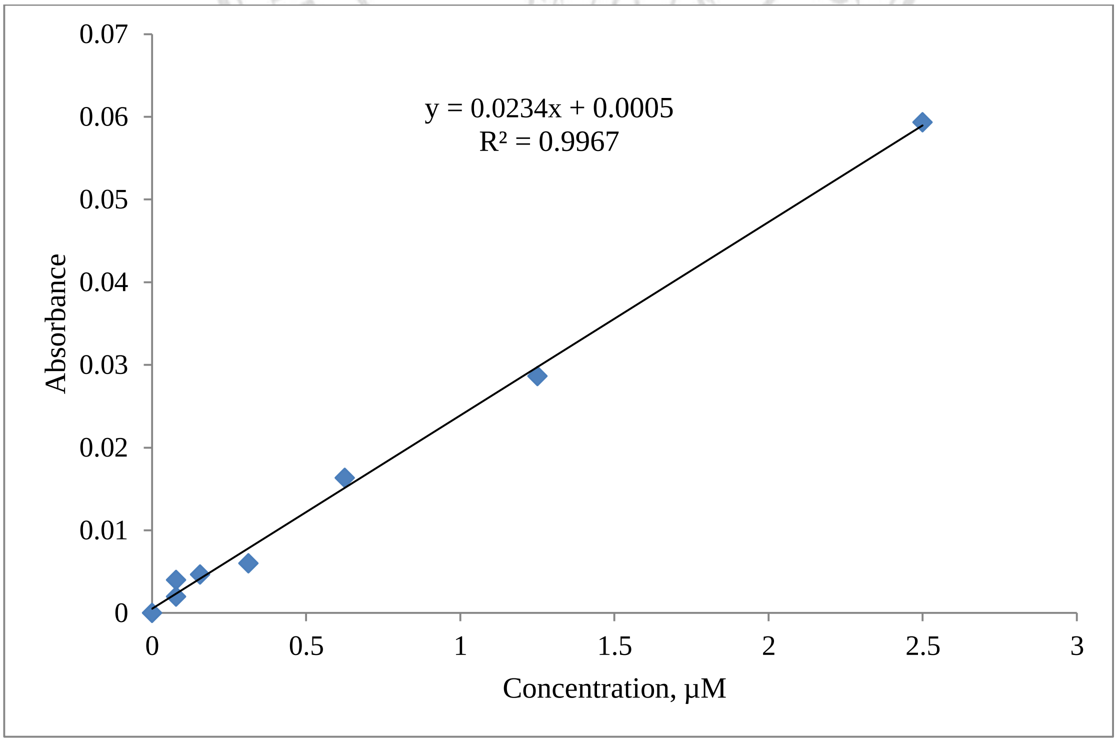

Figure 4.5 Calibration curve of H_2O_2 standard solution

1.2) Calibration curve of MDA standard solution

The calibration curve of MDA standard solution was determined by 8 analytical curves with blank, 0.08, 0.15, 0.30, 0.60, 1.20, 2.40, and 4.80 µM of MDA spiked in purified water. The calibration curve of each MDA standard was constructed using concentration of standard solution versus peak area. The calibration curve for determination of MDA concentration is shown in Figure 4.6.

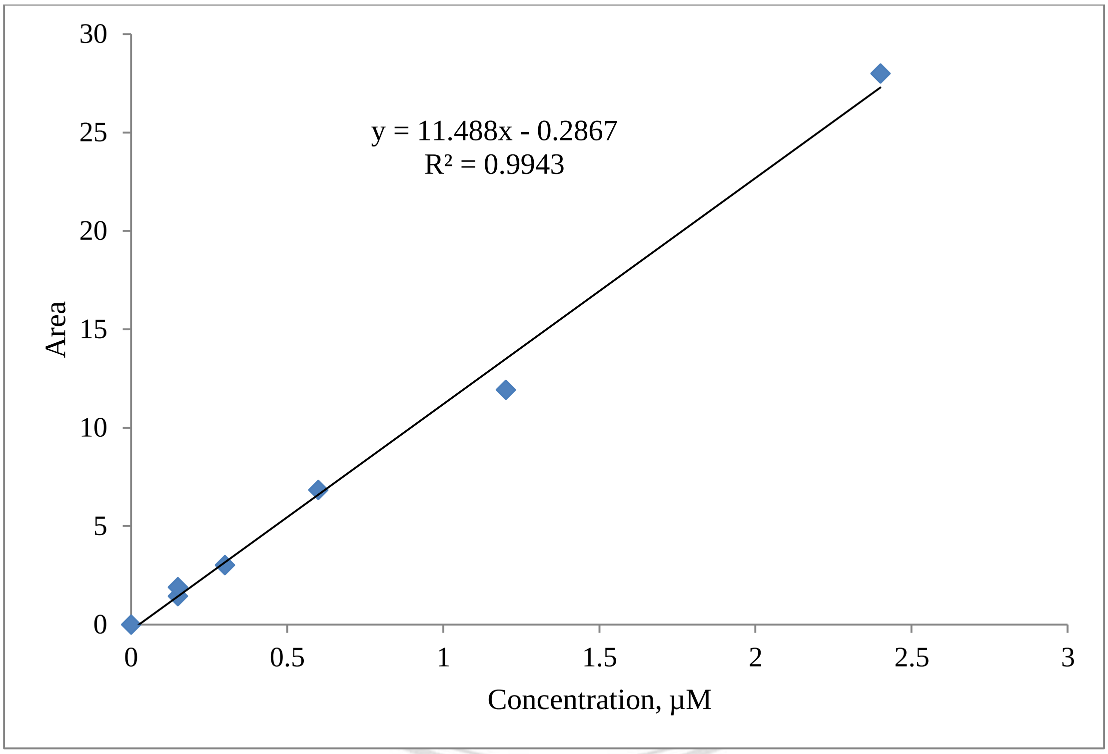

Figure 4.6 Calibration curve of MDA standard solution

2) Repeatability and reproducibility

2.1) Repeatability and reproducibility of H_2O_2

The repeatability of H_2O_2 analysis was obtained by 8 repeated measurements of 1.0 µM H_2O_2 standard solution on a spectrophotometer at 450 nm. Relative standard deviation (RSD) of H_2O_2 concentration was 2.64 %. The reproducibility was obtained by preparing 5 solutions of 1.0 µM H_2O_2 standard solution on a spectrophotometer at 450 nm under the optimum conditions for 5 continuous days. The results of the repeatability and reproducibility were estimate by standard deviation (SD) and the % RSD of H_2O_2 concentration was 3.86 % as shown in Table 4.2.

