Influence of Traffic-Related Air Pollution Exposure on Respiratory Health, *TNFα* and *CYP1A1* Gene and Histone Modifications among School Children in the Klang Valley, Malaysia

Nur Faseeha Binti Suhaimi

Doctor of Philosophy
Universiti Putra Malaysia
2021

INFLUENCE OF TRAFFIC-RELATED AIR POLLUTION EXPOSURE ON RESPIRATORY HEALTH, *TNFα* AND *CYP1A1* GENE AND HISTONE MODIFICATIONS AMONG SCHOOL CHILDREN IN THE KLANG VALLEY, MALAYSIA

By

NUR FASEEHA BINTI SUHAIMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2021

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

INFLUENCE OF TRAFFIC-RELATED AIR POLLUTION EXPOSURE ON RESPIRATORY HEALTH, *TNFα* AND *CYP1A1* GENE AND HISTONE MODIFICATIONS AMONG SCHOOL CHILDREN IN THE KLANG VALLEY, MALAYSIA

By

NUR FASEEHA BINTI SUHAIMI

June 2021

Chair: Juliana Jalaludin, PhD
Faculty: Medicine and Health Sciences

Traffic-related air pollution (TRAP) is a complex mixture of many pollutants, which has adverse health impacts, especially on children who live near heavily-travelled roads. This cross-sectional comparative study was conducted at eight schools in high traffic (HT) and low traffic (LT) areas to investigate the potential risks from TRAP exposure to respiratory health among children with the incorporation of histone H3 level and deoxyribonucleic acid methylation (DNAm) status of *Tumour Necrosis Factor Alpha (TNFα)* and *Cytochrome P450 Family 1 Subfamily A Member 1 (CYP1A1)*. Respondents' background information, personal exposure to TRAP, and respiratory symptoms were obtained from validated questionnaires distributed to randomly selected 7 to 11-year-old children to be filled in by parents or guardians. Portable instruments equipped with integrated sensors for real-time monitoring were used for 6-h measurements of coarse particulate matter (PM_{10}), fine particulate matter ($PM_{2.5}$), extremely fine particulate matter (PM_1), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), carbon monoxide (CO), and total volatile organic compounds (TVOC). Meanwhile, 24-h measurements of $PM_{2.5}$-bound black carbon (BC) in schools and particulate matters in residences were performed using air sampling pumps that utilise the gravimetric method. Data from local air quality monitoring stations were also compared to validate the school findings and proceeded with Principal Component Analysis (PCA) to identify pollution sources. A lung function test was conducted using a spirometer to measure lung performance. Histone H3 modification was captured using an enzyme-linked immunosorbent assay (ELISA) kit, whereas DNAm was quantified using a methylation-specific polymerase chain reaction (MS-PCR) kit on bisulphite-treated DNA; both from saliva samples. The results indicate that HT area had significantly higher concentrations of PM_{10} ($p<0.001$), $PM_{2.5}$ ($p<0.001$), PM_1 ($p<0.001$), BC ($p<0.001$), NO_2 ($p<0.001$), SO_2 ($p<0.001$), O_3 ($p<0.001$), CO ($p<0.001$) and TVOC ($p<0.001$) than LT area. The PCA results highlighted that the air quality in

the HT area had been affected by the combustion of fuel engines. Children who attended schools in the HT area were more prone to get cough (OR=3.0), phlegm (OR=2.3), wheezing (OR=2.3), impairment in forced vital capacity (FVC%)(z= -5.23), impairment in forced expiratory volume in 1 second (FEV_1%)(z= -5.01), higher histone H3 level (z= -5.13), methylated *TNFα* (OR=2.0) and methylated *CYP1A1* (OR=1.7). After controlling the possible confounders, findings from multiple logistic regression show that methylated *TNFα* and *CYP1A1* were mostly influenced by exposure to NO_2 (OR=3.0) and BC (OR=2.0), respectively. Meanwhile, results from multiple linear regression revealed that BC and NO_2 were the most significant factors influencing the FVC% (adjusted R^2=0.405, p<0.001, f^2=0.68) among children. FEV_1% were mostly influenced by BC, PM_1 and $PM_{2.5}$ (adjusted R^2=0.412, p<0.001, f^2=0.70), whereas NO_2 was the most significant factor that influenced the histone H3 level (adjusted R^2=0.337, p<0.001, f^2=0.51) among children. In conclusion, epigenetic mechanisms may govern the relationships between TRAP exposures and respiratory health by acting as mediators. This study also provides the groundwork for future preventive interventions, particularly developing mitigation plans to reduce TRAP exposure in Malaysia.

Keywords: TRAP, children, epigenetic modifications, respiratory health.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGARUH PENDEDAHAN KEPADA PENCEMARAN UDARA BERKAITAN TRAFIK KE ATAS KESIHATAN RESPIRATORI, MODIFIKASI HISTON DAN GEN *TNFα* DAN *CYP1A1* DALAM KALANGAN KANAK-KANAK SEKOLAH DI LEMBAH KLANG, MALAYSIA

Oleh

NUR FASEEHA BINTI SUHAIMI

Jun 2021

Pengerusi: Juliana Jalaludin, PhD
Fakulti: Perubatan dan Sains Kesihatan

Pencemaran Udara Berkaitan Trafik (TRAP) merupakan campuran kompleks beberapa pencemar yang boleh mengakibatkan kesan kesihatan yang buruk, terutamanya terhadap kanak-kanak yang tinggal berhampiran jalan yang sibuk. Kajian perbandingan keratan rentas ini dijalankan di lapan sekolah di kawasan trafik tinggi (HT) dan trafik rendah (LT) untuk menentukan potensi risiko pendedahan TRAP terhadap kesihatan pernafasan dalam kalangan kanak-kanak dengan penggabungan paras histon H3 dan status pemetilan asid deoksiribonukleik (DNAm) *Tumour Necrosis Factor Alpha (TNFα)* dan *Cytochrome P450 Family 1 Subfamily A Member 1 (CYP1A1)*. Maklumat latar belakang responden, pendedahan peribadi terhadap TRAP dan gejala pernafasan telah diperoleh daripada soal selidik yang diedarkan kepada kanak-kanak berusia 7 hingga 11 tahun yang dipilih secara rawak untuk diisi oleh ibu bapa atau penjaga. Instrumen mudah alih yang dilengkapkan dengan sensor bersepadu untuk pemantauan masa nyata digunakan untuk pengukuran bahan zarahan terampai (PM_{10}), bahan zarahan halus ($PM_{2.5}$), bahan zarahan sangat halus (PM_1), nitrogen dioksida (NO_2), sulfur dioksida (SO_2), ozon (O_3), karbon monoksida (CO), dan sebatian organik meruap sepenuhnya (TVOC) selama 6 jam. Sementara itu, pengukuran karbon hitam (BC) pada $PM_{2.5}$ di sekolah, dan bahan zarahan di kediaman dijalankan dengan menggunakan pam pensampelan udara yang menggunakan kaedah gravimetrik. Data dari stesen pemantauan kualiti udara tempatan telah dibandingkan untuk mengesahkan hasil penemuan di sekolah. Analisis Komponen Utama (PCA) telah digunakan untuk mengenal pasti punca pencemaran. Ujian fungsi paru-paru dilakukan dengan menggunakan spirometer untuk mengukur keupayaan paru-paru. Modifikasi histon H3 telah dikesan menggunakan kit imunosorben berkaitan enzim (ELISA), sementara DNAm telah dikesan dengan menggunakan kit tindak balas berantai polimerase spesifik untuk pemetilan (MS-PCR) pada DNA yang diubah suai dengan bisulfit; kedua-duanya daripada sampel air liur. Hasilnya

menunjukkan bahawa kawasan HT mempunyai kepekatan PM_{10} (p<0.001), $PM_{2.5}$ (p<0.001), PM_1 (p<0.001), BC (p<0.001), NO_2 (p<0.001), SO_2 (p<0.001), O_3 (p<0.001), CO (p<0.001) dan TVOC (p<0.001) yang jauh lebih tinggi berbanding dengan kawasan LT. Hasil PCA mendapati bahawa kualiti udara di kawasan HT telah dipengaruhi oleh pembakaran enjin bahan api. Kanak-kanak yang bersekolah di kawasan HT lebih mudah mendapat batuk (OR=3.0), kahak (OR=2.3), nafas berdehit (OR=2.3), pengurangan kapasiti vital terpaksa (FVC%) (z= -5.23), pengurangan isipadu ekspirasi paksa dalam 1 saat (FEV_1%) (z= -5.01), paras histon H3 lebih tinggi (z= -5.13), pemetilan *TNFα* (OR=2.0) dan pemetilan *CYP1A1* (OR=1.7). Setelah mengawal kemungkinan wujudnya risiko sampingan, penemuan daripada regresi logistik berganda menunjukkan bahawa pemetilan *TNFα* dan *CYP1A1* kebanyakannya dipengaruhi oleh pendedahan terhadap NO_2 (OR=3.0) dan BC (OR=2.0). Sementara itu, hasil regresi linear berganda menunjukkan bahawa BC dan NO_2 adalah faktor paling signifikan yang mempengaruhi FVC% (R^2 diubah=0.405, p<0.001, f^2=0.68) dalam kalangan kanak-kanak. FEV_1% adalah paling dipengaruhi oleh BC, PM_1 dan $PM_{2.5}$ (R^2 diubah=0.412, p<0.001, f^2=0.70), manakala NO_2 adalah faktor yang paling signifikan yang mempengaruhi paras Histon H3 (R^2 diubah=0.337, p<0.001, f^2=0.51) dalam kalangan kanak-kanak. Secara ringkasnya, mekanisme epigenetik berkemungkinan terlibat dalam hubungan antara pendedahan TRAP dan kesihatan pernafasan dengan bertindak sebagai pengantara. Kajian ini juga menyediakan panduan untuk intervensi pencegahan di masa depan, terutamanya pelan tindakan mitigasi untuk mengurangkan pendedahan kepada TRAP di Malaysia.

Kata Kunci: TRAP, kanak-kanak, perubahan epigenetik, kesihatan pernafasan.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful.

Alhamdulillah, all praises to the Almighty Allah for granting me His blessings in every beat of my heart, good health, and great vitality in completing my PhD journey. I would like to acknowledge and extend my gazillion thanks to many people who had been there with me during the whole process of this research, making it possible for me to complete this research successfully. I am grateful to have had the privilege of contributing to the nation.

I wish to express my profound appreciation to my supervisor, Prof. Dr Juliana Jalaludin, for guiding me in this adventure. She generously granted me time to learn new subjects related to my study and is always available during and after office hours for consultations, despite her busy work schedule. Her significant expertise, supportive advice, continuous encouragement, and productive criticism have shaped me with the right mould in this field. My special gratitude also goes to my co-supervisors, Dr Suhaili Abu Bakar, Dr Nor Eliani Ezani and Prof. Dr Mohd. Talib Latif for sharing their useful knowledge and constructive comments throughout my research venture.

Besides that, I am indebted to these bodies for financial supports; the Ministry of Higher Education Malaysia for the FRGS grant in funding my research project, the Ministry of Higher Education Malaysia and Universiti Putra Malaysia for full scholarships in financing my PhD study. Many thanks are also extended to the Study Leave Section, Registrar Office of UPM, for enormous supports and sponsored workshops during my candidacy.

Furthermore, I appreciate my fellow graduate friends who cannot be all named here. Thank you for making my research journey more enjoyable, supporting me through thick and thin, and inspiring me at all times. Many thanks to Nur Hazirah Hisamuddin, Ernie Syazween Junaidi, Aina Nadirah Yusof, Muhammad Afif Mohd Juhari, Nur Intan Shafiqah Roslan, Nur Ain Danisya Mohd Yusri, Syhidatul Farhana Othman and Faris Syahmi Mohd Shahidin for accompanying me during data collection. Special thanks also go to Dr Nor Ashikin Sopian, Dr Noor Fatihah Mohd Fandi and Dr Noor Haziqah Kamaludin for their assistance in data analysis.

Moreover, I would like to give my most profound appreciation to all dear respondents, their parents and guardians, the school teachers, and the management of primary schools for their willingness and support to engage in this research despite the inconvenience they had experienced.

My sincere gratitude also goes to these laboratory and support staffs for providing me worthy information and excellent assistance while handling the research samples and instruments, also while performing the laboratory works; from the Department of Environmental and Occupational Health, and Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, UPM; from Faculty of Forestry and Environment, UPM; from Faculty of Science and Technology, UKM; from Institute of Oceanography and Environment, UMT.

Last but not least, I am very grateful to my beloved family members who have been very understanding with my commitments, always helping me to look after my daughter, continuously praying to Allah for my success, and continually motivating me throughout my life from the moment I was born; my mother Ms Normayati Sulaiman, my father Mr Suhaimi Hasan, my sister Nur Azeema Suhaimi, my mother-in-law Ms Asiah Idris, and all of my family members. My special gratitude to my supportive husband, Mr Muhammad Fauzi Idris, for his endless love, patience, companionship and motivation throughout this doctoral journey. To my adorable daughter Fauzana Aneesa, you have been an excellent source of love and relief from my scholarly endeavour. To my son and PhD baby Fauzan Ameen, having you during the final year of my PhD journey is a blessing. I love you all to the moon and back.

Not to forget my sincere gratitude to those who contributed either directly or indirectly to complete my research. May Allah rewards all of you.

I certify that a Thesis Examination Committee has met on 21 June 2021 to conduct the final examination of Nur Faseeha binti Suhaimi on her thesis entitled "Influence of Traffic-Related Air Pollution Exposure on Respiratory Health, *TNFα* and *CYP1A1* Gene and Histone Modifications among School Children in the Klang Valley, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Sarva Mangala Praveena, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Abdah binti Md Akim, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Budi Haryanto, PhD
Professor
Faculty of Public Health
Universitas Indonesia
Indonesia
(External Examiner)

Zaleha Md. Isa, PhD
Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 September 2021

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Juliana binti Jalaludin, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Suhaili binti Abu Bakar@Jamaludin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Nor Eliani binti Ezani, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Mohd Talib Latif, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 October 2021

Declaration by Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from the supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before the thesis is published (in the form of written, printed or in electronic form), including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: 11/11/2021

Name and Matric No.: Nur Faseeha binti Suhaimi, GS50755

Declaration by Members of Supervisory Committee

This is to confirm that:

- this research conducted and the writing of this thesis was under our supervision;
- the supervision responsibilities, as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013), are adhered to.

Signature: 
Name of Chairman of Supervisory Committee: Prof. Dr Juliana binti Jalaludin

Signature: 
Name of Member of Supervisory Committee: Dr Suhaili binti Abu Bakar@Jamaludin

Signature: 
Name of Member of Supervisory Committee: Dr Nor Eliani binti Ezani

Signature: 
Name of Member of Supervisory Committee: Prof. Dr Mohd. Talib Latif

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

<	Less than
>	More than
≥	At least
%	Per cent
°C	Degree Celsius
x g	Times gravity
µm	Micrometres
µg/m^3	Microgram per metre cubic
ADT	Average Daily Traffic
AM	Traffic counts that passed by the schools from 7 to 8 a.m.
AT	Ambient Temperature
Alu	Short-interspersed nucleotide element
API	Air Pollutant Index
ATS	American Thoracic Society
BC	Black Carbon
bp	Base pair
CAQM	Continuous Air Quality Monitoring
CI	Confidence Interval
CO	Carbon Monoxide
CO_2	Carbon Dioxide
CYP1A1	*Cytochrome P450 Family 1 Subfamily A Member 1*
DNA	Deoxyribonucleic Acid
DNAm	DNA Methylation

DOE	Department of Environment
DOS	Department of Statistics
DOSH	Department of Occupational Safety and Health
EH	Environmental Health
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
FEV_1	Forced Expiratory Volume in 1 Second
FMHS	Faculty of Medicine and Health Sciences
FVC	Forced Vital Capacity
h	hours
HT	High Traffic
IAQ	Indoor Air Quality
I/O	Indoor/Outdoor
IFNγ	Interferon Gamma
IQR	Interquartile Range
ISAAC	International Study of Asthma and Allergies in Childhood
km	Kilometre
L/min	Litre per minute
LINE-1	Long Interspersed Nuclear Element 1
LOS	Level of Severity
LT	Low Traffic
m	Metre
m/s	Metre per second
MAAQS	Malaysia Ambient Air Quality Standard
MCE	Mixed Cellulose Esters

min	minutes
ml	Millilitre
MOE	Ministry of Education
MOH	Ministry of Health
MS-PCR	Methylation Specific Polymerase Chain Reaction
ng/µl	Nanogram per microlitre
ng/ml	Nanogram per millilitre
NIOSH	National Institute for Occupational Safety and Health
nm	Nanometre
NO	Nitric Oxide
NO_2	Nitrogen Dioxide
NO_x	Oxides of Nitrogen
NF-κB	Nuclear Factor Kappa B Cells
O_2	Oxygen
O_3	Ozone
OD	Optical Density
OD/ng	Optical Density/Nanogram
OR	Odds Ratio
PAH	Poly Aromatic Hydrocarbon
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
pg/ml	Picogram per millilitre
PM	Traffic counts that passed by the schools from 12.30 to 1.30 p.m.
PM_{10}	Particulate matter below 10 micrometres aerodynamic diameter

$PM_{2.5}$	Particulate matter below 2.5 micrometres aerodynamic diameter
PM_1	Particulate matter below 1 micrometre aerodynamic diameter
ppb	Parts per billion
ppm	Parts per million
PVC	Poly Vinyl Chloride
RH	Relative Humidity
RT	Room Temperature
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
RTVM	Road Traffic Volume Malaysia
s	Seconds
SD	Standard Deviation
SO_2	Sulphur Dioxide
SR	Solar Radiation
T	Temperature
TC	Traffic Counts
TNFα	*Tumour Necrosis Factor Alpha*
TRAP	Traffic-Related Air Pollution
TVOC	Total Volatile Organic Compounds
UKM	Universiti Kebangsaan Malaysia
UMT	Universiti Malaysia Terengganu
UPM	Universiti Putra Malaysia
URTI	Upper Respiratory Tract Illnesses
USA	United States of America
US EPA	United States Environmental Protection Agency

UV	Ultraviolet
V	Volume
Vel	Velocity
VIF	Variance Inflation Factor
WD	Wind Direction
WHO	World Health Organization
WS	Wind Speed

CHAPTER 1

INTRODUCTION

1.1 Background

In a world where human activities are in full swing, both during the day and at night, air pollution is getting worse, and concerns about the effects of toxins on airways and their correspondence with lung diseases are also increasing. Traffic-generating particulate matter is now known to contribute substantially to ambient particulate matter, particularly in urban areas, and globally it creates a growing burden to us in the form of human disease (Awang, Jalaludin, Latif, & Mohamad Fandi, 2019; Haryanto, 2020; Mohamad Jamil et al., 2020). Apart from that, emissions from road vehicles are some of the most important sources of human exposure to air pollution, which are known to have adverse effects on health (Haryanto & Pratiwi, 2020) with the complex combination of many pollutants. This topic is of pivotal importance in the community. After being exposed to air pollutants, inhalation toxicity within the respiratory system may occur as the respiratory system is physically connected with air pollutants through breathing.