Table 4.2 Repeatability and reproducibility of H_2O_2 measurement

No. of measurement	Repeatability of 1.0 µM H_2O_2 standard solution	Reproducibility of 1.0 µM H_2O_2 standard solution (n=5)
1	1.13	1.16 ± 0.03
2	1.22	1.07 ± 0.02
3	1.17	1.11 ± 0.02
4	1.17	1.06 ± 0.03
5	1.17	1.08 ± 0.04
6	1.13	-
7	1.13	-
8	1.17	-
Mean	1.16	1.10
SD	0.03	0.04
% RSD	2.64	3.86

2.2) Repeatability and reproducibility of MDA

The repeatability was done by 5 injections of 0.15 µM MDA standard solution on to HPLC under the optimum conditions. The repeatability of MDA analysis is shown in Table 4.3. Relative standard deviation (RSD) of MDA concentration was 9.84 %.

The reproducibility was checked by injections of 0.15 µM MDA standard solution on to HPLC analysis under the optimum conditions for 5 continuous days. The results of reproducibility were estimate by standard deviation (SD) and the % RSD of MDA concentration was 8.15 % as shown in Table 4.3.

Table 4.3 Repeatability and reproducibility of MDA measurement

No. of measurement	Repeatability of 0.15 µM MDA standard solution	Reproducibility of 0.15 µM MDA standard solution (n=5)
1	0.17	0.17 ± 0.02
2	0.20	0.19 ± 0.02
3	0.20	0.20 ± 0.02
4	0.16	0.17 ± 0.02
5	0.17	0.20 ± 0.02
Mean	0.18	0.19
SD	0.02	0.02
% RSD	9.84	8.15

3) Limit of detection (LOD) and limit of quantification (LOQ)

3.1) LOD and LOQ of H_2O_2 standard

The present study was obtained with 7 measurements of pooled EBC samples on a spectrophotometer at 450 nm. LOD was calculated from 3 times of SD. LOQ was calculated from 10 times of SD (Ref). Mean concentration and SD of H_2O_2 concentrations as shown in Table 4.4.

Table 4.4 Limit of detection and limit of quantification of H_2O_2 measurement

No. of measurement	Concentration (µM)
1	0.04
2	0.09
3	0.04
4	0.04
5	0.04
6	0.04
7	0.04
Mean	0.05
SD	0.02
LOD	0.06
LOQ	0.20

3.2) LOD and LOQ of MDA standard

The limit of detection and limit of quantification were assessed by 7 injections of pooled EBC samples into the HPLC analysis. LOD and LOQ results as shown in Table 4.5

Table 4.5 Limit of detection and limit of quantification of MDA measurement

No. of measurement	Concentration; μM
1	0.05
2	0.04
3	0.03
4	0.08
5	0.02
6	0.03
7	0.06
Mean	0.04
SD	0.02
LOD	0.06
LOQ	0.20

4) Percent recovery

4.1) Percent recovery of H_2O_2

The analytical EBC recovery was calculated by 10 measurements of pooled EBC samples were unspiked and spiked with 0.1, 1.0, and 2.0 µM of H_2O_2 for low, medium, and high spiked concentration, respectively. The recovery of 0.1, 1.0, and 2.0 µM of H_2O_2 were 91.8 %, 100.7 %, and 107.2 %, respectively as shown in Table 4.6.

Table 4.6 Percent recovery of H_2O_2 in EBC samples

No. of measurement	% Recovery of H_2O_2 determination		
	Low spiked level (0.1 µM)	Medium spiked level (1.0 µM)	High spiked level (2.0 µM)
1	48.5	104.4	108.7
2	87.0	112.5	104.4
3	87.0	88.9	104.4
4	87.0	100.0	106.5
5	130.4	100.0	108.7
6	130.4	100.0	108.7
7	87.0	104.2	108.7
8	87.0	96.0	108.7
9	87.0	91.7	104.4
10	87.0	109.1	108.7
Mean	91.8	100.7	107.2
SD	23.6	7.3	2.0

4.2) Percent recovery of MDA

Percent recovery of MDA was found out from 5 replications at 3 levels which were low, medium, and high concentrations (0.15, 0.60, and 2.4 µM). Mean recoveries and SD of MDA are shown in Table 4.7. The result showed that recoveries of MDA were in the range of 91.2-99.3 %.

Table 4.7 Percent recovery of MDA in EBC samples

No. of measurement	% Recovery of 3 spiked-MDA levels		
	Low (0.15 µM)	Medium (0.60 µM)	High (2.4 µM)
1	84.0	111.2	99.0
2	104.0	102.5	98.3
3	104.0	91.9	100.7
4	80.4	92.5	99.4
5	83.4	94.9	99.0
Mean	91.2	98.6	99.3
SD	11.8	8.2	0.9

5) HPLC Chromatograms of MDA analysis

The chromatograms of solvent blank (A) and MDA (B) are shown in Figure 4.7.