Many countries have taken the necessary steps to combat the adverse effects of particulate matter by implementing more stringent emission controls to reduce emissions from motor vehicles and improve air quality, as awareness of the related health issues has grown in recent years (Lee, Lin, Yuan, Lin, & Chen, 2018; Sofwan & Latif, 2020; Winkler et al., 2018). Nevertheless, the populations residing and working close to busy roads and highways have increased throughout the years due to the expansion of urban areas and economic improvement. Banks have always provided accessible financing for car ownership, and land-use changes promote dependence on motor vehicles. As a result, a large proportion of the human population spends significant amounts of their time on or near roadways as part of their daily activities (Matz, Stieb, Egyed, Brion, & Johnson, 2018) include experiencing significantly long periods on the road during traffic congestion.

Work considering traffic-related air pollution (TRAP) has a rich background. Previous research have indicated that TRAP is linked to toxicity within the respiratory system in distinct types of studies – *in vitro*, *in vivo*, and epidemiology. TRAP triggers diverse respiratory health effects, generates reactive oxygen species (ROS), which then cause oxidative stress (Kim, Choi, Park, & Seo, 2017). Besides that, significant evidence has been found on the mechanisms which connect TRAP to epigenetic modifications triggered by damage to Deoxyribonucleic Acid (DNA) (Ding et al., 2017; Rider & Carlsten, 2019). Besides, there are studies documented the prevalence of respiratory symptoms in persons who live close to busy roads (Hegseth et al., 2019; Yi et al., 2017).

This study aimed to collect the baseline data on exposure levels of indoor and outdoor air pollutants among school children living in Klang Valley areas with high traffic. Also, to study the precise nature and sources of local air pollution components in those areas, which would be informative in developing tailored risk control strategies. By investigating the exposure to TRAP, risk factors can be recognised and taken into account for further actions to control the respiratory diseases associated with the exposure to air pollutants.

1.2 Problem Statement

Traffic congestion is a major problem in Klang Valley, which is a known concern with the community. The fast-growing population mainly causes the problem in Klang Valley in the past ten years (DOS Malaysia, 2019). Traffic and industrial air pollution have long been recognised as the crucial external causes of respiratory problems (Azhari, Latif, & Mohamed, 2018; Sopian, Jalaludin, Tengku Mayusi, & Latif, 2020). The problem of TRAP may be further compounded by the growing vehicle fleet, urban society's dependence and preference for private motorised travel. The growth in the number of private vehicles has resulted in increased emissions.

Malaysia is a tropical country where the weather is humid and warm, with low temperature and humidity variation all year round. The particular apprehension over electricity and operational expenditure has resulted in natural ventilation for all national schools in Malaysia. Moreover, natural ventilation through window openings provides a practical solution to enhance the classrooms' indoor environment. Nevertheless, high concentrations of outdoor air pollutants can also infiltrate the schools' indoor environment via open windows and doors and cracks in the building (Mohammadyan et al., 2017), air exchange rate, and micro-environment (Lv, Wang, Wei, Zhang, & Zhao, 2017). The location of classrooms near the main roads and highways in Malaysia have also contributed to poor air quality in the classrooms. Figure 1.1 shows a typical classroom in national schools in Malaysia. A few local studies pointed that traffic and industrial activity influence the indoor air quality (IAQ) in the classrooms by releasing air pollutants into ambient air, which could penetrate the indoor environment (Kamaruddin, Jalaludin, Hamedon, & Hisamuddin, 2019; Mohd Isa, Hashim, Jalaludin, Lung Than, & Hashim, 2020).

Figure 1.1: A classroom in a national school in Malaysia

One of the adverse environmental issues is air pollution generated by road-side exposures in the vicinity of urban localities. Children are vulnerable to air pollutants due to schools and residential complexes located in the urban and sub-urban areas. By 2030, urban areas are expected to accommodate 60% of the world's population, which means that one in every three people will live in cities with at least half a million residents (United Nations, 2016). There is a large crowd of human in cities because government hubs, offices, business parks, education centres and main crossroads of transportations are situated here. Increasing high-rise buildings and population around the area lead to build up of traffic flow, notably during peak hours, which are in the early morning when most people go to work, and in the late evening when most people come back home. According to the recent list of schools under the Ministry of Education Malaysia management, there are 5,895 public schools (57.7%) located in the urban areas of Malaysia out of a total of 10,218 national primary and secondary schools in Malaysia (Ministry of Education Malaysia, 2021). Out of these, 851 schools (90.5%) out of 940 schools are located in the urban areas in Selangor, and 295 schools (100.0%) are located in Kuala Lumpur. By referring to these figures on the schools located in the urban areas, extra attention should be paid to the IAQ of the school buildings, primarily national schools located near heavily-travelled roads.

Air pollutants released by urban air pollution threaten children's respiratory health (Zainudin, Jalaludin, & Sopian, 2019). Although it is a known issue, it has to be highlighted because children inhale a higher volume of air per unit of body weight than adults. Moreover, their body systems and organs are still growing, which means they are less able to detoxify dangerous pollutants. Diseases of the respiratory system have been reported to be the number 2 cause out of 10 leading sources of hospitalisation in both government and private hospitals after pregnancy, childbirth and the puerperium, accounting for 14.8% of cases (MOH Malaysia, 2020). Since specific environmental factors induce respiratory

problems, attention, and early intervention to these environmental conditions could greatly reduce school children's respiratory health effects.

Even though most of the aberrant changes in gene expression linked to the health effects of environmental agents exposure have been associated with genotoxic mechanisms, non-genotoxic mechanisms may also play a part (Ren, Atyah, Chen, & Zhou, 2017). Previous studies conducted on the epigenetic effects induced by environmental agents have revealed global and gene-specific changes of DNA methylation (DNAm) and histone modification level; these changes are similar or equal to the observed epigenetic changes found in patients whose conditions are induced by that particular environmental agent (Sharavanan et al., 2020; Zheng et al., 2017). Epigenetic research is emerging as a new option to decipher the possible consequences of air pollution on DNA because certain epigenetic mechanisms have been associated to diseases. Children's detoxification enzymes are less efficient, which leads to epigenetic modifications after alterations in DNA or chromatin structure (Alvarado-Cruz, Alegría-Torres, Montes-Castro, Jiménez-Garza, & Quintanilla-Vega, 2018); hence, children are more susceptible to TRAP than adults.

1.3 Study Justifications

Environmental health impacts in Malaysia have achieved the state requiring a paradigm shift and should be granted a high priority. Klang Valley is a highly populated area in Malaysia. The population is expected to multiply faster due to the rapid development of various industries; thus, these activities are partly responsible for the high concentrations of TRAP in the vicinity. Although TRAP exposure may cause health impacts, not everyone would experience those effects immediately because the effects could have implications later in life due to human organs' development process. Primary school children were chosen as the study subjects because they are more susceptible to air pollution, mainly TRAP in this study; hence, they are more remarkably to develop health effects. This research was focused on the exposure to TRAP in Klang Valley among school children by using selected epigenetic mechanisms, which are histone H3 modification and DNAm. Air pollution can affect epigenetics in every life cycle, but only children were focused on this study because TRAP can hinder lung growth in children. Along with these epigenetic mechanisms, respiratory symptoms and lung function status were also investigated in this study.

The school children in Malaysian public primary schools spend about 6 to 7 hours per day in their schools and mostly in their classrooms. These children are exposed to the indoor and outdoor environment in the classrooms most of their time on weekdays. Therefore, there is a need to assess air quality on the school buildings, particularly those near heavily-travelled roads. Despite this issue, insufficient local studies have examined indoor and outdoor air quality at the primary schools for devising the situation. There are also limited local studies performed in school settings and has complemented actual measurements of TRAP with children's daily diary activities. Furthermore, it is noteworthy that

children require different protection levels for environmental health strategies intended to safeguard adults. This study incorporated actual TRAP measurements at schools and residences, which were concurrently collected with respondents' biological samples. This study focused on children's exposure to TRAP in schools and residences. Actual measurements were carried out in schools and residences. Previous similar studies did not include black carbon (BC), carbon monoxide (CO) and ozone (O_3) although these are also pollutants produced by vehicle emissions. Besides, modelling of air pollutants using Principal Component Analysis (PCA) and trend of air quality at the nearby monitoring stations using Pollution Rose were included to validate the actual findings at schools. PCA had also identified the specific sources of air pollution at schools.

Epigenetics is a part of exposome, which is a theory in environmental health that clarifies the relation between health and all the exposures of a human in a lifetime from pre-conception onwards (Sarigiannis, 2019). The degree of environmental exposure at individual level that might be mediated through epigenetic mechanisms is still obscure although these mechanisms are excellent intermediates of environmental effects at molecular level (Ferrari, Carugno, & Bollati, 2019). Previously published epidemiological studies in epigenetic mechanisms and interaction with exposure to air pollution among children as the study population was scarce, even though this is a crucial life phase. Besides, the previous research findings for histone modification are still understudied compared to DNAm (Lu et al., 2016; Reddy, Khade, Pandya, & Gupta, 2017). There are also limited studies which have utilised saliva for monitoring of respiratory diseases although it has a direct anatomic relationship with the lower airway. Besides, there are complex associations between air pollutants and the genetic factors in the course of life. An additional aspect of this study is to associate epigenetic mechanisms with related health effects, mainly respiratory, as children underlined as the target study population in this work are considered the susceptible group. These epigenetic changes are also linked to respiratory health impacts such as respiratory symptoms and reduced lung function. The proposed mechanisms are portrayed in Figure 1.2.

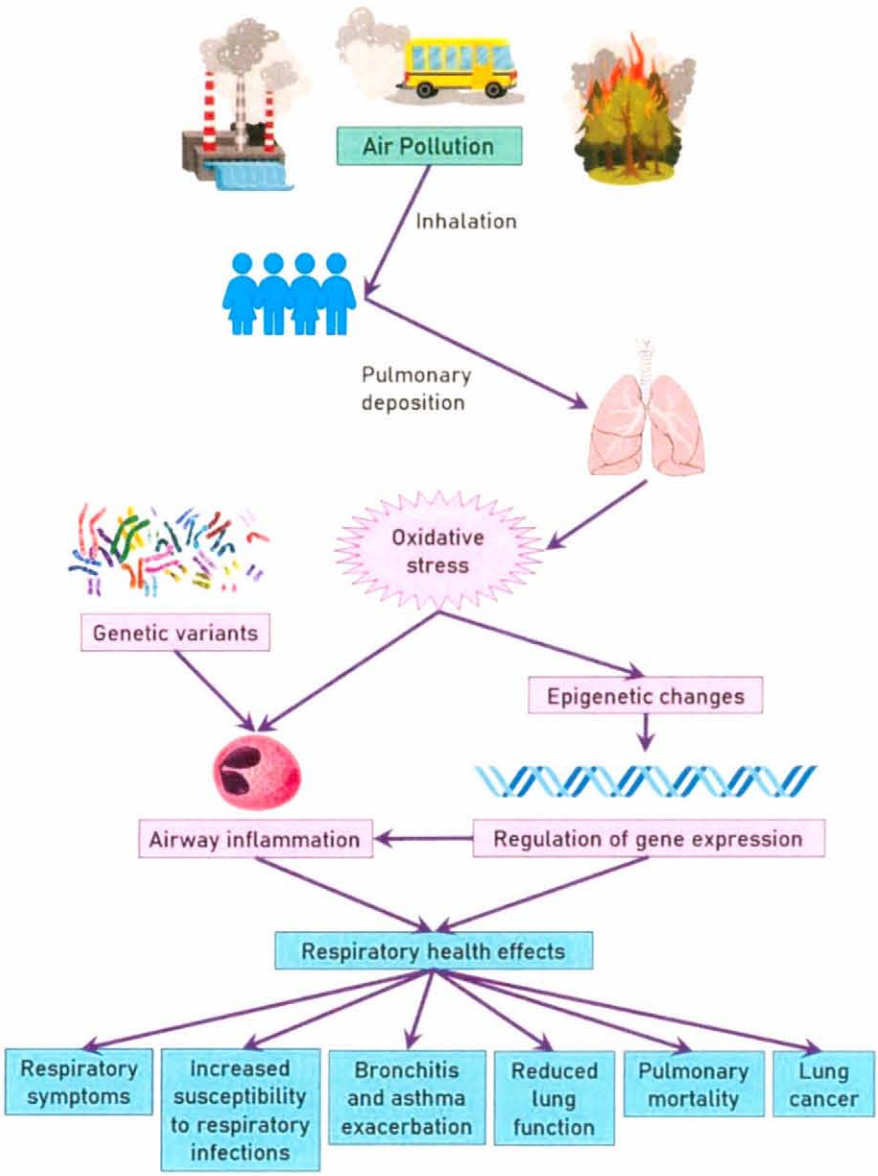


Figure 1.2: A network of a few mechanisms connecting respiratory health effects and exposure to air pollution

This study is also in line with the third goal of the Sustainable Development Goals (SDGs) 2030 by the United Nations Member States of which Malaysia is a signatory; the third goal is to ensure healthy lives and uphold well-being for all ages (United Nations Development Programme, 2021). Moreover, this research covers the issue of children's environmental health, which has been identified as an environmental health threat with the highest priority in Malaysia that call for immediate intervention, according to the Thematic Working Group 10 who are a

team of Environmental Health experts under the National Environmental Health Action Plan (NEHAP) by Ministry of Health Malaysia (Ministry of Health Malaysia, 2021). This study provides data on the formulation of mitigation strategies for managing children's environmental health issues, particularly from the exposure to air pollution at schools and homes by emphasising on multi-pollutant emission reductions and overall air pollution-related risk. This step is a beneficial chance for Malaysia to establish a framework to tackle the emerging health impacts from environmental threats.

In general, this study is applicable to evaluate the potential risks from exposure to air pollutants with the incorporation of epigenetic data into human health risk assessments. This study contributes to the body of knowledge with a specific goal to fill in a gap within the works on epigenetic mechanisms as the mediator in connecting respiratory health effects and air pollution during the childhood phase. Research and data analysis performed in this study have solicited important scientific information, which would allow the related bodies to make strategic, scientific, and evidence-based decision-making. Therefore, this research complements the previous studies by linking epigenetic modification and exposure to air pollution, focusing on children.

1.4 Conceptual Framework

Figure 1.3 shows a diagram that represents the relationship that exists between the variables in the study. Generally, the framework explains the connection between the risk factors that contribute to epigenetic modifications and respiratory health effects among school children in Klang Valley. Both anthropogenic and natural sources could contribute to air pollution. This study focused on air pollution from transportation, which is one of the anthropogenic sources. By selecting transportation as the source of air pollution in the study, respondents' primary schools and residences were chosen for the data collection on TRAP exposure. Those residing, working or studying near the areas with high traffic congestion are the population who might be more affected by the TRAP exposure. The studied respondents were primary school children who were divided into the high-traffic and low-traffic groups. The respondents' exposure to TRAP was investigated by collecting data on respiratory symptoms and biological samples connected to the route of exposure via inhalation. Besides, confounders that may compete with the study's dependent variables were also included in the study.

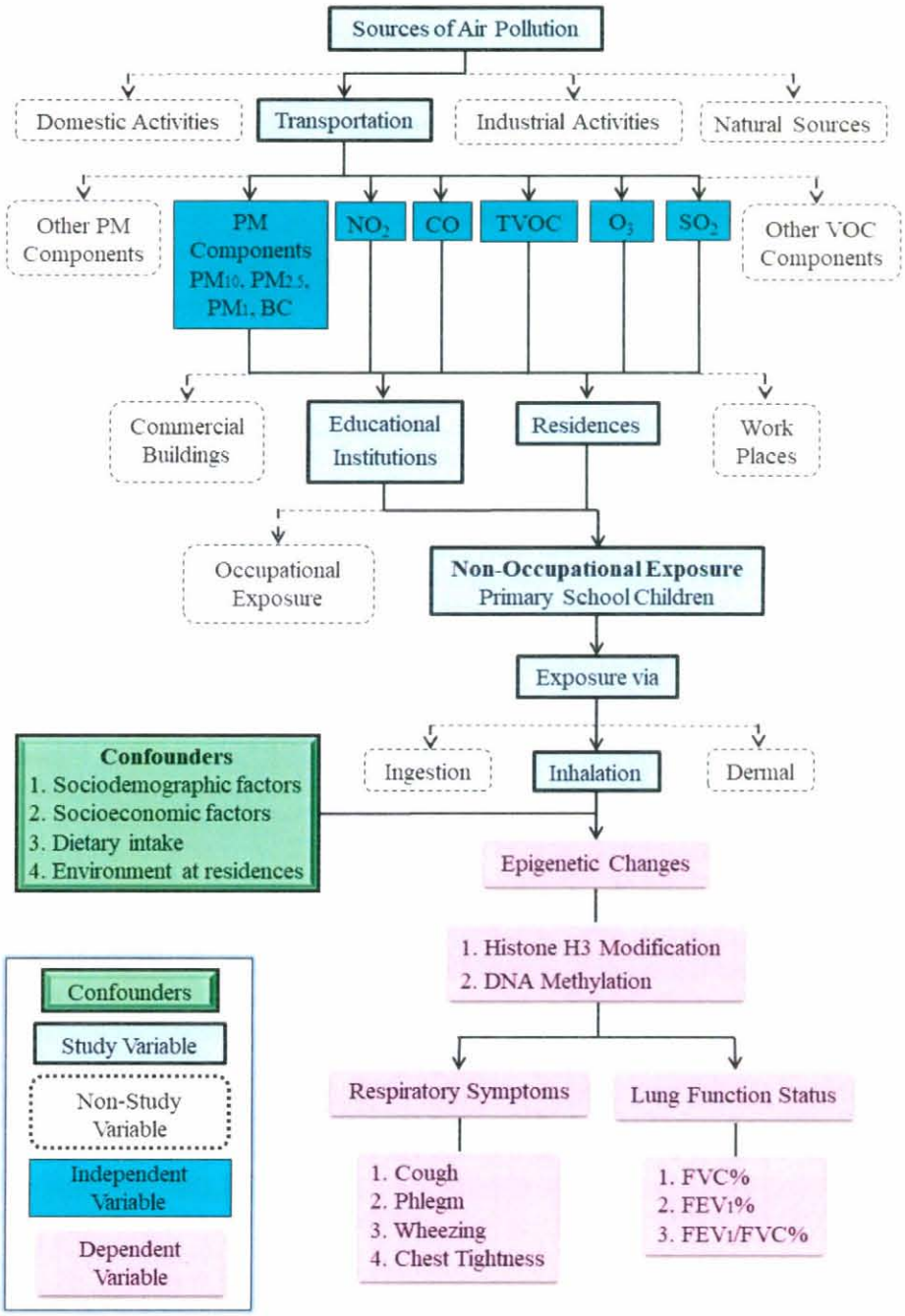


Figure 1.3: Conceptual framework

1.5 Study Objectives

1.5.1 General Objective

To assess the relationship between the magnitude of exposure to TRAP with respiratory health and epigenetic modifications among school children living near major roadways in Klang Valley.

1.5.2 Specific Objectives

1. To determine sociodemographic and socioeconomic information, house condition and location, family background and dietary intake between the respondents in high and low traffic areas.
2. To evaluate the concentrations of TRAP and IAQ parameters inside respondents' classrooms and residences in high and low traffic areas.
3. To compare the reported respiratory symptoms, lung function status, histone H3 level and DNAm status between the respondents in high and low traffic areas.
4. To assess the associations between TRAP exposure with respiratory symptoms, lung function status and epigenetic modifications assessed in the study.
5. To identify the factors that are significantly associated with the respiratory symptoms, lung function status, histone H3 level and DNAm status in response to TRAP-induced systemic inflammation after controlling the confounders.

1.6 Hypotheses

1. There are significant differences in sociodemographic and socioeconomic information, house condition and location, family background and dietary intake between the respondents in high and low traffic areas.
2. There are significant differences in the concentrations of air pollutants inside respondents' classrooms and residences between high and low traffic areas.
3. There are significant differences in reported respiratory symptoms, lung function status, histone H3 level and DNAm status among the respondents between high and low traffic areas.
4. There are significant associations between TRAP exposure with respiratory symptoms, lung function status and epigenetic modifications assessed in the study.
5. TRAP exposure could be the main factor influencing respiratory health and epigenetic modifications among children in response to TRAP-induced systemic inflammation after controlling the confounders.

1.7 Definition of Variables

1.7.1 Conceptual Definitions

1.7.1.1 Traffic-Related Air Pollution (TRAP) Zone

The TRAP zone is described as 500 m on either side of highways with Average Daily Traffic (ADT) of ≥18,000 vehicles, or 100 m on either side of major urban roads with ADT of ≥15,000 vehicles, at least two lanes covering several km, a speed limit of more than 50 km/h (Brauer, Reynolds, & Hystad, 2013). Traffic pollutants travel, but exposures to TRAP are the highest near highways and busy roads.

1.7.1.2 PM_{10}

PM_{10} is a coarse particle with an aerodynamic diameter of 10 μm or less (0.0004 inches or one-seventh the width of a human hair) (United States EPA, 2021). PM_{10} is primarily accumulated on the trachea (United States EPA, 2021).

1.7.1.3 $PM_{2.5}$

$PM_{2.5}$ is a fine particle with an aerodynamic diameter of 2.5 μm or less (approximately one-thirtieth the average width of a human hair) (United States EPA, 2021). $PM_{2.5}$ can penetrate the bronchioles and alveoli (United States EPA, 2021).

1.7.1.4 PM_1

PM_1 is a respirable PM with an aerodynamic diameter of 1 μm or less (United States EPA, 2021). PM_1 may even pass through the bloodstream (United States EPA, 2021).

1.7.1.5 Black Carbon (BC)

BC is the primary light-absorbing element of particulate matter and is generated by the incomplete burning of biofuels, fossil fuels, and biomass (Liu et al., 2016). BC is emitted directly into the atmosphere in the form of fine particles. Its concentrations are usually higher at adjacent sources of emissions and heterogenous because BC has a short lifetime of about one week (Kholod & Evans, 2016).

1.7.1.6 Nitrogen Dioxide (NO_2)

NO_2 is a molecule in the nitrogen oxides (NO_X) group (United States EPA, 2016b). The gas signifies much NO_X that gets into the atmosphere from the fuel-burning of power plants and off-road machinery.

1.7.1.7 Sulphur Dioxide (SO_2)

SO_2 is formed from the combustion of fossil fuels containing sulphur and is one of the significant polluting agents in the atmosphere (United States EPA, 2019). This colourless gas is easily soluble in water and is the determinant for the larger gaseous sulphur oxides (SO_X) group. SO_2 oxidation, primarily at the particulate surface, allows sulphurous and sulphuric acids to form in the presence of metallic catalysts.

1.7.1.8 Carbon Monoxide (CO)

CO is a colourless and odourless gas that can be damaging if inhaled in large quantities (United States EPA, 2016a). It is the product of the incomplete combustion of organic compounds such as petrol and diesel from engines or equipment that burn fossil fuels.

1.7.1.9 Ozone (O_3)

O_3 is a toxic light blue gas with an ordinary smell detected in environments where strong ultraviolet lights are present, and oxygen (O_2) is converted to O_3 (National Center for Biotechnology Information, 2019). As sunlight and air pollution create conditions suitable for reactions that create O_3, the community in polluted areas has more O_3 exposure during windless and dry afternoons.

1.7.1.10 Total Volatile Organic Compounds (TVOC)

TVOCs are a wide variety of organic chemical compounds present in the atmosphere (United States EPA, 2017). They are of great concern due to their adverse effects on human health, as some chemicals can induce cancer directly and are associated with increased long-term health risks due to their carcinogenic and toxic properties.

1.7.1.11 Carbon Dioxide (CO_2)

CO_2 levels determine the amount of ventilation inside buildings. The maximum limit for CO_2 indoors is 1000 ppm, which should not be surpassed at any time (DOSH Malaysia, 2020). Any reading that exceeds the ceiling limit reflects poor ventilation.

1.7.1.12 Air Temperature

Air temperature is one of the IAQ physical parameters. The acceptable range for indoor air temperature is between 23 – 26°C (DOSH Malaysia, 2020).

1.7.1.13 Relative Humidity (RH)

RH is one of the IAQ physical parameters. The acceptable range for indoor RH is between 40 – 70% (DOSH Malaysia, 2020).

1.7.1.14 Air Velocity

Air velocity is an indicator of ventilation indoors. It involves providing or removing air from space to control air pollutant levels, humidity, or temperature within the space. The acceptable range for indoor air velocity is between 0.15 – 0.50 m/s (DOSH Malaysia, 2020).

1.7.1.15 Respiratory Health Symptoms

Respiratory health symptom is a broad term that can refer to a series of conditions that affect the human body's respiratory system (Zimmermann, 2019). Several respiratory diseases can affect humans at different severity levels (Kim, Chen, Zhou, & Huang, 2018). Some of the symptoms last only for a few days, with no medical treatment needed. Meanwhile, some of the symptoms may prolong without appropriate medical treatment.

1.7.1.16 Lung Function Test

A lung function test is applied to assess how well the lungs work and how efficient the lungs can carry oxygen to the rest of the body (Graham et al., 2019). Forced vital capacity (FVC) measurement shows the amount of air a person can forcefully and quickly exhale after taking a deep breath (American Thoracic Society, 2019). Meanwhile, FEV_1 measurement shows the amount of air a

person can forcefully exhale within one second of the FVC test (American Thoracic Society, 2019). FEV_1/FVC measurement is vital to figure out obstructive airways lung diseases.

1.7.1.17 Histone H3 Modification

Histone modifications such as histone H3 modification have been known as epigenetic modifiers. Histone proteins, around which DNA is wrapped, can be chemically modified and alter chromatin structure by recruiting histone modifiers (Ohguchi, Hideshima, & Anderson, 2018). The modifiers are attached to the N-terminal tails of histones.

1.7.1.18 DNA Methylation (DNAm)

DNAm usually governs gene expression via DNA transcription inhibition and silencing DNA, repetitive sequences and transposons (Lin et al., 2016). Decreased DNAm of repetitive elements, associated inflammation and cellular stress have been implicated in health conditions such as respiratory diseases (Ferrari et al., 2019).

1.7.2 Operational Definitions

1.7.2.1 Primary Schools near a TRAP Zone

Primary schools located within TRAP Zone were chosen as high traffic (HT) group, whereas primary schools in low traffic (LT) group were decided from the location at a distance of more than 5 km away from nearby highways, major roadways and industrial sites in Selangor.

1.7.2.2 Particulate Matter

PM_{10}, $PM_{2.5}$ and PM_1 in schools were measured using DustTrak DRX Aerosol Monitor in the unit of $\mu g/m^3$ for 6 h, based on the concept of light scattering. This instrument can recognise aerosol concentration ranges from 0.001 to 150 mg/m^3. As a result, it can concurrently measure both size fraction and mass concentration of PM_{10}, $PM_{2.5}$ and PM_1. The cut-point in bivariate analyses was 97.5 $\mu g/m^3$, 74.0 $\mu g/m^3$ and 63.0 $\mu g/m^3$ for PM_{10}, $PM_{2.5}$ and PM_1, respectively. Meanwhile, PM_{10} and $PM_{2.5}$ in residences were measured gravimetrically in the unit of $\mu g/m^3$ by using an Escort Personal Sampling Pump for 24 h, with samples on a mixed cellulose ester membrane (MCE) filter paper. The cut-point in bivariate analyses was 81.7 $\mu g/m^3$ and 65.4 $\mu g/m^3$ for residential PM_{10} and $PM_{2.5}$, respectively.

1.7.2.3 Black Carbon (BC)

BC in $PM_{2.5}$ was measured gravimetrically by using a low volume sampler in the unit of $\mu g/m^3$ for 24 h, with samples on a quartz microfiber filter paper. Then, these samples were evaluated by using a smoke stain reflectometer. The blackness of a sample correlates to BC composition after calculating absorbed light spots all over the filter paper. The cut-point in bivariate analyses was 28.0 $\mu g/m^3$.

1.7.2.4 NO_2, SO_2, CO and O_3

NO_2, SO_2, CO and O_3 were determined in the unit of parts per billion (ppb) using a portable gas sensor, Aeroqual S500, which quantifies air pollutants in real-time precisely. Various gases can be measured interchangeably using the same body because the sensor head could be easily removed and replaced. Interchangeable sensors were affixed to the monitor base. Each sensor head was set up with active sampling fan that reinforces the measurement accuracy and assures a representative sample. The cut-point in bivariate analyses was 74.7 ppb, 64.5 ppb, 300 ppb and 43.5 ppb for NO_2, SO_2, CO and O_3, respectively.

1.7.2.5 TVOC

TVOC concentrations were measured using a handheld gas monitor, which is specially designed for VOC. It has a Photoionisation Detector (PID) that yields immediate detection and readings for gases, varing from 1 ppb up to 10,000 ppb. The cut-point in bivariate analyses was 210 ppb.

1.7.2.6 CO_2

CO_2 was determined in the unit of parts per million (ppm) using Q-Trak IAQ Monitor. The detection range was from 0 – 5,000 ppm, with an accuracy of ±3% of reading or ±50 ppm CO_2. This equipment used a non-dispersive infrared sensor to detect the concentration of CO_2.

1.7.2.7 Air Temperature

The air temperature was measured in the degree of Celsius (°C) using Q-Trak IAQ Monitor. The range of detection was from -10 – 60 °C, with an accuracy of ±0.5 °C. This equipment used a thermistor sensor to measure the air temperature.

1.7.2.8 RH

RH was measured in percentage (% RH) using the Q-Trak IAQ Monitor. The detection range was from 5 – 95% RH, with an accuracy of ±3% RH. This equipment used a thin-film capacitive sensor to detect the humidity.

1.7.2.9 Air Velocity

The type of ventilation system in selected primary schools was identified and observed through a site visit. The air velocity was measured in metre per second (m/s) using the VelociCalc Multifunction Ventilation Meter. The detection range was from 0 – 50 m/s, with an accuracy of ±3% of reading or ±0.015 m/s. This equipment used a thermal sensor of a pitot tube.

1.7.2.10 Respiratory Symptoms

Respiratory symptoms were detected from questionnaires adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) and the American Thoracic Society (ATS). These questionnaires assessed the distinctions within the prevalence of respiratory symptoms at the population level and their causes by questioning parents or legal guardians. In this study, the respiratory symptoms of interest were cough, phlegm, wheezing and chest tightness.

1.7.2.11 Lung Function Test

Lung function tests, specifically spirometry tests, were done using a spirometer. Respondents breathed multiple times, with regular and maximal effort, through a tube that was connected to a spirometer. A computerised sensor calculates and graphs the results. FEV_1% was determined by dividing FEV_1 of the respondent from the spirometer with the predicted FEV_1. In contrast, the FVC% was determined by dividing the FVC of the respondent with the predicted FVC. FEV_1/FVC ratio was determined by dividing FEV_1 with FVC. All measurements were expressed in litres. In this study, the predicted values applied the reference values reported by Azizi & Henry (1994) for the spirometry test among Malay children in Malaysia.

1.7.2.12 Histone H3 Modification

The level of histone H3 modification in the saliva samples was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit for circulating total histone H3 modification after extraction. This assay showed whether a population of cells

had changed its histone-modification profile in response to some exposure. The ELISA kit had high sensitivity with a detection limit as low as 2 ng/well in a dynamic range from 5 - 200 ng/well of the saliva. Moreover, the kit had high specificity for detecting total histone H3 modification whereby each histone H3 modified at specific sites was captured by an antibody coated on the strip wells and specifically targeted the appropriate histone modification pattern. The level of histone H3 modification in the saliva samples was determined by comparing the optical density (OD) of the samples against the standard curve.

1.7.2.13 DNAm

The level of DNAm in the saliva samples was analysed by Methylation-Specific Polymerase Chain Reaction (MS-PCR) on bisulphite-treated DNA after extraction. Two independent primer sets were used for Polymerase Chain Reaction (PCR) amplification; one pair was designed to recognise the methylated sample and the other pair for the unmethylated versions of the bisulphite-modified sequence. The amplicons were visualised using novel juice staining following agarose gel electrophoresis. Amplicons of the expected size produced from either primer pair were indicative of the presence of DNA in the original sample with its respective methylation status. Because MS-PCR, as described here, was nonquantitative, these results provide a "present-or-absent" insight into methylation

CHAPTER 2

LITERATURE REVIEW

2.1 Traffic-Related Air Pollution (TRAP)

A high density of the human population in most large cities leads to population growth, leading to more highways being built and increased vehicle use, which later produces a higher concentration of air pollutants than less-developed areas. Several megacities with more than 10 million population, such as Jakarta, Beijing and New Delhi, have recorded air pollutants approaching dangerous levels (Haque & Singh, 2017; Haryanto, 2018). Fuel combustion has been identified as a key air pollution source in urban settings, which tends to increase with population size and economic activities, and traffic emission is one significant contributor (Haque & Singh, 2017; Haryanto, 2020). Improvement in vehicle engine technology and fuel quality has been fruitful in tackling TRAP issues worldwide. Nevertheless, such solutions are not widely applied in developing regions, including Malaysia. Malaysia has already implemented Euro 4 for RON 95 and RON 97 gasoline since 2020 and plans to introduce Euro 5 in 2025 (Hirota & Kashima, 2020). Implementation of the new fuel standard is in line with the government's goal to reduce air pollution and its impact on human's health by increasing fuel efficiency.

Besides, meteorological conditions also affect the dispersion of TRAP to the neighbouring regions. Table 2.1 shows previous research on TRAP in Southeast Asian countries, which have similar weather and climate patterns to Malaysia. These countries are all influenced by two monsoons, leading to hot and dry seasons in a year (Sulong et al., 2019).

Table 2.1: Previous research on TRAP in Southeast Asia

Locations	Findings	Authors
Metro Manila, Philippines	• For the 1-year monitoring, O_3 (71 ppb) and NO_2 (32 ppb) had the highest concentrations observed at Luneta Park, while $PM_{2.5}$ (70 $\mu g/m^3$) recorded was the highest at Luneta and Arroceros Park. • Luneta Park is mostly surrounded by major roads compared to other urban parks in the study.	Galvez et al. (2020)
Central Business District, Singapore	• Strikingly high concentrations of $PM_{2.5}$ (36.4 ± 12.5 $\mu g/ m^3$) and BC (18.1 ± 12.0 $\mu g/m^3$) were recorded at a bus stop during the 1-h personal monitoring.	Tran et al. (2020)

Table 2.1: Continued

Locations	Findings	Authors
Bangkok, Thailand	• For the 1-year monitoring, a spatial-wise average of $PM_{2.5}$ (33.6 μg/m^3) and PM_{10} (53.4 μg/m^3) were recorded. • Several busy roads and intersections were recognised as the TRAP source areas.	Kanchanasuta et al. (2020)
West Jakarta, Indonesia	• The monthly variation of Suspended Particulate Matter (SPM) revealed that Glodok had a higher average SPM concentration than Ancol, and the highest concentration was observed in June (512 μg/m^3). • Heavily-travelled roads mostly surround Glodok compared to Ancol, which is located in a coastal area and not as busy as Glodok.	Kusumaningtyas et al. (2018)

2.1.1 TRAP in Klang Valley

Klang Valley is a rapidly growing urban area with the highest growth rate in Malaysia when it became the dominant economic region in Malaysia since the late 20th century. This status, however, has considerably reduced the air quality in this vicinity due to the pollutants released from active commercial and industrial development, heavy traffic flow of motor vehicles and transboundary haze (Amil, Latif, Khan, & Mohamad, 2016; Sulong et al., 2017). With a population of 32.6 million people (DOS Malaysia, 2019), Malaysia has the highest population density of 7,328 people per square km in Kuala Lumpur (DOS Malaysia, 2019), which is a primary concern for TRAP in Klang Valley.