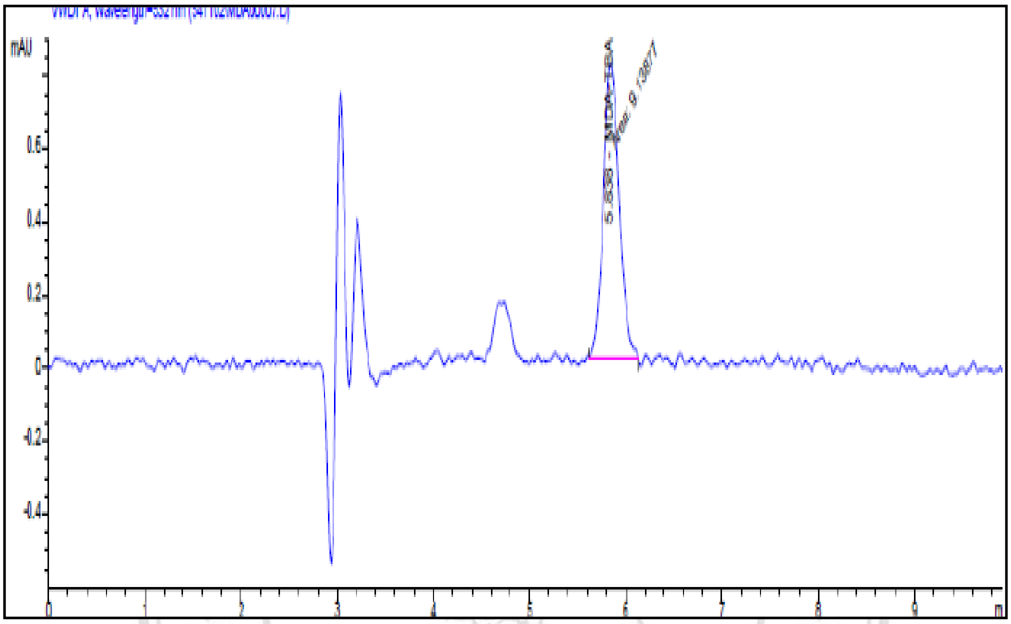

Figure 4.7 HPLC Chromatograms of solvent blank and MDA

4.3.2 Subject characteristics

The first 126 school children, from 2 schools, who agreed to participate in the study, were invited for first visit, at which they received further information and were requested to give their informed consent. Among them, 2 drop outs during the study because they were changed school during the study, and 124 subjects were finally included. Sixty three (50.8%) healthy subjects are study at ST school which locates at central Chiang Mai city and 61 (49.2%) healthy subjects are study at SN school which locates at highland area of Chiang Mai province. Table 4.8 shows the characteristics of the study subjects. The first evaluation was performed on June 2011 and no significant difference in age and sex were found between groups. Majority of the subjects at study start were 11 years old. The median height and weight of subjects at ST school were higher than SN school.

Table 4.8 Basic characteristic of the study subjects by school.

Characteristics	ST school (N=63) n (%)	SN school (N=61) n (%)	p-value
Age (years)			
10	23 (36.5)	21 (34.4)	0.075
11	37 (58.7)	24 (39.3)	
12	3 (4.8)	15 (24.6)	
13	0 (0.0)	1 (1.6)	
Median, (range)	11.00, (10-12)	11.00, (10-13)	
Sex			
Male	31 (49.2)	35 (57.4)	0.325
Female	32 (50.8)	26 (42.6)	
Height (cm)			
≤ 130	2 (3.2)	33 (54.1)	0.000*
131-140	22 (34.9)	18 (29.5)	
141-150	24 (38.1)	9 (14.8)	
151^+	15 (23.8)	1 (1.6)	
Median, (range)	144, (130-164)	130.0, (113-155)	
Weight (kg)			
≤ 30	15 (23.8)	39 (63.9)	0.000*
30-40	30 (47.6)	20 (32.8)	
41-50	12 (19.0)	2 (3.3)	
51^+	6 (9.5)	0 (0.0)	
Median, (range)	35.0, (25-60)	27.5, (18-50)	

4.3.3 Exposure assessment

1) Outdoor PM_{10} levels

During July 2011 to March 2012 for 8 months of PM_{10} sampling, 38 days were recorded by YP station to represent PM_{10} concentrations at ST school and 29 days were monitored by portable air sampler at SN school. Five-day mean levels of daily outdoor PM_{10} of 2 study sites are shown in Table 4.9. The PM_{10} levels at ST school in July 2011 – March 2012 were varied from 15.0 to 194.6 µg/m^3, meanwhile at the SN school varied from 19.7 µg/m^3 to 63.0 µg/m^3. During study period, the means PM_{10} level at ST school were highest in March 2012 and lowest in July 2011. At SN school, the means PM_{10} level were highest in March 2012 and lowest in September 2011.

To get a single daily outdoor PM_{10} level in each season, the PM_{10} levels at July to September 2011 were averaged to present the PM_{10} level in wet season, November 2011 was present transitioned period, and December 2011 to March 2012 were average to present the PM_{10} level in dry season. Summarize distribution of outdoor PM_{10} level in each school during wet season, transitioned period, and dry season are shown in Table 4.10. The seasonal averages of PM_{10} levels at ST school were in the range from 20.4 µg/m^3 in wet season, 21.2 µg/m^3 in transitioned period to 77.7 µg/m^3 in dry season. At the SN school, the seasonal average of PM_{10} levels were in the range from 21.6 µg/m^3 in wet season, 35.0 µg/m^3 in transitioned period to 37.6 µg/m^3 in dry season. The PM_{10} levels in both study sites were low in wet season and increasing in dry season.

The PM_{10} levels at ST and SN school were significantly different ($p < 0.05$) in each season. PM_{10} levels at ST school were gently increased from wet season to transitioned period but in dry season increased rapidly. The variation of outdoor PM_{10} levels at SN school was similar to ST school but in dry season increased slowly. The PM_{10} levels were compared between ST and SN school in each season. Outdoor PM_{10} level at SN school was significantly higher than ST school in wet season and transitioned period; however in dry season this pattern was inversed when outdoor PM_{10} level increased significantly at ST school.

Table 4.9 Five-day means level of daily outdoor PM_{10} by school during study period

Study site	Season	Month	No. obs.	Outdoor PM_{10} level; µg/m^3	
				Mean ± SD	Range
ST school	*Wet*	July 11	5	16.7 ± 1.1	15.0-18.2
		August 11	5	23.2 ± 2.7	19.2-27.4
		September 11	5	21.4 ± 2.8	18.1-26.2
	Transitioned	November 11	3	21.2 ± 1.0	19.8-22.2
	Dry	December 11	5	30.8 ± 3.2	27.8-36.9
		January 12	5	33.9 ± 3.0	29.5-37.0
		February 12	5	90.7 ± 24.3	63.6-125.3
		March 12	5	150.1 ± 28.6	105.1-194.6
SN school	*Wet*	July 11	0	NA	NA
		August 11	5	22.6 ± 1.0	21.5-24.0
		September 11	5	20.8 ± 0.7	19.7-21.7
	Transitioned	November 11	5	35.0 ± 3.1	31.3-39.7
	Dry	December 11	5	35.3 ± 2.7	32.1-38.0
		January 12	5	26.8 ±3.0	22.3-29.6
		February 12	0	NA	NA
		March 12	5	50.6 ± 8.8	38.6-63.0

NA: Not available

Table 4.10 Five-day means level of daily outdoor PM_{10} by school during the different seasons