The cause of air pollution in Klang Valley is primarily from local anthropogenic activities such as vehicles' emission (Mohamad Fandi, Wan Mansor, & Jalaludin, 2018; Sulong et al., 2017) during non-haze periods. Traffic emissions were reported to be emitted from both vehicles and road dust and accounted for 22.4% of the total $PM_{2.5}$ mass, also the second largest contributor to $PM_{2.5}$ in Kuala Lumpur (Sulong et al., 2017). Moreover, the extreme reduction of NO_2 during the Movement Control Order (MCO) in Kuala Lumpur (Abdullah et al., 2020) has proven that TRAP contributes the most to air pollution in the urban areas in Malaysia, particularly Klang Valley. Motor vehicle ownership has increased significantly every year, with Malaysia's economic boost (Chu, Law, Hamid, Law, & Lee, 2019). Malaysian vehicle registration data up to the second quarter of 2019 recorded that the total number of vehicles on the roads have reached 30 million units, and these numbers are increasing continually (Bernama, 2019).

In 2017, motor vehicles were the primary source attributed to the emission of air pollutants (70.4%) with 2.3 million tonnes (DOS Malaysia, 2018b). From these emission figures of motor vehicles, 2.0 million tonnes were contributed by CO, followed by 0.22 million tonnes by NO_2, 14.3 thousand tonnes by SO_2 and 3.9

thousand tonnes by particulate matter (DOS Malaysia, 2018b). Moreover, Batu Muda station in Kuala Lumpur recorded 15 days, the highest number of days for air quality to be categorised as unhealthy in Malaysia in 2017, with the Air Pollutant Index (API) valued between 101 – 200 (DOS Malaysia, 2018b).

There exists an extensive literature on the topic of TRAP. Table 2.2 compiles previous research on TRAP in Klang Valley. The high number of vehicles, particularly private motorcycles and cars on the road, had contributed to the highest PM_{10} concentrations in Kuala Lumpur during peak hours between 7 a.m. to 9 a.m. and 5 p.m. to 8 p.m. (Azhari et al., 2018). On the whole, air pollution sources in Klang Valley are mostly influenced and defined by vehicular emissions. The increasing numbers of buildings in the urban areas have sealed these pollutants and restricting them from escaping the urban areas; hence, forming the urban heat island of Klang Valley.

Table 2.2: Previous research on TRAP in Klang Valley

Study Descriptions	Findings	Authors
Relationship between the composition of air pollutants and road traffic volume in an industrial environment, in which the authors chose two sampling stations at 1 m and 100 m from the road-side.	• The concentrations of PM_{10} and CO at the sampling point of 1 m from the road-side were significantly higher than at the sampling point of 100 m from the road-side, while O_3 concentrations were observed to show the opposite results. • When comparing working and non-working days, the levels of PM_{10}, CO and SO_2 were significantly higher on a working day compared to a non-working day ($p \leq 0.05$). • The concentration of pollutants increased as the number of vehicles increased and decreased as the number of vehicles decreased. • The results were confirmed by the data recorded for PM_{10}, CO, NO_2, SO_2 and O_3 from a Continuous Air Quality Monitoring (CAQM) Station nearby, which showed diurnal pattern during peak hours when vehicles utilised the roads to the maximum. • The authors concluded that the concentrations of pollutants were related to vehicular traffic emissions, even though the study sites were located in an industrial area.	Azhari et al. (2018)
Sampling was taken on the roof of the Malaysian Meteorological Department (MET) located in Petaling Jaya to represent Klang Valley on the western side of the Malaysian Peninsular.	• $PM_{2.5}$ is substantial as an air pollutant in the ambient air of Petaling Jaya, which is a hotspot for heavy traffic in the Klang Valley. • The average $PM_{2.5}$ mass was 28 ± 18 $\mu g/m^3$, almost three times (2.8-fold) the amount stipulated within the annual WHO guideline. • Klang Valley recorded higher results than other parts of Peninsular Malaysia but showed lower results than many large Asian cities. • $PM_{2.5}$ daily mass ranged between 6 and 118 $\mu g/m^3$ with a 43% exceedance of the daily WHO guideline, while $PM_{2.5}$ weekend results were recorded at a lower mass (26 $\mu g/m^3$) compared to weekdays (29 $\mu g/m^3$).	Amil et al. (2016)

2.1.2 Children as A Population at Risk for Exposure to TRAP

There is no universally agreed age range for the characterisation of children. According to WHO, a child is a person who is 19 years old or younger, unless a country's national law considers a person to be an adult at an earlier age (WHO, 2013). Children make up 9.4 million people in Malaysia, which is about 29% of the total population (DOS Malaysia, 2018a). Living within 100 m of a major road or within 500 m of a highway is considered within the TRAP exposure area (United States EPA, 2015) and is associated with the onset and worsening of asthma in children and other adverse health outcomes. Motor vehicle traffic is the largest air pollution source emitted in Klang Valley, with TRAP exposures being the highest near highways and busy roads. Exposure to TRAP pollution over short periods can aggravate respiratory diseases, leading to respiratory symptoms such as coughing, wheezing, and breathing difficulty.

Many studies have examined the subjects exposed to real traffic conditions, such as a busy road, which justified the premise that vulnerable populations could be more susceptible to detrimental health effects from such exposure (Abdul Rahman, Ismail, Sahani, Firuz, & Latif, 2017; Abdul Wahab, Razak, Sahani, & Khan, 2020; Latif et al., 2019). In line with this finding, healthy individuals would also experience mild acute inflammatory responses from TRAP exposure (Awang et al., 2019; Tajudin et al., 2019). Moreover, there has been sufficient evidence to conclude that the populations living or working within 300 to 500 m from major roads, or those spending a significant amount of time in traffic such as taxi, bus or lorry drivers, commuters and school children, were potentially at the extreme health risk (American Lung Association, 2020).

2.1.3 Guidelines and Government Policies

Environmental concern among the public has raised awareness about environmental and air quality issues and is a power that drives regulatory forces. In order to replace the old Malaysia Ambient Air Quality Guidelines (MAAQG), which has been applied since 1989, a new Malaysia Ambient Air Quality Standard (MAAQS) was established in 2015. The new standard has adopted six air pollutant criteria that include five existing air pollutants which are PM_{10}, SO_2, NO_2, O_3, and CO, with the addition of $PM_{2.5}$. These standards are then revised in 2018 with Interim Target 2 (IT-2) (DOE Malaysia, 2020). The average concentration of these pollutants is used for calculating API values. The API value is determined by the air pollutant found at the highest concentration (dominant pollutant). Table 2.3 shows the standards comparison of air pollutants exposure between MAAQS, US EPA and WHO. Malaysia is undergoing stricter air quality guidelines in stages to improve the local air quality.

Table 2.3: Comparison of ambient air quality guidelines

Pollutants	IT-2 of MAAQS (2018)	US EPA	WHO
PM_{10}	1 Year: 45 $\mu g/m^3$ 24 Hours: 120 $\mu g/m^3$	24 Hours: 150 $\mu g/m^3$	1 Year: 20 $\mu g/m^3$ 24 Hours: 50 $\mu g/m^3$
$PM_{2.5}$	1 Year: 25 $\mu g/m^3$ 24 Hours: 50 $\mu g/m^3$	1 Year: 15 $\mu g/m^3$ 24 Hours: 35 $\mu g/m^3$	1 Year: 10 $\mu g/m^3$ 24 Hours: 25 $\mu g/m^3$
NO_2	24 Hours: 300 $\mu g/m^3$ 1 Hour: 75 $\mu g/m^3$	1 Year: 53 ppb 1 Hour: 100 ppb	1 Year: 40 $\mu g/m^3$ 1 Hour: 200 $\mu g/m^3$
SO_2	24 Hours: 300 $\mu g/m^3$ 1 Hour: 90 $\mu g/m^3$	3 Hours: 0.5 ppm 1 Hour: 75 ppb	24 Hours: 20 $\mu g/m^3$ 10 min: 500 $\mu g/m^3$
O_3	8 Hours: 200 $\mu g/m^3$ 1 Hour: 120 $\mu g/m^3$	8 Hours: 0.070 ppm	8 Hours: 100 $\mu g/m^3$
CO	8 Hours: 35 mg/m^3 1 Hour: 10 mg/m^3	8 Hours: 9 ppm 1 Hour: 35 ppm	

Besides, there are several relevant government policies related to air pollution and health impacts, as shown in Table 2.4. These policies are designed to deal with environmental concerns, especially with the increasing evidence on the health effects of exposure to air pollution (Abdullah, 2020; Othman & Latif, 2020). The success of environmental management and protection does not depend on these policies only, but also the support from Malaysian citizens and other occupants in Malaysia. However, a local study reported that the community perceived that they have less control in reducing environmental issues, although they are aware and fully support the government's movement (Chin, De Pretto, Thuppil, & Ashfold, 2019).

Table 2.4: Relevant government policies

Policies	Agencies	Descriptions
National Environmental Health Action Plan (NEHAP)	Engineering Services Division, MOH Malaysia	Air Quality is one of the critical environmental health areas of concern based on the Regional Initiative on Environment and Health in Southeast and East Asian countries.
National Clean Air Action Plan (NCAAP)	Department of Environment (DOE), Ministry of Environment and Water, Malaysia	• Developing NCAAP improves air quality in urban areas by reducing emission from motor vehicles and increasing public awareness. • To establish a baseline database for developing the strategic model and action plan on air quality management, specifically on TRAP, which can provide guidelines for the government and stakeholders in implementing pollution control measures.

Table 2.4: Continued

Policies	Agencies	Descriptions
National Policy on the Environment	DOE, Ministry of Environment and Water, Malaysia	Addressing issues in line with Core Strategy No.3 in National Policy on the Environment: Ensure continuous improvement in the productivity and quality of the environment while pursuing economic growth and human development objectives.
National Strategic Plan for Non-Communicable Disease	Department of Public Health, MOH Malaysia	Providing a roadmap for all relevant stakeholders in Malaysia to reduce the preventable and avoidable burden of morbidity and disability due to non-communicable diseases through multi-sectoral collaboration and cooperation at national and state levels.

2.2 Monitoring Exposure to Air Pollution in Schools and Residences

There are three categories for measuring TRAP, which are optical sensors, media-based and continuous monitoring. Each has its advantages and disadvantages. Some provide researchers with direct readings, while some require laboratory analysis. In recent years, air pollution monitoring has shifted from being based on the costly air quality monitoring networks operated by the government to various systems comprising reference-grade technologies and air monitoring sensors that are more affordable to wider groups of users. New air quality monitoring technologies with increased spatial and temporal resolution include low-cost and remote-sensing technologies. Besides that, calculations of motor-vehicle emissions are crucial when evaluating their impact on local air quality and traffic-related exposures; these also involve collecting the data of travel-activity over space and time, along with the development of emissions inventories (Neves & Brand, 2019).

Air pollution monitoring in schools and residences has accelerated in the past few years, and plentiful monitoring approaches have been implemented. Table 2.5 summarises several measurements of air pollutants by previous local studies. The methods used to monitor air pollution were chosen according to project goals and monitoring conditions. These studies have examined and reported sensor performance under a series of distinct circumstances, and the performance was deduced to be satisfactory. Local air monitoring studies were conducted in schools and preschools, which utilised sensor-based method to measure particles over several hours a day (Jalaludin, Syed Noh, Suhaimi, & Md Akim, 2014; Kamaruddin et al., 2019; Mohd Nor Rawi, Jalaludin, & Chua, 2015; Othman, Latif, & Matsumi, 2019; Yang Razali et al., 2015). Contrarily, air monitoring in residences for 24 h was performed using a media-based method (Fadzir & Jalaludin, 2013; Kamaruddin et al., 2019).

Table 2.5: Compilation of measurements of air pollutants by previous research

Sampling Sites	Monitoring Methods	Findings	Authors
• 5 primary schools in Kemaman, Malaysia. • 162 residences. • Local background activities include residential areas and industrial complexes.	Indoor PM_{10} and $PM_{2.5}$ in schools • Sensor-based. • Used an aerosol monitor (DustTrak DRX 8534) by TSI. • 6-hour monitoring. Indoor PM_{10} and $PM_{2.5}$ in residences • Media-based. • Used personal air sampling pumps (Gilian 5000) by Sensidyne. • Filter papers used were PVC and MCE. • 24-hour monitoring. Indoor NO_2 and SO_2 in schools and residences • Media-based. • Used air sampling pumps (BD 1949) by LaMotte. • 6-hour monitoring. Indoor VOCs in schools and residences • Sensor-based. • Used a VOC monitor (PpbRAE 3000) by RAE Systems. • 6-hour monitoring. Indoor CO, CO_2, temperature and RH in schools • Sensor-based. • Used an IAQ monitor (Q-Trak Plus 8554) by TSI. • 6-hour monitoring. • The equipment was placed at the height of 0.6 to 1.5 m above the floor to simulate the location of a breathing zone and was not closer than 1 m to a door, window and wall. • Whenever possible, all the equipment were placed at the back of the classroom to ensure no sound disruption from equipment during learning sessions.	• The exposure of the exposed group from the concentrations of $PM_{2.5}$, NO_2, and SO_2 in schools and residences was higher than the comparative group and the Malaysian ambient standard. • Both VOC concentrations in the exposed and comparative areas were within the recommended levels. • Air pollutants in schools and residences were influenced by different activities, indoors or outdoors.	Kamaruddin et al. (2019)

Table 2.5: Continued

Sampling Sites	Monitoring Methods	• Findings	Authors
• Primary schools in Kuala Lumpur, Malaysia. • Local background activities include traffic sources and residential estates.	$PM_{2.5}$ • Sensor-based. • Used $PM_{2.5}$ sensors developed by Nakayama et al. (2018). • The sensor for indoor monitoring was placed at 1 m above the floor in the middle of the classroom, while the sensor for outdoor monitoring was placed in the corridor. • The average $PM_{2.5}$ concentrations collected for 8 h (7.30 a.m. – 3.30 p.m.) and 24 h (midnight to midnight) were determined from 1 min data intervals. • The classroom located on the first floor of the school building that faced the main road was chosen for $PM_{2.5}$ measurement.	• There were similar trends of $PM_{2.5}$ concentration between the indoor and outdoor measurements. • Indoor concentrations for 24 h and 8 h sampling were somewhat higher during weekdays. • $PM_{2.5}$ concentration abruptly elevated at 10.00 a.m. when the school children walked around the classroom and left for recess. Then, when the children returned to the classroom after recess, the highest $PM_{2.5}$ concentration was recorded at 10.30 a.m. A swift reduction in $PM_{2.5}$ concentration was observed after recess time when teaching continued. Then, the $PM_{2.5}$ concentration slowly reduced until 1.30 p.m., when school time ended. • Indoor and outdoor $PM_{2.5}$ concentrations measured on the weekends showed higher concentrations for the 24 h sampling duration than weekdays, whereas not much disparity was observed between indoor and outdoor monitoring for an 8 h sampling.	Othman et al. (2019)

Table 2.5: Continued

Sampling Sites	Monitoring Methods	• Findings	Authors
• 1 secondary school in Putrajaya and 2 secondary schools in Bandar Baru Bangi, Malaysia. • Local background activities include residential estates and construction sites.	CO, CO_2, temperature and RH: • Sensor-based. • Used a particulate matter monitor (IQ-410 IAQ Probe) by GrayWolf Sensing Solutions. PM_{10}, $PM_{2.5}$ and PM_1 • Sensor-based. • Used an aerosol spectrometer (model 1.108 with a flow rate of 1.2 L/min) by Grimm Technologies. • Instruments were fixed at the centre of the classroom at a height of 1 m from the floor to tailor the average height at which students breathe. In contrast, outdoor air quality instruments were placed near the main entrance gate of the school at the same height as the height of IAQ instruments. • All measuring instruments were set to measure a reading of 1 min interval for 8 h. • One classroom from each school was selected for measurement from 7.30 a.m. to 1.30 p.m. for two days.	• Several factors affected the particulate matter concentration in the classroom: the floor level of the classroom, the number of occupants in the classrooms, the classroom conditions and cleanliness. • The average concentrations of PM_{10}, $PM_{2.5}$ and PM_1 measured in the classrooms did not inevitably adhere to PM_{10}, $PM_{2.5}$ and PM_1 outside the school buildings as outdoor air pollutants have minimal impact on the indoor air pollutants in the classroom.	Yang Razali et al. (2015)

Table 2.5: Continued

Sampling Sites	Monitoring Methods	• Findings	Authors
• 4 preschools in Balakong and Bangi, Selangor. • Local background activities include residential areas and traffic sources.	PM_{10} and $PM_{2.5}$ • Sensor-based. • Used an aerosol monitor (DustTrak 8520) by TSI. VOCs • Sensor-based. • Used a VOC monitor (PbbRAE 3000) by RAE Systems. CO, CO_2, temperature and RH • Sensor-based. • Used an IAQ monitor (Q-Trak Plus 8554) by TSI. Air Velocity • Sensor-based. • Used an IAQ monitor (VelociCalc Plus 8386) by TSI. • Instruments were installed at the height of about 0.6 - 1.5 m above the floor, roughly at the children's breathing zone level. The chosen site was not nearer than 1 m to a wall, a door, or an active heating system. • The air monitoring was performed for at least 3- to 4-h periods during regular preschool activities.	• The highest concentrations of $PM_{2.5}$ (94 $\mu g/m^3$) and PM_{10} (131 $\mu g/m^3$) were reported in Classroom A, which was in the exposed location. • The highest VOCs (0.12 ppm) was recorded in Classroom F, and the highest CO_2 (1073 ppm) was found in Classroom C. • Classroom D measured the highest CO, with a concentration of 2.60 ppm. • T, RH, and air velocity were revealed at higher levels within preschools in the exposed area than preschools in the comparative area. • Unlike preschools in the comparative area, both preschools in the studied area were located nearby busy roads. Heavy traffic and vehicle fossil fuel combustion might have contributed to high particulate matter levels in the exposed areas.	Mohd Nor Rawi et al. (2015)