Season	Outdoor PM_{10} level **, µg/m^3		p-value $^{\#}$
	ST school	SN school	
Wet season	20.4 ± 3.6^{a}	21.7 ± 1.2^{a}	0.000^{*}
Transitioned period	21.2 ± 1.0^{b}	35.0 ± 3.1^{b}	0.000^{*}
Dry season	77.7 ± 52.3^{ab}	37.6 ± 11.0^{ab}	0.000^{*}
p-value $^{\#\#}$	0.000^{*}	0.000^{*}	

$^{\#}$ p-value for differences between schools

$^{\#\#}$ p-value for differences between seasons

**The outdoor PM_{10} level with different superscripts within the same column was significant difference ($p < 0.05$)

2) Classroom PM_{10} levels

During July 2011 to March 2012 for 8 months of the PM_{10} sampling, 156 days were monitored at ST school and 117 days were monitored at SN school. The number of PM_{10} sampling days at ST school was 56, 20 and 80 samples in wet season, transitioned period and dry season, respectively. Meanwhile, the number of PM_{10} sampling days at SN school was 45, 15 and 57 samples in wet season, transitioned period and dry season, respectively. Five-day means level of daily classroom PM_{10} at ST and SN school through 8 months from July 2011 to March 2012 as shown in Table 4.11.

The classroom PM_{10} levels at ST school in July 2011-March 2012 were varied from 30.4 µg/m^3 to 114.1 µg/m^3, meanwhile at the SN school varied from 49.1 µg/m^3 to 77.9 µg/m^3. During study period, the means classroom PM_{10} levels at ST and SN school were highest in January 2012, lowest in August 2011 for ST school and July 2011 for SN school. The seasonal average of classroom PM_{10} levels at ST school were in the range from 50.3 µg/m^3 in wet season, 71.2 µg/m^3 in transitioned period to 77.7 µg/m^3in dry season. While at SN school were 62.3, 63.1, and 66.8 µg/m^3 in wet, transitioned period, and dry season, respectively.

The levels of classroom PM_{10} in each school during wet season, transitioned period, and dry season are shown in Table 4.12. The means of classroom PM_{10} level at SN school significantly exceeded ST school in wet season but in transitioned period and dry season the means of classroom PM_{10} level at ST were significantly higher than SN school.

Comparison of classroom PM_{10} level during 3 different seasons at ST and SN school revealed that the means classroom PM_{10} level at ST school had increased in transitioned period and dry season. At SN school the means PM_{10} level in dry season were significantly higher than transitioned period and wet season ($p<0.05$). The classroom PM_{10} levels were compared between ST and SN school in each season. Classroom PM_{10} level at SN school was significantly higher than ST school in wet season; however this pattern was inversed in transitioned period and dry season when classroom PM_{10} level increased significantly at ST school.

Table 4.11 Five-day means level of daily classroom PM_{10} by school during the study period.

Study site	Season	Month	No. obs.	Classroom PM_{10} level; µg/m^3	
				Mean ± SD	Range
ST school	*Wet*	July 11	16	48.8± 7.3	30.4-61.0
		August 11	20	46.0 ± 4.7	38.7-52.6
		September 11	20	55.8± 8.2	40.9-72.6
	Transitioned	November 11	20	71.2± 21.0	46.8-114.1
	Dry	December 11	20	72.1± 13.2	51.2-93.7
		January 12	20	81.9± 10.6	57.7-97.4
		February 12	20	81.5± 13.1	61.8-105.7
		March 12	20	75.0 ± 8.8	61.4-89.9
SN school	*Wet*	July 11	15	59.7 ± 5.6	49.1-69.1
		August 11	15	61.9 ± 4.6	51.7-69.3
		September 11	15	65.2 ± 6.0	54.6-76.5
	Transitioned	November 11	15	63.1 ± 6.6	50.1-73.6
	Dry	December 11	15	66.7 ± 7.9	52.6-77.6
		January 12	12	69.3 ± 6.0	58.2-77.9
		February 12	15	65.5 ± 4.7	53.3-73.3
		March 12	15	65.7 ± 6.6	53.0-75.2

Table 4.12 Five-day means level of daily classroom PM_{10} by school during the different seasons

Season	Classroom PM_{10} level[**], $\mu g/m^3$		p-value [#]
	ST school	SN school	
Wet season	50.3 ± 8.0^{a}	62.3 ± 5.7^{a}	0.000[*]
Transitioned period	71.2 ± 21.0^{b}	63.1 ± 6.6^{a}	0.000
Dry season	77.7 ± 12.3^{ab}	66.8 ± 6.3^{b}	0.000[*]
p-value [##]	0.000[*]	0.000[*]	

[#] p-value for differences between schools

[##] p-value for differences between seasons

[**] The classroom PM_{10} level with different superscripts within the same column was significant difference ($p < 0.05$)

4.3.4 Health outcomes

1) Pulmonary function

All study subjects were visited at the school to perform pulmonary function test every month during the follow-up period. Overall, the 124 subjects underwent a total 920 measurements. The average number of repeated observations was 7.5 in ST subjects and 7.3 among SN subject. The number of pulmonary function test of ST subjects were 175, 61 and 237 measurements in wet season, transitioned period and dry season, respectively. Meanwhile, the number pulmonary function test of SN subjects were 165, 54 and 228 measurements in wet season, transitioned period, and season, respectively.

1.1) FVC predicted value

Table 4.13 shows means FVC predicted value of ST and SN subjects through 8 months from July 2011 to March 2012. The FVC predicted values of ST subjects through 8 months were varied from 72.1% to 126.2%, meanwhile the SN subjects were varied from 78.8% to 128.9%. The means FVC predicted value were highest in July 2011 for ST subjects, February 2012 for SN subjects and lowest in March 2012 for both subject groups.

The seasonal average FVC predicted value of ST subjects were in the range from 99.7% in wet season, 100.2% in transitioned period and 97.7 % in dry season. The seasonal average of FVC predicted value of SN subjects were in the range from 101.1% in wet season, 100.9% in transitioned period and 102.2% in dry season. There were no significantly different in the FVC predicted value between ST and SN subjects in wet season and transitioned period, the FVC predicted value of SN subjects were significantly higher than ST subjects in dry season. Comparison of the FVC predicted value of ST and SN subjects during 3 seasons of study periods, shown that the means FVC predicted value of ST subjects were decreased in dry season otherwise SN subjects were not significantly different in any season. The comparison of FVC predicted value of the subjects group during the different seasons as shown in Table 4.14.

Table 4.13 Mean FVC predicted value of the subjects by school during study period

Study site	Season	Month	No. obs.	FVC predicted value, % Mean ± SD	Range
ST school	*Wet*	July 11	61	100.1 ± 7.4	90.1-117.2
		August 11	60	99.6 ± 4.6	89.2-109.7
		September 11	54	99.5 ± 5.4	87.8-113.3
	Transitioned	November 11	61	100.2 ± 7.2	84.9-125.2
	Dry	December 11	60	99.9 ± 4.8	90.6-112.3
		January 12	61	98.9 ± 9.7	72.1-126.2
		February 12	55	96.9 ± 5.3	82.9-105.8
		March 12	61	95.3 ± 5.6	81.6-103.8
SN school	*Wet*	July 11	54	99.9 ± 10.7	78.8-127.3
		August 11	57	99.9 ± 10.4	82.8-118.2
		September 11	54	103.8 ± 11.1	83.5-126.7
	Transitioned	November 11	54	100.9 ± 10.3	82.0-121.0
	Dry	December 11	59	102.9 ± 9.4	83.9-124.8
		January 12	58	101.7 ± 6.6	88.4-116.8
		February 12	57	104.7 ± 9.9	81.1-128.9
		March 12	53	99.3 ± 8.2	81.5-118.3

Table 4.14 The FVC predicted value of the subjects by school during the different seasons

Season	FVC predicted value ** ,%		p-value $^{\#}$
	ST subjects	SN subject	
Wet season	99.7 ± 4.0^{a}	101.2 ± 9.0	0.139
Transitioned period	100.2 ± 7.2^{a}	100.9 ± 10.3	0.709
Dry season	97.7 ± 4.3^{b}	102.2 ± 4.9	0.000*
p-value $^{\#\#}$	0.043*	0.645	

$^{\#}$ p-value for differences between schools

$^{\#\#}$ p-value for differences between seasons

**The FVC predicted value with different superscripts within the same column was significant difference ($p < 0.05$)

1.2) FEV$_1$ predicted value

The FEV$_1$ predicted values of ST subjects through 8 months from July 2011 to March 2012 were varied from 83.1% to 144.0%, meanwhile at the SN school were varied from 81.7% to 138.0%. The highest of means FEV$_1$ predicted value was appeared in September 2011 whereas the lowest value of mean FEV$_1$ predicted values was shown in March 2012 for both subject groups (Table 4.15).

The seasonal average of FEV$_1$ predicted value of ST subjects were in the range from 110.1% in wet season, 110.7%in transitioned period and 101.1% in dry season. The seasonal average of FEV$_1$ predicted value of SN subjects were in the range from 109.8% in wet season, 109.4% in transitioned period and 110.6 % in dry season. The diurnal FEV$_1$ variability did not differ between seasons for SN subjects whereas FEV$_1$ predicted value of ST subjects significantly decreased in dry season.

Comparison of FEV$_1$ predicted value of ST and SN subjects during 3 seasons of study periods, appeared lower value of ST subject in dry seasons, but no statistical difference was observed in other seasons. The comparison of FEV$_1$ predicted value of the subjects group during the different seasons as shown in Table 4.16.

Table 4.15 Mean FEV$_1$ predicted value of the subjects by school during study period

Study site	Season	Month	No. obs.	FEV$_1$ predicted value, %	
				Mean ± SD	Range
ST school	*Wet*	July 11	61	109.6 ± 8.2	96.1-133.5
		August 11	60	109.8 ± 6.1	95.2-123.1
		September 11	54	110.8 ± 7.3	90.3-124.4
	Transitioned	November 11	61	110.7 ± 8.5	93.8-135.6
	Dry	December 11	60	110.0 ± 6.6	90.7-121.6
		January 12	61	110.0 ± 11.5	83.1-144.0
		February 12	55	103.0 ± 5.4	92.9-115.9
		March 12	61	101.1 ± 5.6	88.2-113.8
SN school	*Wet*	July 11	54	108.3 ± 11.2	84.8-132.3
		August 11	57	107.7 ± 10.0	83.4-126.4
		September 11	54	113.4 ± 11.4	90.1-138.0
	Transitioned	November 11	54	109.4 ± 10.3	88.5-131.4
	Dry	December 11	59	111.5 ± 9.6	87.1-128.1
		January 12	58	111.6 ± 5.9	96.9-122.6
		February 12	57	112.2 ± 9.6	88.0-134.1
		March 12	54	106.9 ± 9.6	81.7-123.7

Table 4.16 The FEV_1 predicted value of the subjects by school during the different seasons

Season	FEV_1 predicted value ** ,%		p-value $^{\#}$
	ST school	SN school	
Wet season	110.1 ± 4.9^a	109.8 ± 9.0	0.782
Transitioned period	110.7 ± 8.5^a	109.4 ± 10.3	0.470
Dry season	101.1 ± 4.4^b	110.6 ± 4.7	0.000^*
p-value $^{\#\#}$	0.000^*	0.706	

$^{\#}$ p-value for differences between schools

$^{\#\#}$ p-value for differences between seasons

**The FEV_1 predicted value with different superscripts within the same column was significant difference ($p < 0.05$)

1.3) FEV_1 /FVC ratio

The means FEV_1 /FVC ratio between subject groups from July 2011 to March 2012 are given in Table 4.17. The means FEV_1 /FVC ratio of ST subjects were varied from 82.6% to 99.6%, meanwhile at the SN school were varied from 84.5% to 99.6%. The means FEV_1 /FVC ratio of ST subjects were highest in September 2011, lowest in February 2012 while SN subjects were highest in January 2012 and lowest in February 2012.

The seasonal average of FEV_1 /FVC ratio did not differ between seasons for SN subjects, meanwhile FEV_1 /FVC ratio of ST was significantly decreased in dry season. Comparison of ST and SN subject groups in each season did not show any variation (Table 4.18).