Table 2.5: Continued

Sampling Sites	Monitoring Methods	• Findings	Authors
• 3 primary schools in Sri Petaling, Bandar Tun Razak, and Beranang, Malaysia. • Local background activities include residential estates, agricultural activities and traffic sources.	PM_{10} and $PM_{2.5}$ • Sensor-based. • Used an aerosol monitor (DustTrak) by TSI. NO_2 • Media-based. • Used air sampling pumps (BD 1949) by LaMotte. • The measurement of indoor air pollutants was conducted in the classrooms during the school period of 5 h depending on the school session (morning or evening). • Both instruments were placed on desks at 1.5 m above floor level at the back of the classrooms and located at least 0.5 m away from the children.	• The particles penetrated from the outside could have accumulated inside and settled down on the curtains, shelves and ceiling fans. • $PM_{2.5}$, together with NO_2 trends, seems to be the peak in the morning and afternoon sessions due to traffic congestion of school buses, which led to the higher amount of fine particles and NO_2 in ambient pollution. • The selected schools in the urban area were situated near the main roads (approximately 50 to 100 m), which allowed the traffic emission pollutants to be scattered inside the classrooms.	Jalaludin et al. (2014)
• 108 homes in Cheras, Malaysia. • Local background activities include residential estates and traffic sources.	Indoor $PM_{2.5}$ in residences • Media-based. • Used personal air sampling pumps (Gilian 5000) by Sensidyne. • Filter papers used were MCE. • 24-hour monitoring. • Traffic counts were used to differentiate between busy and less busy roads. • The cyclone attached to the instrument was put at the height of the respondents' breathing area. In contrast, the instrument itself was positioned at the area of everyday routine activities performed by children inside the house to measure $PM_{2.5}$ indoors.	• There were significant differences in indoor $PM_{2.5}$ concentrations between residences near busy roads and residences near less busy roads. • Children living near busy roads have significantly higher indoor $PM_{2.5}$ concentrations than those living near less busy roads.	Fadzir and Jalaludin (2013)

For example, DustTrak DRX 8534, as an optical sensor for particles measurement, employ a laser diode as the light source to illuminate particles (Wang et al., 2016). Then, the light scattered by the particles is measured by a photodetector (Wang et al., 2016). Unlike particulate matter sensors, the principle of gaseous sensor such as PpbRAE 3000 uses ultraviolet light as a sensing material to split the VOC into positive and negative ions (Spinelle, Gerboles, Kok, Persijn, & Sauerwald, 2017). Then, VOC concentration is measured by the charge of the ionised gas (Spinelle et al., 2017). This method has lower operating costs and takes a shorter time to get the results than the media-based method. However, issues have been raised for its lower sensitivity and precision; hence, it is deemed not appropriate for compliance monitoring (Morawska et al., 2018).

On the other hand, an example of the media-based method is when the air is drawn through a pre-weighed filter, and the filter collects the particles (Amil et al., 2016). After a pre-determined sampling period, the filter is reweighed to discover the concentration of the particles (Amil et al., 2016). For example, $PM_{2.5}$ collected in the filter can be analysed chemically for various $PM_{2.5}$-bound components such as heavy metals and Poly Aromatic Hydrocarbon (PAH). Nevertheless, this method is not sufficient for modelling functions because the concentrations are not captured in real-time and produce only average data within the period the filter was deployed (Wang et al., 2016).

2.3 Deposition of TRAP in Respiratory System

Respirable particles and gases can distress any of the different organs and the respiratory system, starting from the nose, finally the alveoli. More injury happens here because respiratory organs act as the first line of defence and the first body parts that encounter air pollutants. A figurative representation of the deposition of air pollutants in a child's respiratory system is shown in Figure 2.1. Although ambient air pollution triggers airway inflammation, measurement of the intensity of acute or chronic inflammation in children's airways is often difficult to be determined because some of the methods involved in assessing airway inflammation are invasive, for example, bronchoalveolar lavage and blood samples. Children are vulnerable, so we have a special obligation to ensure their safety when they become research subjects.

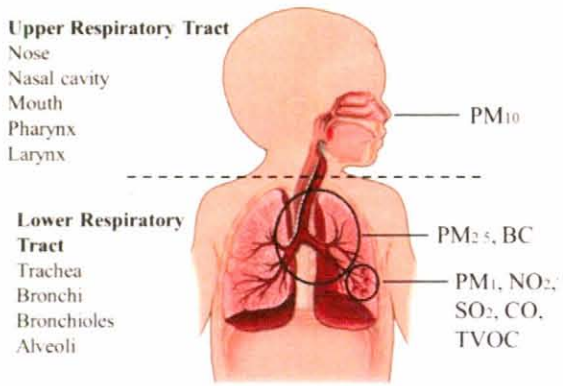


Figure 2.1: Diagrammatic representation of deposition of TRAP in the respiratory system of a child (adapted from Nucleus Medical Media, 2016)

TRAP have health effects even at low concentrations. No threshold has been recognised, below which no damage to health is observed. Several studies have shown that exposure to air pollution could cause a wide range of health effects from long-term exposure, including premature deaths in people with heart or lung disease, non-fatal heart attacks, abnormal heartbeat, worsened asthma, reduced lung function, as well as increased respiratory symptoms from short-term exposure, such as inflammation of the airways, coughing or breathing difficulty (Khan et al., 2019; Tan, Praveena, Abidin, & Cheema, 2018). These effects are contributed by exposure over the short term in hours or days and the long term in months or years.

2.4 Lung Function Test

2.4.1 Interpretation of Lung Function Test

ATS defines acceptable spirometry results. Reduction in the amount of FEV_1 may reflect the reduction in the lungs' maximum inflation, obstruction of the airways or respiratory muscle weakness (McCarthy, 2018). Lung function abnormality can be divided into two main categories, which are obstructive and restrictive. Airway obstruction is the most common cause of the reduction in FEV_1. Airflow obstruction may be secondary to bronchospasm, airway inflammation, loss of lung elastic recoil, increased secretions in the airway, or any combination of these causes (McCarthy, 2018). Respondents who have had problems with their lungs will have a lower airflow from expiration than normal lungs. This condition will usually happen when there is an airway constriction. Normal results for a spirometry test vary from person to person because they are based on age, height, race, and gender. The predicted normal value will be calculated before the test. Once the test has been done, the test score will be compared to the predicted value. The result is considered normal if the score is 80% or more of the predicted value (Sampson, 2017).

2.4.2 Lung Function Test as An Indicator of Respiratory Health

Lung function test has been widely used to study the association between respiratory health and exposure to TRAP. In a local study by Asrul & Juliana (2017), they performed a cross-sectional comparative study among 120 pre-schoolers aged 5 and 6 years old using study locations in Puchong (representing the urban area) and Hulu Langat (representing the suburban area). They found that FVC and FEV_1 among studied groups were significantly lower than the comparative group (t= -3.710, p<0.001) and (t= -4.027, p<0.001), respectively. Besides, FVC% (z= -2.866, p=0.004) and FEV_1% (z= -3.139, p=0.002) among studied groups were significantly lower compared to the comparative group, respectively. However, only the value of FEV_1/FVC% between the groups was not significantly different (z= -1.205, p=0.228). They concluded that the exposures to poor IAQ in the urban area might increase the risk of reducing lung function among the pre-schoolers.

In a different study by Rice et al. (2016), they investigated a cohort of children residing in Boston, USA and followed them from birth through to their childhood years (mean age 7.9 years). They reported that estimates of long-term exposure to ambient pollution, including proximity to a major roadway, BC and particulate matter, were associated with lower lung function. Proximity to the nearest major roadway at the time of the exam, past 365-day and lifetime estimates of BC and particulate matter exposure were all associated with lower FVC. As for the FEV_1, there were weaker associations for most exposures. Living <100 m from a major roadway was associated with a -71.6 ml (95% CI= -145.9, 1.8) lower FEV_1 and a -86.8 ml (95% CI= -164.9, -8.7) lower FVC, compared to living 400 m from a major roadway.

2.5 Epigenetics

2.5.1 Basics of Histone Modifications and DNA Methylation (DNAm)

Genes transcription depends on the transcription machinery that describes and examines proper regulatory zones within the gene, including promoter regions. As observed, in-depth, double-stranded DNA (dsDNA) in the nucleus is wrapped in increasingly intricate protein scaffolds, collectively known as chromatin (Gsell, Richly, Coin, & Naegeli, 2020). DNA is arranged twice around the protein core at its lowest level, forming a unit called a nucleosome consisting of 8 proteins, two copies of H2A, HB2, H3 and H4 histones (Ichikawa et al., 2017). Nucleosomes are then enveloped in increasing complexity to form chromosomes eventually, and this is where epigenetic modification takes place, which is described to occur at the nucleosome level (Schalch & Steiner, 2017).

Epigenetics determines developmental and cell-specific gene transcription, gene silencing and alteration of the transcription level of genes. Accurately timed

regulation of gene transcription is needed during normal development. Genes that are specific to a definite cell type and developmental stage are the only transcriptionally active genes, whereas the rests are silenced. Modifications are classified as active or repressive, depending on their occurrence in either active or silent genes (Bintu et al., 2016). The self-illustrated Figure 2.2 shows the organisational network of epigenetic components in the cell, including DNAm and histone modification. Different molecules can attach to histone tails, thus altering the DNA activity wrapped around them. Meanwhile, for DNA, marks that indicate methylation are added to certain DNA bases, which repress gene transcription.

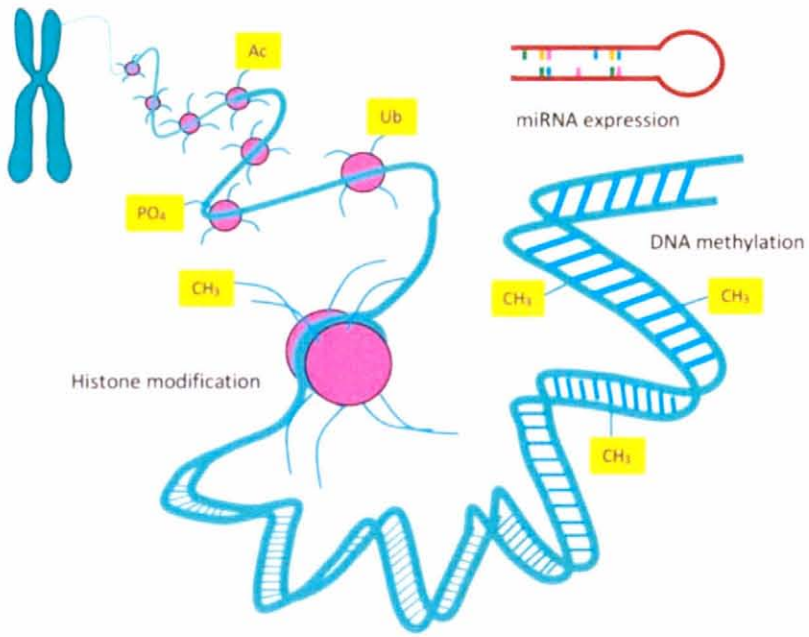


Figure 2.2: The organisational network of epigenetic components in the cell

2.5.2 Histone H3 Modification

Altered histone modifications are one aspect of respiratory epigenetics that may coordinate the early-life programming of immune T-cell response, dendritic cell function, macrophage activation, and rupture of the epithelial barrier in the airways that increases asthma risk and reduces lung function in a future life (Wright & Brunst, 2013). Histone H3 is a protein-coding gene that is arginine-rich (UniProt, 2020). Alterations to the histones change the normal regulation of DNA wrapped around them.

2.5.3 DNA Methylation (DNAm)

DNAm is the most broadly characterised epigenetic mechanism involved in the regulation of gene expression. DNAm contributes to silencing gene expression by adding a methyl (−CH3) group to cytosine to form 5-methyl-cytosine (5mC). 5mC controls gene activity in a heritable way without changing the primary DNA sequence in several biological processes across the evolutionary hierarchy (Chowdhury et al., 2017).

2.6 Associations between TRAP and Epigenetic Mechanisms

Human exposure studies depend upon the associations between health effects in humans and air pollutant inhalation compared with cell and animal models. As highlighted in Figure 2.3, the epigenetic field includes several mechanisms. Exposure to environmental pollution modulates the organism to become more susceptible to the respiratory effects of air pollution. The associated changes alter chromatin organisation and condensation, gene expression and eventually disease risks. The resulting detrimental health impacts include the generation of oxidative stress and inflammation (Baccarelli & Bollati, 2009).

Generally, there are limited epidemiological studies that examined the childhood stage, although such a phase being important in life. Several systematic reviews contribute a compilation of evidence of how air pollution can cause epigenetic modifications throughout several lifespan phases involving preconception to the elderly phase (Alfano et al., 2018; Shukla et al., 2019). Table 2.6 compiles previous studies that link TRAP with epigenetic mechanisms. Although their results' immediate pathological effects are currently ambiguous, their studies present some scientific direction and confirmation for subsequent air pollution studies concerning epigenetics. From these findings, it can be proposed that air pollutants can induce changes within the epigenome of an organism and cause long-lasting phenotypic modifications over generations (Rider & Carlsten, 2019; Sun et al., 2018).

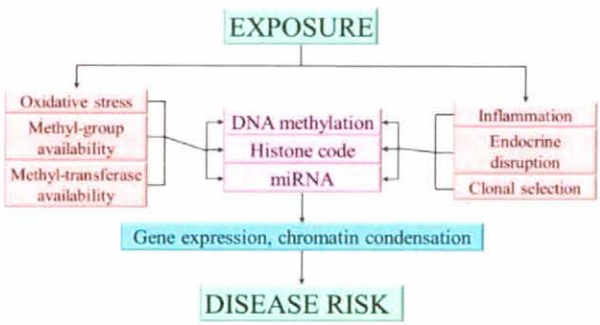


Figure 2.3: Potential mechanisms linking environmental exposures to epigenetic effects (adapted from Baccarelli & Bollati, 2009)

Table 2.6: Previous research that links TRAP with epigenetic mechanisms

Study Descriptions	Authors
Mechanism: Histone Modification **Subjects:** Repeated measures study on 60 lorry drivers and 60 office workers in Beijing within 14 days. **Findings:** • Each μg/m^3 increase in 14-day average ambient PM_{10} exposure was associated with lower H3K27me3 (β= −1.1%, 95% CI= −1.6, −0.6) and H3K36me3 levels (β= −0.8%, 95% CI= −1.4, −0.1) in all participants, which suggest the possible role of global histone H3 modification in the effects of traffic-derived particulate exposures. • H3K9ac and H3K9me3 were observed in office workers but not in truck drivers. These results could be due to the epigenetic 'ceiling' effect of TRAP exposure regardless of additional exposure to high concentrations. **Limitations:** Future studies of the epigenetic effects of PM_{10} should attempt to avoid cross-sectional designs in populations whose exposure to particulate matter could be highly variable over time, as such designs may be unable to account for the delayed effects of PM_{10} exposure. **Conclusion:** The authors had testified the first human study that established the possible role of global histone H3 modification in the effects of traffic-derived particulate matter exposures, particularly BC exposure.	Zheng et al. (2017)
Mechanism: Histone Modification **Subjects:** Rats in their study were placed in cages and exposed to three TRAP levels – high, moderate and low, at various locations in Zhejiang, China, during spring and autumn. **Findings:** • Rats exposed to TRAP for 7 days at both high and low exposure sites showed a dose-dependent increase of H3K9 acetylation levels. • H3K9 acetylation levels were still significantly higher than the control group, but there were no statistically significant differences between the rats exposed for 7, 14, or 28 days. • $PM_{2.5}$ and PM_{10} levels were positively associated with H3K9 acetylation levels in both lung tissues and PBMCs when the rats were exposed for 7 days. However, there was no relationship between H3K9 acetylation levels and NO_2 levels. **Limitations:** The study only focused on describing the effects of TRAP on the epigenetic marks in both rat blood and lung tissues, while the consequences of such changes were not investigated. **Conclusion:** The authors uncovered an association between inhaling diesel exhaust fumes from TRAP and a notable increase in epigenetic marks found on histone proteins. Their study helps to explicate the epigenetic changes caused by TRAP exposure and could be used to understand the connection between pollutants and lung diseases.	Ding et al. (2016)

Table 2.6: Continued

Study Descriptions	Authors
Mechanism: DNAm **Subjects:** A study among 163 children of African American and Dominican descent (9 to 14-year-olds) living in New York City. **Findings:** • Discovered links between higher accumulated personal exposure to BC, physical activity and lower methylation levels of the *FOXP3* promoter. • There was an association between physical exercise in children with increased exposure to BC (≥1200 ng/m^3) and 2.37% reduced methylation in promoter 2, but not among those with decreased BC exposure. • Even though physical activity may escalate air pollutant accumulation in the lungs and increase airway inflammation, everyday physical activity with high intensity may initiate adequate protective immune balance to relieve this impact on children. **Limitations:** The cross-sectional study design that restricted the authors to interpret causality and an average of DNAm across 2 measurements of 5 days apart could argue the difference in methylation. **Conclusion:** Urban children who were physically active had an association with lower *FOXP3* promoter methylation; this indicates a more prominent Treg role under high BC exposure. These results recommend that physical activity may be related to an immune defence within the context of high exposure to pollutants, although the mechanisms are unknown.	Lovinsky-Desir et al. (2017)
Mechanism: DNAm **Subjects:** 150 children between the age of 7 to 10 were recruited from the northern region of the Mexico City Metropolitan Area for this cross-sectional study. **Findings:** • There was no significant association in an unadjusted model between PM_{10} exposure and LINE-1 methylation. • However, PM_{10} constituents individually demonstrated that the methylation of LINE-1 and the concentrations of benzo[b]fluoranthene were significantly positive and kept in the modified model. • There was a significant connection between the DNA repair gene and methylation with specific PM_{10}-associated PAH and Vanadium. **Limitations:** The authors' study was limited by their study design, which measured outcomes and exposure simultaneously. It is challenging to deduce causal relationships from the cross-sectional technique. **Conclusion:** Overall, the research revealed novel discoveries in children who lacked protection from PM_{10} components chronically, showing how the methylation makeup of repair genes in DNA was connected to oxidative damage.	Alvarado-Cruz et al. (2017)

CHAPTER 3

METHODOLOGY

3.1 Study Design

This is a cross-sectional comparative study intending to assess the influence of TRAP on respiratory health and epigenetic modifications among children in the high traffic (HT) and low traffic (LT) areas, as shown in the Gantt Chart (Appendix 1). Exposure and outcome were determined simultaneously (Appendix 2). Study subjects were selected based on their exposures to TRAP, which were divided into those who attended schools and resided near HT areas, also those who attended schools and resided further away from HT areas.