Table 4.17 Means FEV_1/FVC ratio of the subjects by school during study period

Study site	Season	Month	No. obs.	FEV_1 /FVC ratio, %	
				Mean ± SD	Range
ST school	*Wet*	July 11	61	93.6 ± 3.2	84.9-99.6
		August 11	60	94.2 ± 2.6	87.2-99.1
		September 11	54	95.5 ± 2.3	89.5-99.2
	Transitioned	November 11	61	94.3 ± 2.2	88.7-99.5
	Dry	December 11	60	95.2 ± 2.4	89.7-98.5
		January 12	61	94.9 ± 2.4	89.2-98.7
		February 12	55	91.2 ± 4.7	82.6-99.6
		March 12	61	93.0 ± 4.6	83.0-99.8
SN school	*Wet*	July 11	54	93.2 ± 3.1	86.7-99.3
		August 11	57	93.6 ± 3.5	85.7-99.5
		September 11	54	94.3 ± 3.0	86.6-99.5
	Transitioned	November 11	54	93.4 ± 3.8	85.2-99.0
	Dry	December 11	59	93.3 ± 3.6	84.5-99.6
		January 12	58	94.5 ± 3.6	85.4-99.6
		February 12	57	92.5 ± 4.1	85.2-99.6
		March 12	53	92.8 ± 2.7	86.6-98.2

Table 4.18 The FEV_1 /FVC ratio of the subjects by school during the different study seasons

Season	FEV_1 /FVC ratio[**], %		p-value [#]
	ST school	SN school	
Wet season	94.4 ± 2.0^a	93.7 ± 2.2	0.087^*
Transitioned period	94.3 ± 2.2^a	93.4 ± 3.8	0.112
Dry season	93.6 ± 1.7^b	93.3 ± 2.1	0.333
p-value [##]	0.045^*	0.767	

[#] p-value for differences between schools

[##] p-value for differences between seasons

[**] The FEV_1/FVC with different superscripts within the same column was significant difference ($p < 0.05$)

2) The concentration of H_2O_2 and MDA in EBC

All study subjects were visited at the school to collect EBC in every month during the follow-up period. Overall, the 124 subjects underwent a total 920 EBC samples. The numbers of EBC samples of ST subjects were 175 samples in wet season, 61 in transitioned period, and 237 in dry season. Meanwhile, the numbers of EBC samples of SN subjects were 165, 54 and 228 samples in wet season, transitioned period and dry season, respectively.

2.1) Exhaled H_2O_2 concentration

The exhaled H_2O_2 concentrations of the subjects through 8 months from July 2011 to March 2012 are shown in Table 4.19. The means exhaled H_2O_2 concentrations of ST subjects were highest in March 2012 and lowest in August and September 2011 whereas the exhaled H_2O_2 concentrations of SN subjects were 20 µM in December 2011 to February 2012 before decreased to 0.19 µM in March 2012.

The seasonal average of exhaled H_2O_2 concentration of ST subjects were in the range from 0.17µM in wet season and transitioned period to 0.20 µM in dry season, while SN subjects were in the range from 0.18 µM in wet season and transitioned period to 0.19 µM in dry season. Comparison of exhaled H_2O_2 concentration of each subject group during 3 seasons of study periods revealed that no significant difference were observed in any seasons in SN subject. Otherwise in ST subjects, an increased mean of exhaled H_2O_2 concentrations was observed in dry season.

Comparison of exhaled H_2O_2 concentration of between ST and SN subjects in each season, shown exhaled H_2O_2 concentration of SN subjects significantly exceeded ST school in wet season and transitioned period but exhaled H_2O_2 concentration of SN subjects were significantly lower than ST school in dry season. Summarize distribution of exhaled H_2O_2 concentration of the subjects in each school during wet season, transitioned period, and dry season as shown in Table 4.20.

Table 4.19 Mean exhaled H_2O_2 concentration of the subjects by school during study period

Study site	Season	Month	No. obs.	Exhaled H_2O_2 concentration, µM	
				Mean ± SD	Range
ST school	*Wet*	July 11	61	0.17 ± 0.07	0.07-0.30
		August 11	60	0.16 ± 0.05	0.07-0.28
		September 11	54	0.16 ± 0.06	0.07-0.33
	Transitioned	November 11	61	0.17 ± 0.07	0.07-0.38
	Dry	December 11	60	0.17 ± 0.07	0.07-0.32
		January 12	61	0.20 ± 0.07	0.07-0.38
		February 12	55	0.21 ± 0.08	0.07-0.33
		March 12	61	0.23 ± 0.08	0.07-0.34
SN school	*Wet*	July 11	54	0.17 ± 0.05	0.07-0.33
		August 11	57	0.17 ± 0.05	0.07-0.30
		September 11	54	0.19 ± 0.08	0.07-0.33
	Transitioned	November 11	54	0.19 ± 0.07	0.07-0.33
	Dry	December 11	59	0.20 ± 0.08	0.07-0.37
		January 12	58	0.20 ± 0.09	0.07-0.37
		February 12	57	0.20 ± 0.07	0.07-0.33
		March 12	53	0.19 ± 0.07	0.07-0.33

Table 4.20 Exhaled H_2O_2 concentration of the subjects by school during the different seasons

Season	exhaled H_2O_2 concentration**, µM		p-value $^{\#}$
	ST school	SN school	
Wet season	0.17^{a}	0.18	0.033^{*}
Transitioned period	0.17^{a}	0.18	0.833
Dry season	0.20^{b}	0.19	0.000^{*}
p-value $^{\#\#}$	0.000^{*}	0.437	

$^{\#}$ p-value for differences between schools $^{\#\#}$ p-value for differences between seasons

** H_2O_2 with different superscripts within the same column was significant difference ($p < 0.05$)

2.2) Exhaled MDA concentration

The mean exhaled MDA concentrations of ST and SN subjects from July 2011 to March 2012 are given in Table 4.21. The mean exhaled MDA concentrations of ST subjects were highest from January to March 2012; lowest MDA concentrations were appeared in August 2011 while SN subjects were highest in February 2012 and lowest in July 2011.

Both study groups did not differ significantly in log-transformed exhaled MDA concentrations when compared between wet season and transitioned period, however ST subjects had significantly higher log-transformed of exhaled MDA compared to those in dry season. Comparison between ST and SN subject groups in each season revealed no significant differences were observed for any seasons (Table 4.22).