3.2 Study Location

The HT area was Klang Valley, which centred in Kuala Lumpur, and covered its neighbouring cities and towns in Selangor, whereas the LT area was in Selangor. Klang Valley is also known as the Greater Kuala Lumpur. Titiwangsa Mountains are located to the north and east of Klang Valley, while the Strait of Malacca is situated to its west. Car traffic started to fill major highways as more traffics were originated from newly developed urban areas. Furthermore, nearly all trips were generated from urban areas and concentrated towards the central part of Klang Valley.

There is no specific distance set by any regulation in Malaysia regarding the distance of schools from major roadways, which requires health assessment to be conducted. The primary schools' selection was based on their distances from the traffic source. Children from four primary schools located in the TRAP zone were chosen as the HT group for this study. Meanwhile, four primary schools in the LT group were selected from the location at a distance of more than 5 km away from nearby highways, major roadways and industrial complexes in Selangor. This definition was adopted to classify whether a particular school belongs to either an HT or LT area and to compare these two categories throughout this study. The average distance of traffic exposure was designated by a distance of 5 km (Amin, Tamima, & Amador Jimenez, 2017; Muhamad Daud, Jalaludin, & Sopian, 2018).

The transport statistics were obtained from ADT and Road Traffic Volume Malaysia (RTVM) as tabulated by the Road Safety Department of the Ministry of Works, Malaysia, in their RTVM for 2017 and 2016. The selected routes and schools were then confirmed by using Google Maps and Google Earth. Another factor to look for from data captured by census stations was the Level of Severity (LOS), which was used to categorise the traffic volume on the road. It started

with LOS A, which meant no congestion, free flow with low traffic volumes and high speeds, and the list went up to LOS F when there was extreme congestion with stop-and-go traffic and forced flow. Table 3.1 shows the list name of selected primary schools and Continuous Air Quality Monitoring (CAQM) stations nearby selected schools.

Table 3.1: List of selected primary schools

School ID	Coordinates	Nearby CAQM Stations
	HIGH TRAFFIC	
H1	3°11'31.9" N 101°41'24.7" E	Batu Muda and Cheras
H2	3°10'05.4" N 101°42'24.8" E	Batu Muda and Cheras
H3	3°11'58.5" N 101°45'40.8" E	Batu Muda and Cheras
H4	3°09'36.8" N 101°41'41.1" E	Batu Muda and Cheras
	LOW TRAFFIC	
L1	3°09'02.4" N 101°50'19.9" E	None
L2	3°10'38.1" N 101°51'21.2" E	None
L3	3°09'55.9" N 101°53'01.6" E	None
L4	3°12'41.6" N 101°52'15.7" E	None

Figure 3.1 and Figure 3.2 present the study sites. According to Alam Sekitar Malaysia Sdn. Bhd., a company that formerly managed the air monitoring sites in Malaysia, a station can represent the area with a radius of 15 km (Zamri et al., 2012). From June 2016 until now, Pakar Scieno TW Sdn. Bhd was awarded the concession to develop, establish and implement the Environmental Quality Monitoring Program (EQMP) by the DOE Malaysia.

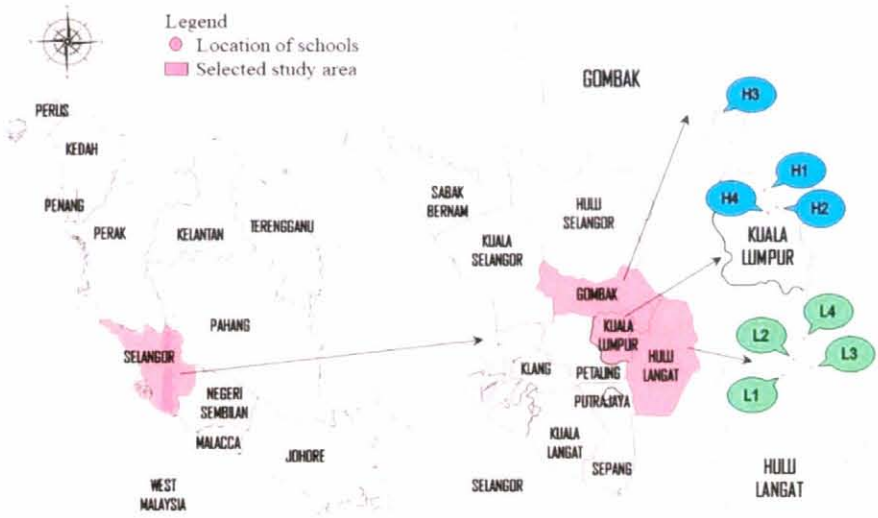


Figure 3.1: Locations of the selected primary schools

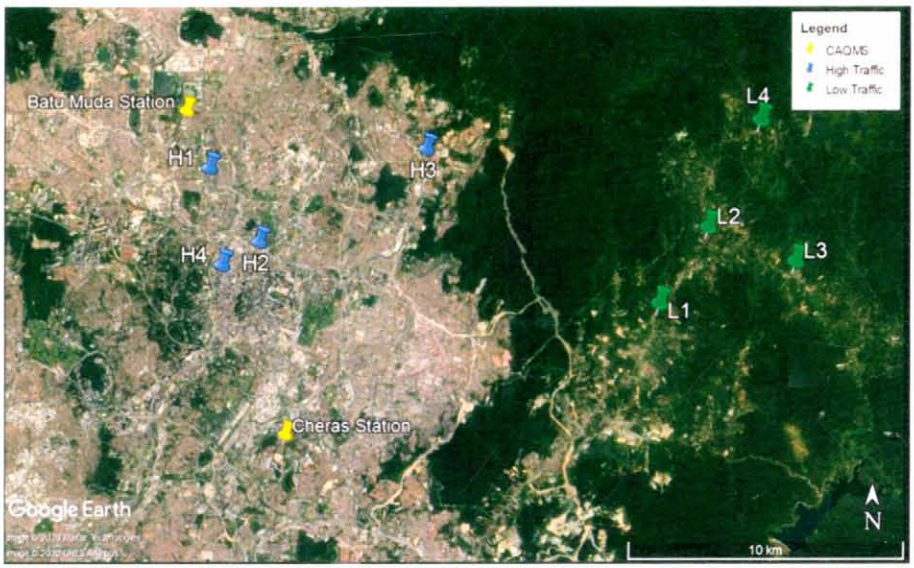


Figure 3.2: Locations of the selected primary schools and nearby CAQM stations as generated by Google Earth

The HT and LT schools and their local surrounding environment are shown in Figure 3.3 and Figure 3.4, respectively.

Figure 3.3: HT schools and surrounding local environment as generated by Google Earth (a) H1, (b) H2, (c) H3, (d) H4

Figure 3.4: LT schools and surrounding local environment as generated by Google Earth (a) L1, (b) L2, (c) L3, (d) L4

Table 3.2 shows the physical characterisation of each school in HT and LT areas and surrounding local activities. This information was used for site selection purposes.

Table 3.2: Physical characterisation of classrooms in both areas and surrounding local activities

	H1	H2	H3	H4	L1	L2	L3	L4
Sampling Date	25 Feb 2019 – 1 Mar 2019	13 May 2019 – 17 May 2019	11 Mar 2019 – 15 Mar 2019	6 May 2019 – 10 May 2019	8 Apr 2019 – 12 Apr 2019	22 Jan 2019 – 25 Jan 2019	11 Feb 2019 – 15 Feb 2019	29 Apr 2019 – 3 May 2019
Ventilation System	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)	Natural/ mechanical ventilation (ceiling fans)
Floor Type	Cement	Cement	Cement	Cement	Cement	Cement	Cement	Cement
Window Type	Louvre window – open during school hours only	Louvre window - open most of the time	Louvre window – open most of the time	Transom window - open most of the time	Louvre window – open most of the time	Louvre window – open most of the time	Louvre window – open during school hours only	Louvre window – open most of the time
Number of Fans	3	3	3	3	3	3	3	3
Number of Doors	2	2	2	2	2	2	2	2
Distance to Main Roads	40 m	30 m	70 m	40 m	50 m	110 m	160 m	110 m
Traffic Condition	Busy at peak time	Busy at peak time	Busy at all times	Busy at all times	Busy at peak time	Busy at peak time	Not busy at all times	Not busy at all times
Age of Building	33 years	43 years	26 years	89 years	29 years	35 years	32 years	30 years

3.3 Study Sampling

3.3.1 Sampling Duration

The environmental and biological sampling was performed between January to May 2019. The collection of 6-h environmental data at schools and biological samples took place during school hours, whereas the 24-h environmental data at schools and residences were collected during and outside school hours.

3.3.2 Target Population

There were 126,729 and 512,717 enrolments in government primary schools in Kuala Lumpur and Selangor, respectively (DOS Malaysia, 2018a). The study population was all year 1 to 5 children whose age was between 7 to 11 years old in 2019. Meanwhile, the target population included all male and female children aged 7 to 11 years old who were both studying and residing in the areas of interest either in Kuala Lumpur or Selangor. The HT group comprised of those children studying and living in the HT areas in Klang Valley. They may have higher exposure to TRAP than the children from another group. In contrast, children who were both studying and living in the LT areas in Selangor from the same age category as those in the HT group were the LT group. They may have lower exposure to TRAP than the children in the LT group. Both groups were matched in terms of inclusion criteria.

3.3.3 Sampling Frame

The sampling frame included all males and females of year 1 to 5 who attended the selected eight primary schools as listed in Table 3.1 in 2019. The list name of the school children was obtained from the school management.

3.3.4 Sampling Unit

The sampling unit was the individual child who attended the selected schools, fulfilled the inclusion criteria and received permission from parents or guardians to engage in this study.

Inclusion criteria for the selection of respondents:

- Children who were aged between 7 and 11 years old (according to birthday) during the school year 2019
- Children who were Malaysian citizen
- Children who were free from the history of doctor-diagnosed chronic respiratory illnesses or allergies

- Children who did not have surgery, unstable heart condition or severe respiratory infection within the past six months

Exclusion criteria for the selection of respondents:
- Children whose parents refused to fill in the questionnaires completely
- Children who experienced symptoms of upper respiratory tract infections such as cough, sore throat, runny nose and nasal congestion during the sampling period of saliva samples
- Children who experienced influenza symptoms, nausea, dizziness or vomiting during the lung function test

3.3.5 Sampling Method

Based on the purposive sampling method, primary schools located in high and low traffic areas were chosen based on the MOE Malaysia list. The primary schools were selected based on their distances from nearby highways or major roadways, which was presumed to contribute to the emission of TRAP. Those who met the inclusion criteria were enrolled through stratified random sampling proportionate to size based on the criteria listed previously on inclusion criteria. The entire population of school children in selected schools were divided into different strata; then, the children were randomly selected as the final respondents proportionally from the different strata. The school was the strata for this study.

3.3.6 Sample Size

Objective 1: To compare the reported respiratory symptom among school children in the high and low traffic areas

Equation 3.1 was applied based on hypothesis testing for two population proportions by Lemeshow et al. (1990).

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}}\sqrt{2\bar{P}\,(1-\bar{P})} + Z_{1-\beta}\sqrt{P_1\,(1-P_1) + P_2\,(1-P_2)}\right)^2}{(P_1 - P_2)^2} \quad \textbf{(Equation 3.1)}$$

where:

n = Sample size

$Z_{1-\frac{\alpha}{2}}$ = Standard error associated with confidence interval, 95% CI = 1.96

$Z_{1-\beta}$ = Standard error associated with power, 90% of power = 1.28

P_1 = Proportion of primary school children with cough in exposed area = 55.9% = 0.56 (Kamaruddin et al., 2016)

P_2 = Proportion of primary school children with cough in comparative area = 30.3% = 0.30 (Kamaruddin et al., 2016)

$\overline{P} = \frac{P_1 + P_2}{2} = \frac{0.56 + 0.30}{2} = 0.43$

therefore:

$$n = \frac{\left(1.96\sqrt{2\,(0.43)\,(0.57)} + 1.28\sqrt{0.56\,(0.44) + 0.30\,(0.70)}\right)^2}{(0.56\text{-}0.30)^2}$$

$n = 74.2 \approx 75$ respondents each from HT and LT group

Objective 2: To compare the lung function status among the school children in the high and low traffic areas

Equation 3.1 above was applied based on hypothesis testing for two population proportions by Lemeshow et al. (1990).

where:

n = Sample size

$Z_{1-\frac{\alpha}{2}}$ = Standard error associated with confidence interval, 95% CI = 1.96

$Z_{1-\beta}$ = Standard error associated with power, 90% of power = 1.28

P_1 = Proportion of FEV_1 abnormality among exposed group = 21.7% = 0.22 (Ab Jamil, Jalaludin, Kamaruddin, & Ibrahim, 2015)

P_2 = Proportion of FEV_1 abnormality among comparative group = 5% = 0.05 (Ab Jamil et al., 2015)

$\overline{P} = \frac{P_1 + P_2}{2} = \frac{0.22 + 0.05}{2} = 0.14$

therefore:

$$n = \frac{\left(1.96\sqrt{2\,(0.14)\,(0.86)} + 1.28\sqrt{0.22\,(0.78) + 0.05\,(0.95)}\right)^2}{(0.22\text{-}0.05)^2}$$

$n = 84.3 \approx 85$ respondents each from HT and LT group

Objective 3: To compare the histone H3 level and DNAm status among the school children in the high and low traffic areas

Equation 3.2 was applied based on hypothesis testing for two population means by Lemeshow et al. (1990).

$$n = \frac{2x2\sigma^2\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2}{(\mu_1 - \mu_2)^2}$$ **(Equation 3.2)**

where:
n = Sample size
σ^2 = Estimated pooled standard deviation (assumed to be equal to each group)
$Z_{1-\frac{\alpha}{2}}$ = Standard error associated with confidential interval, 95% CI = 1.96
$Z_{1-\beta}$ = Standard error associated with power, 90% of power = 1.28
μ_1 = Mean of biomarker concentration among exposed group= 7.4 pg/ml (Jalaludin et al., 2014)
μ_2 = Mean of biomarker concentration among comparative group = 5.6 pg/ml (Jalaludin et al., 2014)

therefore:

$$n = \frac{4(2.73)^2[1.96 + 1.28]^2}{[7.4 - 5.6]^2}$$

n = 96.6 ≈ 97 respondents each from HT and LT group

This study aimed to estimate the respiratory health implication among school children with different air exposure in two different areas; high and low traffic. The largest sample size out of the three calculations was 97 respondents. After considering the 20% non-response rate among the respondents, it was then decided that the sample size for this study were 117 respondents, each from the high and low traffic group. The total sample size was 234 school children.

Equation 3.3 was applied to calculate the sample size needed from each stratum. The number of children needed to represent the strata population is calculated, as shown in Table 3.3 and Table 3.4.

$$n = \frac{Size\ of\ strata\ population}{Size\ of\ entire\ population}\ x\ Size\ of\ entire\ sample$$ **(Equation 3.3)**

where:
n = Sample size of strata

Table 3.3: The sample size of each stratum in the HT group

School ID	Number of Children in Strata	Number of Children in Sample
H1	555	$\frac{555}{2104} \times 117 = 31$
H2	664	$\frac{664}{2104} \times 117 = 37$
H3	675	$\frac{675}{2104} \times 117 = 37$
H4	210	$\frac{210}{2104} \times 117 = 12$
Total	**2104**	**117**

Table 3.4: The sample size of each stratum in the LT group

School ID	Number of Children in Strata	Number of Students in Sample
L1	669	$\frac{669}{1565} \times 117 = 49$
L2	448	$\frac{448}{1565} \times 117 = 33$
L3	336	$\frac{336}{1565} \times 117 = 26$
L4	112	$\frac{112}{1565} \times 117 = 9$
Total	**1565**	**117**

3.4 Data Collection and Instrumentations

3.4.1 Questionnaires

Standardised and validated questionnaires from ISAAC and ATS (ATS-DLD-78-C) were adapted and developed to accommodate Malaysia's environment. The questionnaires, as attached in **Appendix 6,** were filled in by parents or legal guardians of the children. Moreover, the questionnaires were translated from English to Malay and validated by a previous local study by Jalaludin et al. (2002). The final version of the translated questionnaire was pretested on about 10% of the intended respondents, who were the parents or guardians of 7- to 11-year-old children in a school in Selangor. There were 28 questionnaires collected for this pre-test procedure, and the answers were measured for reliability using the Cronbach α test with values of 0.812, which were deemed acceptable for internal consistency.

A prior arrangement was made with the school management to determine the school teachers in charge of distributing and collecting the questionnaires. The children were then expected to give the questionnaires to their parents or guardians. Those interested parents or guardians were required to fill in the questionnaires, signed the consent form given completely and returned them to the school teachers by passing them to their children. To maintain the privacy of the information provided, the completed questionnaires were returned in the envelope provided, sealed and signed on top of the envelope opening at the back of the envelope. The data obtained from the questionnaires were recorded for the next procedures in the study. For those subjects who did not meet the criteria, they were excluded from this study. The questionnaires were divided into seven parts, as follow:

Part A: Sociodemographic and Socioeconomic Information
Part B: Health Status
Part C: History of Respiratory Symptoms
Part D: Exposure to Environmental Tobacco Smoke (ETS)
Part E: Environment in Residences
Part F: Current Eating Habits
Part G: Exposure to TRAP in Residences

3.4.2 Air Quality

3.4.2.1 Measurements of IAQ

School children spend most of their time in the classroom compared to other particular-purposes room such as science lab and library; hence, classrooms were chosen for air monitoring in each school. The IAQ measurements in classrooms were conducted once an hour for 6 h during the school session for a 1-min interval (7.20 a.m., 8.20 a.m., 9.20 a.m., 10.20 a.m., 11.20 a.m., 12.20 p.m., 1.20 p.m.). This step was important to determine that the dispersion of air pollutants was not affected by other factors and the temperature of the building was consistent (Othman et al., 2019). Some of the inspected characteristics were the type of ventilation of the classrooms, type of building materials for the classrooms, age of school buildings, history of renovation or maintenance works, and the number of occupants per classroom. Interviews were conducted verbally with the school teachers to gain the necessary information during the site inspection.

Measurements of the parameters of interest were carried out based on the method suggested in the manual of each instrument and earlier studies. Q-Trak IAQ Monitor (TSI, USA; model 7565), as shown in Figure 3.5, was used to measure air temperature, RH and CO_2. VelociCalc Multifunction Ventilation Meter (TSI, USA; model 9565) shown in Figure 3.6 was used for air velocity measurements. These battery-operated instruments were set at 1.0 m above the floor at the back of the classrooms, and away from obvious sources of potential

pollutants such as window and door, as adapted from Yang Razali et al. (2015) and are shown in Figure 3.7.

Figure 3.5: Q-Trak IAQ Monitor

Figure 3.6: VelociCalc Multifunction Ventilation Meter

Figure 3.7: The placement of the instruments indoors

3.4.2.2 Measurements of Meteorological Parameters

The measurements of ambient temperature (AT), RH and wind speed (WS) were obtained from the National Climate Centre of the Malaysian Meteorological Department at Parlimen station (3°08'55" N 101°40'40" E). Only the data during the sampling period was taken. The meteorological factors, particularly the AT and WS were vital as they could influence the concentration of air pollutants outdoors. Meanwhile, the Q-Trak IAQ Monitor was used to measure CO_2 outside classrooms.

3.4.2.3 Measurements of TRAP in Schools

TRAP, including PM_{10}, $PM_{2.5}$, PM_1, BC, NO_2, SO_2, TVOC and CO were carried out based on the method suggested in the manual of each instrument and earlier studies. Measurements of PM_{10}, $PM_{2.5}$ and PM_1 in classrooms were performed using DustTrak DRX Aerosol Monitor (TSI, USA; model 8534), as shown in **Figure 3.8**. A correction factor of 1.6 was applied to DustTrak readings based on a previous study, which presented that DustTrak recorded $PM_{2.5}$ concentrations 1.59-1.70 times higher than the filter-based methods (McNamara, Noonan, & Ward, 2011). The data were collected in real-time in each site for a 1-min interval during the 6-h school period from 7.20 a.m. to 1.20 p.m., which was done with the function of a data logger. Each classroom was measured twice. NO_2, SO_2 and CO were measured using Air Quality Monitor (Aeroqual, New Zealand; model Series 500), a portable gas sensor, as shown in Figure 3.9. TVOC was measured using a Handheld VOC monitor (RAE Systems, USA; model PpbRAE 3000), as shown in Figure 3.10. These instruments also accurately measured air pollutants in real-time and accompanied by a data logger.

Figure 3.8: DustTrak DRX Aerosol Monitor

Figure 3.9: Aeroqual Air Quality Monitor

Figure 3.10: PpbRAE 3000

This cross-sectional study assessed TRAP exposure in multilevel classrooms from the ground to the first and second floor because TRAP mixes to a different height in the school building. Hence, TRAP measurements were varied from floor to floor due to deposition behaviours of air pollutants. For indoor monitoring, the instruments were positioned at the back of the classrooms to minimise interference with everyday activities in the classroom; they were explicitly placed at 1.0 m above the floor at the back of the classrooms, and away from obvious sources of potential pollutants such as window and door, as adapted from Yang Razali et al., (2015) and shown previously in Figure 3.7. Meanwhile, for outdoor monitoring, they were installed at the spot nearest to the traffic source (e.g. guard post, school field), as shown in Figure 3.11.

Figure 3.11: The placement of the instruments outdoors

Measurements of BC in $PM_{2.5}$ were taken using MiniVol Air Sampler (Airmetrics, USA; model 4.2) as shown in Figure 3.12 with samples on 47 mm quartz microfibre filters (Whatman, USA; catalogue number 1851-047). This battery-operated instrument was placed on its stand on the ground to capture air at 5 L/min from Monday to Friday. For indoor monitoring, this sampler was placed for 24 h per filter sample at placed at 1.0 m above the floor at the back of the classrooms, and away from obvious sources of potential pollutants such as window and door, as adapted from Yang Razali et al., (2015). Meanwhile, for outdoor monitoring, it was placed 24 h simultaneously with the indoor monitoring at the location closest to the traffic source (e.g. school field, guard post).

Figure 3.12: MiniVol Air Sampler

3.4.2.4 Gravimetric Analysis of BC in Schools

Filter papers used were weighed before and after exposure to figure out the concentration in $\mu g/m^3$. There was no pre-cleaning involved for the filter media used for sample collection. Nevertheless, each filter was covered with aluminium foil and pre-baked for 4 h at 500°C inside a furnace (Carbolite, United Kingdom; model CWF 11/23) before sampling at Graduate Laboratory (Wet), Faculty of Forestry and Environment, UPM. The filter media were put in Petri pad dishes (Merck Millipore, Germany; catalogue number PD1504700). Loaded and unloaded filters were kept in a desiccator and below 25% RH for 48 h before weighing to lessen the effect of water adsorption. $PM_{2.5}$ mass was measured by weighing filter papers before and after sampling using a semi-micro analytical balance (A&D, USA; model GR-202) with 0.01 mg sensitivity and five-digit at Physiology Laboratory, Faculty of Medicine and Health Sciences (FMHS), UPM. There were 72 filters collected, including 8 fields blank; one blank for each school. The samples were stored in a desiccator before being analysed. The calculation of 24-h $PM_{2.5}$ measurement in schools is shown in Equation 3.4 and Equation 3.5 below, adhering the manual by Airmetrics.

$$V\ (m^3) = \frac{\frac{60\ min}{h} x \frac{5\ L}{min} x\ 24\ h}{\frac{1000\ L}{m^3}} \qquad \textbf{(Equation 3.4)}$$

$$Particulate\ Matter\ (\mu g/m^3) = \frac{(W_2 - W_1)}{V} x\ 10^6 \qquad \textbf{(Equation 3.5)}$$

where:

W_1 = mass of filter paper before sampling (g)
W_2 = mass of filter paper after sampling (g)
V = volume of air sampled (m^3)

3.4.2.5 Reflectometric Analysis of BC

BC samples were analysed using a Smoke Stain Reflectometer (Diffusion Systems, England; model EEL 43), as shown in Figure 3.13. Secondary standards of known BC concentrations were used to calibrate the smoke stain reflectometer. Dark spots on the filter papers were measured via their reflectance of white light, with an assumption of 100% reflection. Afterwards, the absorbed light was converted into real BC mass concentration after computing average of several spots throughout the filter paper. The blackness of a sample resembled BC content. This procedure took place at Chemical Oceanography and Marine Pollution Laboratory, Institute of Oceanography and Environment, UMT. The calculation of BC measurement in schools is shown below in Equation 3.6, as Quincey (2007) explained following ISO 9835 for BC standard.

$$BC\ (\mu g/m^3) = 3.462 \times 10^9 \left(\frac{A}{V}\ ln\left(\frac{R_b}{R_s}\right)\right)^2 + 4.438 \times 10^5\ \frac{A}{V}\ ln\left(\frac{R_b}{R_s}\right) \qquad \textbf{(Equation 3.6)}$$

where:
R_b = intensity of light reflected from a clean filter
R_s = intensity of light reflected from a sampled filter
A = exposed filter area (m^2)
V = volume of air sampled (m^3)

Figure 3.13: Smoke Stain Reflectometer

3.4.2.6 Measurements of TRAP in Residences

PM_{10} and $PM_{2.5}$ in residences were measured using Escort Personal Sampling Pump (Zefon International, USA; model LC) as shown in Figure 3.14, with samples on 0.8 µm of 37 mm MCE filter (Zefon International, USA; catalogue no. FMCE837). The method followed a modified NIOSH Manual of Analytical Methods 0600 for $PM_{2.5}$ (NIOSH, 1998) and NIOSH Manual of Analytical Methods 0500 for PM_{10} (NIOSH, 1994). For this study, the 2.5-micron impactor was used instead of the 4-micron cyclone to collect the $PM_{2.5}$ sample. This battery-operated instrument was placed on the wall of respondents' residences at 1.7 L/min.

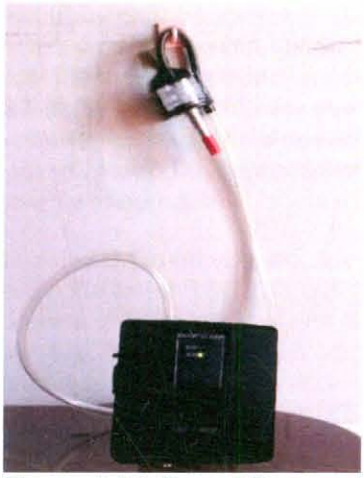


Figure 3.14: Escort Personal Sampling Pump

3.4.2.7 Gravimetric Analysis of PM_{10} and $PM_{2.5}$ in Residences

Filter papers used were weighed before and after exposure to figure out the concentration in µg/m^3. There was no pre-cleaning involved for the filter media used for sample collection. Nevertheless, each filter was covered with aluminium foil and pre-baked for 4 h at 500°C inside a furnace (Carbolite, United Kingdom; model CWF 11/23) before sampling at Graduate Laboratory (Wet), Faculty of Forestry and Environment, UPM. The filter media were put in Petri pad dishes (Merck Millipore, Germany; catalogue number PD1504700). Loaded and unloaded filters were kept in a desiccator and below 25% RH for 48 h before weighing to lessen the effect of water adsorption. Sampling was left for 24 h in the children's residences and was placed at the child's breathing level. There were 60 filters collected, including 8 fields blank; one blank for each school. The calculations of 24-h PM_{10} and $PM_{2.5}$ measurements in residences are shown in Equation 3.4, as mentioned previously except replacing 5 L to 1.7 L, and Equation 3.7 below.

$$Particulate\ Matter\ (\mu g/m^3) = \frac{(W_2 - W_1) - (B_2 - B_1)}{V} \times 10^6 \quad \textbf{(Equation 3.7)}$$

where:
W_1 = tare weight of filter before sampling (g)
W_2 = post-sampling weight of sample-containing filter (g)
B_1 = mean tare weight of blank filter (g)
B_2 = mean post-sampling weight of blank filter (g)
V = volume of air sampled (m^3)

3.4.2.8 Traffic Counts (TC)

Traffic volumes by vehicle types were manually counted using a video camera and tally counters. This step was performed simultaneously with the measurements of the TRAP. The type of vehicles was classified according to the size of vehicles, which were light-sized (motorcycles), medium-sized (cars, taxis and vans) and heavy-sized (buses, lorries, light- and heavy-good vehicles).

The TC surveys were conducted at two-time intervals per day from 7.00 a.m. to 8.00 a.m. as shown in Figure 3.15 and from 12.30 p.m. to 1.30 p.m. as shown in Figure 3.16 when school children arrived at school and left school. This method was adapted from a previous local study conducted by Ezani et al. (2018). From this vehicle count per hour, there was also a TC for idling vehicles near the school gate in the morning from 7.00 a.m. to 7.20 a.m. and in the afternoon from 1.00 p.m. to 1.30 p.m.

Figure 3.15: Traffic counts in the morning

Figure 3.16: Traffic counts in the afternoon

3.4.3 Human Samples

3.4.3.1 Measurements of Anthropometry

Anthropometry measurements involve the measurement of height and weight of the respondents, as shown in Figure 3.17. Therefore, each respondent's height and weight were measured before the lung function test. This step was performed at rooms designated by the school management during school hours. The height was measured by a body meter (SECA, Deutschland; model 206), whereas the weight was measured by a weighing scale (SECA, Deutschland; model 803). The weighing scale was calibrated before performing the measurements and placed on a hard-floor surface. In order to measure the height of the respondents, the back of their head, back, buttocks, calves and heels were ensured to touch the upright feet together. Then, they were asked to look straight. The sliding part of the body meter was lowered so that the hair (if present) was pressed flat. As for measuring the respondents' weight, they were required to take off their shoes and any heavy outer garments. Then, they were asked to stand in the centre of the platform so that their weights were distributed evenly to both feet.

Figure 3.17: Anthropometry measurements

3.4.3.2 Spirometry Test

The respondents performed spirometry test using a spirometer (Chestgraph, Japan; model HI-105), as shown in Figure 3.18 and Figure 3.19 under certified medical practitioners' guidance. This step was performed at rooms designated by the school management after the anthropometry test. The spirometer and its accessories were disinfected with alcohol wipes, and mouthpieces were changed between respondents to prevent the spread of infectious diseases. Gloves were also worn when handling the spirometer.

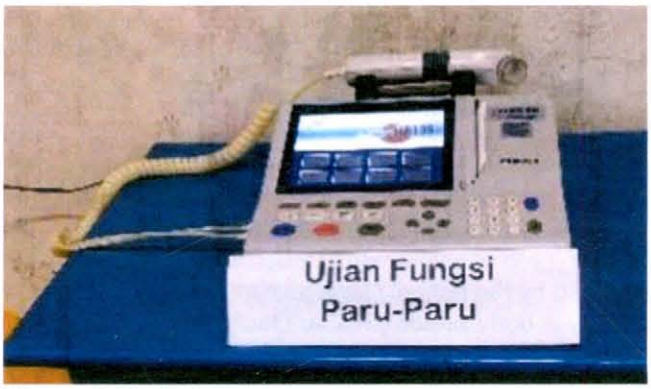


Figure 3.18. Spirometer

Figure 3.19. Spirometry test

The spirometer was calibrated using a 3 L syringe, where the air from the syringe was injected into the spirometer. The respondents were checked on these items before the lung function test can proceed; 1) do not wear tight clothes; 2) do not eat at least 30 minutes before the procedure. They were also explained about the objective of the test and demonstrated on the procedure of the test. Before measurement was taken, information such as age, gender, race, weight, and height was keyed into the spirometer. For full expiration, the respondents were asked to stand up still in their comfortable position. A disposable mouthpiece was connected to the spirometer's sensor. Then, it was inserted into the respondents' mouths, and they were asked to seal around the mouthpiece. They were reminded to do these three phases of the test; 1) maximal inspiration; 2) a maximal exhalation; 3) continue complete exhalation to the end of the test around 6 s. The test was carried out according to the ATS procedure until a satisfactory reading was taken; a minimum of 3 and a maximum of 6. Spirometry sessions consisting of the three best manoeuvres were retained, and three

indicators were included in the study: FEV_1, FVC, FEV_1/FVC ratio. This step is important to achieve good repeatability (reproducibility) of FEV_1 and FVC within a spirometry test session because poor repeatability reduces the best manoeuver.

The results were printed out on a chart called a spirogram. The spirometry test evaluation was calculated by comparing the measured value with a predicted value as outlined in the updated technical spirometry standards developed by ATS and the European Respiratory Society (ERS) (Graham et al., 2019). For spirometry test among Malay children in Malaysia, the reference values reported by Azizi & Henry (1994) were referred to as shown in Table 3.5. These values were further used to classify the respondents' lung function status according to ATS, as shown in Table 3.6. If an obstructive, restrictive or mixed pattern is observed, the severity of the lung function test abnormality was then graded based on the grades of FEV_1 by ATS as summarised in Table 3.7.

Table 3.5: Reference values of lung function test among Malay children (Source: Azizi & Henry, 1994)

Lung Function Test Item	Boys	Girls
FVC	4.1120×10^{-6} Height$^{2.6421}$	6.0777×10^{-7} Height$^{3.0112}$
FEV_1	6.2523×10^{-6} Height$^{2.5388}$	5.7588×10^{-7} Height$^{3.0067}$

Table 3.6: Lung function test interpretation (Source: Pellegrino et al., 2005)

FVC	FEV_1/FVC Ratio	Suggested Diagnosis
≥ 80 %	≥ 80 %	Normal
≥ 80 %	< 80 %	Obstructive
< 80 %	≥ 80 %	Restrictive
< 80 %	< 80 %	Mixed Pattern

Table 3.7: The severity of lung function test abnormality (Source: Pellegrino et al., 2005)

FEV_1	Suggested Diagnosis
≥ 80 %	Normal
70-79 %	Mild
60-69 %	Moderate
50-59 %	Moderately Severe
35-49 %	Severe
<35 %	Very Severe

3.4.3.3 Saliva Collection and Processing

Children who were granted permission were chosen to proceed with saliva collection to collect epigenetic biomarker of interest, histone H3 and DNA methylation (DNAm). The technique is uncomplicated and practical to carry out among children. This procedure took place during school hours at rooms determined by the school management after the lung function test was performed. Saliva collection has the potential to transmit infectious diseases through active organisms suspended in droplets and aerosolised particles. To prevent the spread of infectious diseases, gloves were donned when managing the sample containers. The outer side of the containers was disinfected with alcohol wipes after the saliva had been collected. The set up of saliva collection is shown in Figure 3.20.

Figure 3.20: Collection of saliva samples

Saliva samples were collected in sterile 60 ml sample containers (VWR, USA; catalogue no. KART5571). Before saliva collection, the respondents had to wash their mouth with running water. Then, the respondents were asked to rub their tongue against the inside of the mouth for 15 s and expectorated saliva every 30 s into the container on ice until roughly 10 ml of the whole saliva was collected. The sample containers were labelled with each respondent's names, identification number, date, and time the sample was taken. Saliva samples were kept at 4°C in an icebox (Coleman, USA; catalogue no. 2000033010) with ice packs (Coleman, USA; catalogue no. 1237167 and 1237168) until being transferred to Environmental Health (EH) Laboratory, FMHS, UPM. Once the samples had reached the laboratory, the samples were aliquoted into sterile 1.5 ml microcentrifuge tubes (Eppendorf, Germany; catalogue no. 0030120086). After that, the samples were centrifuged at 14,000 x g for 15 min at 4°C (MPW, Poland; model MPW-352R) at Cell Signalling Laboratory, FHMS, UPM to remove all debris, such as insoluble material, cell debris and food debris as mentioned in previous studies (Jasim et al., 2016; Winck et al., 2015). The supernatant from each sample was collected and frozen at -80°C ultra-low temperature freezer (Sanyo, Japan; model MDF 192) until extraction and quantification procedure.