Table 4.21 Mean exhaled MDA concentration of the subjects by school during study period

Study site	Season	Month	No. obs.	Exhaled MDA concentration, µM	
				Mean ± SD	Range
ST school	*Wet*	July 11	61	0.18 ± 0.06	0.08-0.31
		August 11	60	0.16 ± 0.06	0.08-0.26
		September 11	54	0.18 ± 0.05	0.08-0.30
	Transitioned	November 11	61	0.19 ± 0.06	0.08-0.30
	Dry	December 11	60	0.16 ± 0.06	0.08-0.30
		January 12	61	0.22 ± 0.07	0.10-0.32
		February 12	55	0.22 ± 0.06	0.12-0.33
		March 12	61	0.22 ± 0.07	0.08-0.34
SN school	*Wet*	July 11	54	0.16± 0.05	0.08-0.30
		August 11	57	0.17± 0.06	0.08-0.28
		September 11	54	0.18 ± 0.05	0.08-0.30
	Transitioned	November 11	54	0.18 ± 0.06	0.08-0.30
	Dry	December 11	59	0.20 ± 0.06	0.08-0.32
		January 12	58	0.21 ± 0.06	0.08-0.35
		February 12	57	0.22 ± 0.06	0.11-0.35
		March 12	53	0.21 ± 0.07	0.10-0.33

Table 4.22 Exhaled MDA concentration of the subjects by school during the different seasons

Season	exhaled MDA concentration[**], µM		p-value [#]
	ST school	SN school	
Wet season	0.18^{a}	0.17^{a}	0.130
Transitioned period	0.19^{b}	0.18^{a}	0.455
Dry season	0.21^{ab}	0.21^{b}	0.695
p-value [##]	0.000^{*}	0.000^{*}	

[#] p-value for differences between schools

[##] p-value for differences between seasons

[**] Exhaled MDA concentration with different superscripts within the same column was significant difference ($p < 0.05$)

3) The prevalence of respiratory symptoms

The simple questionnaire adapted by the researcher was used to collect the data on respiratory symptoms of the study subjects. The subjects will be defined as having respiratory symptom if they had at least one symptom of respiratory symptom such as cough, sputum induction, shortness of breath, wheezing and chest discomfort in the concurrent week of PM_{10} monitoring. Table 4.23 shows the number of subjects with respiratory symptoms on different seasons.

Out of a sample of 124 subjects, a total 99 subjects of various respiratory symptoms were recorded during the study period. Among the subjects 62.6% or 62 subjects were ST school children while the remaining 37.4% (37 subjects) were SN school children. A comparative analysis of the study subject group shows that in wet season 25 subjects of ST school children suffered from respiratory symptoms while it fell to 6 subjects in transitioned period and then progressed to 31 subjects in dry season. The reversed then was observed in SN school children who respiratory symptoms decrease from 23 subjects in wet season, to 6 subjects in transitioned period, and then to 8 subjects in dry season. In dry season, the respiratory symptoms were founded to be significant increase in ST subjects as compared to SN subjects while other seasons were not significant difference.

Table 4.23 Number of subjects with selected respiratory symptoms by school during the different seasons

Season	No. of subjects with respiratory symptoms[**]		p-value [#]
	ST (N=63)	SN (N=61)	
Wet	25^a	23^a	.822
Transitioned period	6^b	6^b	.876
Dry	31^a	8^b	.000*
p-value [##]	.000*	.000*	

[#] p-value for differences between schools

[##] p-value for differences between seasons

[**] Number of subjects with respiratory symptoms with different superscripts within the same column was significant difference ($p < 0.05$)

Table 4.24 shows the comparison of risk of the selected respiratory symptoms among ST and SN school children. The odd ratio (OR) for cough and sputum induction was above 1. Subject from ST school are 2.88 times more likely to suffer from cough and 3.09 times more likely to suffer from sputum induction than those in SN school.

Table 4.24 Comparison of risk of the selected respiratory symptoms among ST and SN school children

Symptom	Subject	No. of subject with respiratory symptom		OR	95% CI
		Yes	No		
Cough	ST	18	43	2.88*	1.143-7.247
	SN	8	55		
Sputum induction	ST	17	44	3.09*	1.178-8.111
	SN	7	56		
Shortness of breath	ST	9	52	0.92	0.345-2.440
	SN	10	53		
Wheeze	ST	14	47	2.38	0.888-6.392
	SN	7	56		
Chest discomfort	ST	4	57	0.814	0.208-3.186
	SN	5	58		

* $p < 0.05$

4.3.5 Correlation analysis among PM_{10} exposure and health outcomes across 3 seasons

Pearson correlation coefficients (r) were calculated using SPSS for window (version 17) software that is significant at the level 0.01. The correlation between outdoor PM_{10} and health outcomes cannot be computed because the outdoor PM_{10} level is constant for each study subject.

The correlation among classroom PM_{10} exposure and health outcomes across 3 seasons of ST and SN subjects as shown in Table 4.25. Pearson's correlation among classroom PM_{10} levels and health outcomes including FVC predicted value, FEV_1 predicted value, FEV_1/FVC ratio, exhaled H_2O_2 concentration, and exhaled MDA concentration were analyzed to find out correlation of each pair.

The present study found that classroom PM_{10} were positively correlated with exhaled MDA in ST and SN children. The levels of classroom PM_{10} was moderately correlated with exhaled MDA concentration ($r = 0.422$ for ST subjects and 0.459 for SN subjects).

Table 4.25 Pearson's correlation among classroom PM_{10} levels and health outcomes by school

Site	Correlation among classroom PM_{10}levels and health outcomes				
	FVC	FEV_1	FEV_1/FVC	H_2O_2	MDA
ST	-.066	-.047	.253	.100	$.422^*$
SN	-.162	-.035	.341	.173	$.459^*$

4.4 Discussion of part 2: Applying the developed EBC collecting device to collect EBC samples from school children who expose to PM_{10}.

The main aim of part 2 study was to evaluate the effects of PM_{10} air pollution on respiratory health among children using pulmonary function and the concentration of H_2O_2 and MDA in EBC. The subjects of the follow-up study were the school children in urban (ST school) and highland areas (SN school). PM_{10} exposure was estimate by measuring level of PM_{10} at school and subject's classroom. The results of this study in urban area were showed the high level of PM_{10} in dry season especially in February and March. During study period the PM_{10} has a highest level on March 9th 2012 at 194.6 $\mu g/m^3$ that exceed 24 hours PM_{10} standard of Thailand (120 $\mu g/m^3$). This is probably due to the effect from the large scale of forest fires and open burnings in the agricultural setting, which is mostly occurred in the dry season (Vinitketkumnuen *et al.*, 2002; Pengchai *et al.*, 2009; Wiriya *et al.*, 2013). While the levels of PM_{10} at highland area ranged from 19.7-63.0 $\mu g/m^3$, with mean concentration of 31.8 $\mu g/m^3$. This result was similar to the level of PM_{10} at Bhubing Palace measured by mobile station of PCD. The PM_{10} concentration measured at Bhubing Palace was lower than other air quality monitoring station in Chiang Mai. This is may result from the station located on a high mountain above the temperature inversion. SN school is situated in the Doi Suthep Pui National Park near Bhubing Palace, with the strict control of forest fires and open burnings.

The classroom PM_{10} level as conducted in 4 classrooms at ST school and 3 classrooms at SN school. In these classrooms, 7 - 8 hours averages during school time were measured. The current results revealed that most of classroom PM_{10} levels exceeded the outdoor PM_{10} levels. Results of the current study are in agreement with findings of the previous studies. Several studies found that human activity can cause increased indoor particle levels (Janssen *et al.*, 1997; Lee and Chang, 2000). The most probable cause of the increased classroom levels was re-suspension of settled dusts or suspension of soil material brought in the classroom by the children' shoes. Use of chalk for writing on the blackboard could also be a dust source.

Comparison between ST and SN school, higher outdoor and classroom PM_{10} levels were measured in dry season compared to wet season. This finding is indicate of increased PM_{10} levels in dry season. This result was agreed with the study of Pengchai and co-workers (2009) which reported that the PM_{10} levels were high in dry season (February and March) before decreasing in April. In the present study, classroom PM_{10} were significantly correlated with increments the concentration of MDA in exhaled breath condensate in both subject groups, but no significant effect of PM_{10} on pulmonary function or exhaled H_2O_2 in both subject groups. The school children in the urban area had significantly more respiratory symptoms (cough and sputum induction) when compared with those living in the highland area. The result is consistent with this finding in that inhaled PM_{10} was significantly associated with an increase in an oxidative stress marker in EBC. Oxidative stress (OS) is the condition that occurs when there is the imbalance between oxidant and antioxidant on a cellular or individual level. OS can arise for many reasons, including consumption of alcohol, medications, trauma, toxins, radiation and air pollution (Kelly, 2003; Romieu *et al.*, 2008). Exposure to PM_{10} pollutant gives rise to OS within the lung, and this appears to initiate responses that are harmful to susceptible population. The generation of reactive oxygen species can cause oxidative damage to DNA, proteins, or lipid in the body. MDA is one of the major final products of lipid peroxidation. MDA are a group of low molecule-weight chemicals that are formed during the decomposition of lipid peroxidation products and thus are often used as an index of lipid peroxidation and OS (Valenzuela, 1991; Nielsen *et al.*, 1997).

The present study was observed an increased in MDA but not H_2O_2 in EBC in correlation to increased classroom PM_{10}, it appears that MDA may be more sensitive biomarker than H_2O_2 in EBC as a useful tool for investigating air pollution-related OS among healthy school children. This finding suggested that MDA concentration in EBC give good information of early biological effect of PM_{10} exposure before clinical symptoms appear.

This result on exhaled MDA changes was similar to those published previously (Romieu *et al*., 2008a). EBC has been used in clinical settings to study pathological mechanisms of respiratory illness such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease (van de Kant *et al*., 2009; Taylor, 2011; Tohda and Higashimoto, 2011; Po *et al*., 2012). Biomarkers of inflammation and OS in EBC were associated with occupational exposure such as asbestos (Lehtonen *et al*., 2007), silica (Pelclová *et al*., 2007), and chromium (Caglieri *et al*., 2006). Barregard and co-workers (2008) exposed adult human subjects to wood smoke in a controlled environment and reported an increase in MDA levels in EBC. However, little work has been done to use biomarkers in EBC to examine the effects of air pollutants on airway inflammation and OS in school children. Recently, Epton and co-workers (2008) reported a cohort study on healthy and asthmatic students in New Zealand school children, where they found small effects of PM_{10} in ambient air on FEV_1, but no effect on pH and exhaled H_2O_2.

In contrast to some earlier results, the present study was no significant effect of PM_{10} on pulmonary function in both subject groups. Other studies have shown that PM_{10} levels adversely affect the pulmonary function of children (Kim *et al.,* 2005; Kasamatsu *et al*., 2006). The present study findings agree with several previous studies of ambient air pollution from Helsinki, Athens, Amsterdam and Birmingham (Hartog *et al*., 2010), Bangkok (Lungkulsen *et al*., 2006) and New Zealand (Epton *et al*., 2008). Researchers of those studies conclude that elevated in PM_{10} levels had no relationship with pulmonary function of school children, but a small effect on respiratory symptoms. There are several possible reasons for the lack of relationships of PM_{10} and pulmonary function observed in the present study. Firstly, in wet season, respiratory infections are common and may confound the relationship with PM_{10}, making effects difficult to be detected. Secondly, this study had only 124 participants; therefore, our study did not find statistically significantly adverse change in pulmonary function between different seasons. For this reason, we might need a larger number of participants to detect significant changes in pulmonary function. Moreover, considering from their characteristics i.e. heights, the subjects in the present study were rather healthy school children; therefore, the effects of PM_{10} to the subject may not be detected by pulmonary function testing. This finding may be confounded by some other environment factors, like environmental tobacco smoke and cooking smoke at home. In addition, there may be other potential confounders such as socio-economic factors including household crowding, number of smoker in family, fuel used for cooking, air-conditioned bedroom, presence of pet indoor, traffic level in the neighborhood and dietary habits. Eroshina *et al*, (2004) showed that environmental and social factors affected health condition, respiratory dysfunction and impaired lung function of school children in Moscow. However, to decrease these confounders, this study was designed to follow up the health outcomes of the same group of school children in different season.

In this study, we did not find statistically significant adverse changes in exhaled H_2O_2 associated with PM_{10}. The possible explanation might be that low sensitivity of the assay. Similarly, Barregard and co-workers (2008), though they describe statistically significant alterations in exhaled MDA after exposure to wood smoke, suggest that the results be interpreted with caution due to very low overall levels in EBC, with many measurements below detection limit of the assay.