3.4.3.4 Histone Extraction and Histone Modification Analysis

The approach to histone H3 modification can be seen as a two-stage process; there were histone extraction and ELISA. Histones were extracted using a histone extraction kit (Epigentek, USA; catalogue no. OP-0006-100), which used acid precipitation to isolate the highly basic histone from samples. The protocol was adapted from the manufacturer's protocol. Firstly, saliva samples were pre-lysed with a pre-lysis buffer on ice, centrifuged at 10,000 rpm for 1 min at 4°C, and the supernatant was removed. After that, the samples were lysed with a lysis buffer, incubated on ice for 30 min and centrifuged at 12,000 rpm for 5 min at 4°C. Finally, a pH balance buffer was added to the supernatant immediately. This procedure was carried out at Cell Signalling Laboratory, FMHS, UPM. Circulating histone H3 were measured using a histone quantification kit (Epigentek, USA; catalogue no. P-3091-96). The protocol was adapted from the manufacturer's protocol, and Figure 3.21 shows one of the ELISA steps.

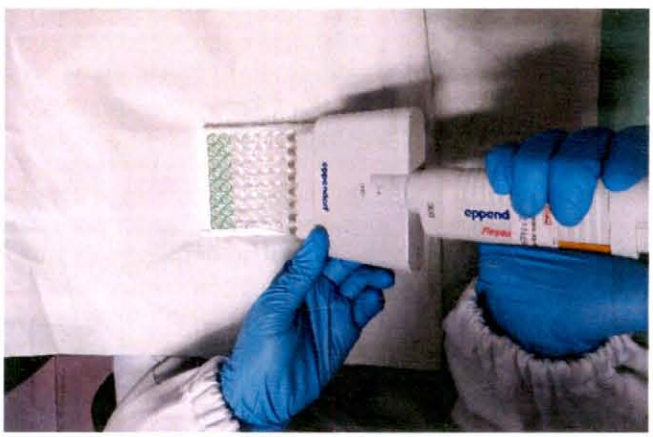


Figure 3.21: One of the steps in ELISA using a multichannel pipette

Firstly, the samples, blank and positive controls, were run in duplicates in the 96-well ELISA plate. Then, the whole plate was covered and incubated at 37°C for 60 min in an incubator (Hach, USA; model 2569900). Histone H3 modified at specific sites were captured on the strip wells coated with anti-histone H3 antibody. The reaction solution was removed, and the wells were washed 3 times with a wash buffer. Then, a detection antibody solution was added to each well. The whole plate was wrapped and incubated at room temperature (RT) for 60 min. The reaction solution was removed, and the wells were washed 4 times with a wash buffer. Subsequently, a colour development reagent was added to each well, and the plate was incubated at RT for 1 to 10 min away from light. Finally, a stop solution was added to each well to cease the enzyme reaction. The absorbance or intensity of the colour was measured on a microplate reader (Tecan, Switzerland; model Infinite M200) within 2 to 10 min at 450 nm with an optional wavelength of 655 nm, as shown in Figure 3.22. This procedure was carried out at Physiology Laboratory, FMHS, UPM.

Figure 3.22: ELISA samples to be analysed by a microplate reader

A standard curve was generated with OD versus positive controls at each concentration point, as shown in Figure 3.23. The slope was determined as OD/ng. The amount of circulating histone H3 was calculated using Equation 3.8 below.

$$\textit{Histone H3 (ng/ml)} = \frac{(\textit{Sample OD} - \textit{Blank OD})}{\textit{Slope x Sample Amount } (\mu l)} \textit{ x 1000} \qquad \textbf{(Equation 3.8)}$$

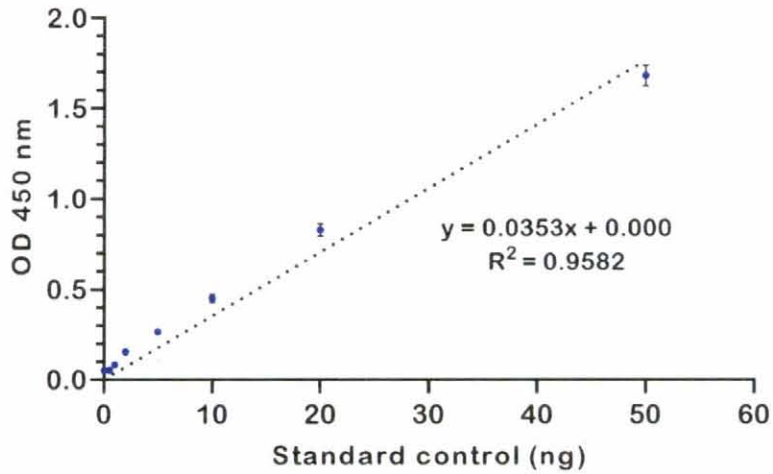


Figure 3.23: Calibration curve of ELISA for histone H3

3.4.3.5 DNA Extraction

This method is the standard technique used in any DNA study. DNA was extracted using a DNA extraction kit (Qiagen, Germany; catalogue no. 51106). The protocol was adapted from the manufacturer's protocol and performed at Molecular Biology and Bioinformatics Laboratory, FMHS, UPM. The procedure was carried out in a laminar airflow cabinet (Erla Technologies, Malaysia; model CFM4). Firstly, saliva samples were lysed at RT with a lysis buffer to digest protease and isolate DNA. The reactions solution was mixed with a vortexer (Heidolph, Germany; model Reax top) and incubated at 56°C for 10 min in a water bath (Memmert, Germany; model WNB 7). Upon incubation, the solution was briefly mixed in a centrifuge (Hettich, Germany; model Mikro 120). After that, undenatured 99.8% ethanol (R&M Chemicals, United Kingdom; CAS no. 64-17-5) was added to the solutions to precipitate DNA into a solid form. The solution was mixed with a vortexer and a centrifuge.

Subsequently, the solution was transferred to spin columns placed in collection tubes and centrifuged at 6000 x g for 1 min. Both the flow-through and collection tubes were dumped. Next, the spin columns were put in new collection tubes and washed with wash buffer 1. The solution was centrifuged at 6000 x g for 1 min. Both the flow-through and collection tubes were removed. Later, the spin columns were fixed in new collection tubes and washed with wash buffer 2. The solution was centrifuged at 20,000 x g for 3 min. Both the flow-through and collection tubes were disposed of. After that, the spin columns were inserted in 1.5 ml microcentrifuge tubes (Eppendorf, Germany; catalogue no. 0030120086) and centrifuged at 20,000 x g for 1 min. Both the flow-through and collection tubes were discarded. Next, the spin columns were placed in new microcentrifuge tubes, added with an elution buffer and incubated at RT for 1 min. The solution was centrifuged at 6000 x g for 1 min. Finally, the extracted DNA was shifted to 0.2 ml PCR tubes (Thermo Fisher Scientific, USA; catalogue no. AB0620).

A nanophotometer (Implen, Germany; model P300) at wavelengths of 260 nm and 280 nm was used to quantify and analyse the condition of each extracted DNA sample from saliva. 2 µl of samples were used. Two measures were observed on each sample: 1) concentration (ng/µl); 2) purity of DNA (via the relative absorbance ratio). Three measurements were taken and averaged for each DNA to be used. Samples with a ratio closer to 1.8 indicated a relatively pure DNA sample, whereas samples between 1.6 to 2.0 were considered pure. The samples were stored in a -80°C freezer for long-term storage.

3.4.3.6 Bisulphite Conversion and DNAm Analysis

Alternative DNAm analysis techniques explored in the literature include quantitative PCR, bisulphite pyrosequencing assay, and genomewide 27K assay. However, the method applied in this study results in a simpler and more

cost-effective approach for DNAm analysis. This procedure was carried out at Molecular Biology and Bioinformatics Laboratory, FMHS, UPM. With the bisulphite treatment, unmethylated cytosines of DNA were converted to uracil, whereas methylated cytosines remained unmodified. Extracted genomic DNA was modified using a bisulphite conversion kit (Qiagen, Germany; catalogue no. 59826). The protocol was adapted from the manufacturer's protocol. The procedure was carried out in a laminar airflow cabinet. Firstly, the samples were thawed, and the bisulphite reaction solution was prepared in 0.2 ml PCR tubes at RT. The solution was mixed with a vortexer. A thermal cycler with a heated lid (Labnet International, USA; model Multigene 96-Well) at Biochemistry Laboratory, FMHS, UPM was programmed accordingly, and the solution was placed in the thermal cycler for the conversion process.

Upon completion of the bisulphite conversion, the solution was briefly centrifuged. Then, the solution was transferred to clean 1.5 ml microcentrifuge tubes. A buffer that promotes binding of the converted single-stranded DNA to the tube was added to the solution, and the solution was mixed with a vortexer for 15 s and briefly centrifuged. Undenatured 99.8% ethanol was added to the solution to precipitate DNA into a solid form. The solution was mixed with a vortexer and briefly centrifuged. Subsequently, the solution was transferred to spin columns fixed in collection tubes and centrifuged at 20,000 x g for 1 min. The flow-through was disposed of, and the spin columns were inserted back into the collection tubes. Next, the membrane-bound DNA was washed with a wash buffer to remove residual sodium bisulphite and centrifuged at 20,000 x g for 1 min. The flow-through was discarded, and the spin column was placed back into the collection tubes.

After that, a buffer for desulfonation was added into the spin columns, and the solution was incubated for 15 min at RT. The spin columns were centrifuged at 20,000 x g for 1 min. The flow-through was dumped, and the spin columns were put back into the collection tubes. Subsequently, the spin columns were washed twice with a wash buffer and centrifuged at 20,000 x g for 1 min. The flow-through was discarded, and the spin column was placed back into the collection tubes. After that, undenatured 99.8 % ethanol was added to the spin columns and centrifuged at 20,000 x g for 1 min. The spin columns were placed into new collection tubes and centrifuged at 20,000 x g for 1 min. Next, the spin columns were placed into clean 1.5 ml microcentrifuge tubes and added with an elution buffer. The spin columns were incubated at RT for 1 min and centrifuged at 15,000 x g for 1 min. In the end, the purified DNA was transferred to new 0.2 ml PCR tubes.

A nanophotometer (Implen, Germany; model P300) at wavelengths of 260 nm and 280 nm was used to quantify and analyse the condition of each bisulphite-converted DNA sample. 2 µl of samples were used. Two measures were observed on each sample: 1) concentration (ng/µl); 2) purity of DNA (via the relative absorbance ratio). Three measurements were taken and averaged for each DNA to be used. Samples with a ratio closer to 1.8 indicate a relatively pure

DNA sample, whereas samples between 1.6 to 2.0 were considered pure. The DNA was stored in a -80°C freezer for long-term storage.

DNAm was quantified using an MS-PCR kit (Qiagen, Germany; catalogue no. 59305) on bisulphite-treated DNA. The protocol was adapted from the manufacturer's protocol. The procedure was carried out in a laminar airflow cabinet at Molecular Biology and Bioinformatics Laboratory, FMHS, UPM. The reaction compositions were dispensed into 0.2 ml PCR tubes according to the manual. After a thermal cycler at Biochemistry Laboratory, FMHS, UPM was programmed, the PCR tubes were placed in the thermal cycler, and the cycling program was initiated. The promoter regions of choice were *TNF-α* and *CYP1A1*, where methylation is potentially responsive to TRAP or has been implicated in allergy, asthma, and airway inflammation (Jung et al., 2017; Sofer et al., 2013). PCR amplification was performed in two separate reactions with two different primer pairs specific for either the methylated or the unmethylated sequence. Primers for the assay and respective PCR conditions are tabulated in Table 3.8.

Table 3.8: Primers for DNAm analysis

Genes and References		Sequence (5' – 3')	PCR Conditions
TNF-α (Cordero et al., 2011)	Uf	GGTTTAGAAGATTTTTTTT GGAATT	Initial denaturation: 95°C for 10 min Denaturation: 95°C for 30 s Annealing: 57°C for 1 min Extension: 72°C for 1 min (38 cycles) Final extension: 72°C for 7 min Product Size: 120 bp
	Ur	TCTATCTCAATTTCTTCTC CATCAC	
	Mf	TTAGAAGATTTTTTTCGGA ATC	
	Mr	TATCTCGATTTCTTCTCCA TCG	
CYP1A1 (He et al., 2015)	Uf	GGATTATTTTTTGGTTTGG ATTAGT	Initial denaturation: 95°C for 5 min Denaturation: 95°C for 30 s (60 cycles) Annealing: 56°C for 30 s Extension: 72°C for 30 s Final extension: 72°C for 7 min Product Size: 194 bp
	Ur	AACCTAACTACCTACCTCC AACACT	
	Mf	GATTATTTTTTGGTTTGGA TTAGC	
	Mr	TAACCTAACTACCTACCTC CGACG	

Uf: Unmethylated forward; Ur: Unmethylated reverse; Mf: Methylated forward; Mr: Methylated reverse

The reactions were performed in a thermal cycler at Biochemistry Laboratory, FMHS, UPM, as shown in Figure 3.24. The amplicons were visualised on gel documentation and image analysis system (Alpha Innotech, USA; model AlphaImager 2200) using novel juice staining (GeneDireX, USA; catalogue no. SL001-1000) following agarose gel electrophoresis made from 3% agarose gel (1st Base, Singapore; catalogue no. BIO-1000-100g). Figure 3.25 shows the set-up for the agarose gel electrophoresis. This procedure was carried out at

Molecular Biology and Bioinformatics Laboratory, FMHS, UPM. Amplicons of the expected size produced from either primer pair determine the presence of DNA in the original sample with the respective methylation status. Positive amplification only for unmethylated primers was interpreted as unmethylation. In contrast, positive amplification only for methylated primers or for both methylated and unmethylated primers was considered as methylation (Ma et al., 2014).

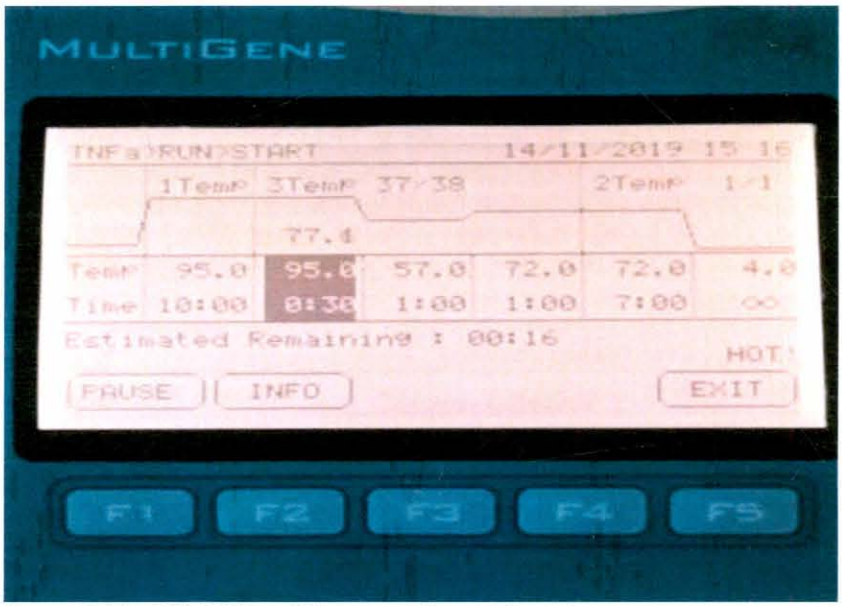


Figure 3.24: MS-PCR setting on a thermal cycler

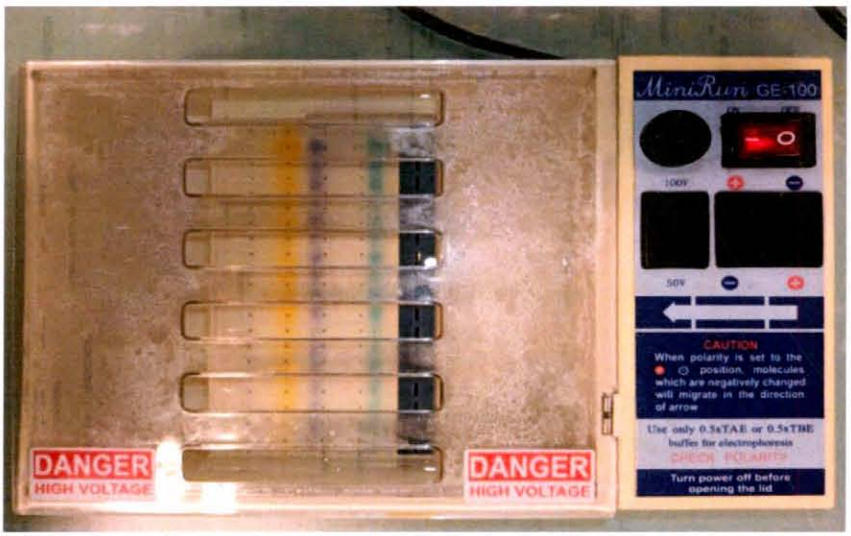


Figure 3.25: Set-up for agarose gel electrophoresis

3.4.4 Statistical Analyses

Data collected were analysed using Statistical Package for Social Science (SPSS) Version 23, RStudio Version 1.2.1335 and GraphPad Prism 8 Version 8.4.2. Descriptive analyses were done to convey the important aspects of the data collected, including screening and organising the data. Data normality of continuous variables were determined based on Shapiro Wilks. This analysis was done to identify whether the data were normally distributed or not normally distributed. Data were normally distributed when the p-value of Shapiro Wilks > 0.05. Then, an appropriate test was determined for further data analysis. Normally-distributed data were analysed using parametric tests, while the data that were not normally distributed were analysed using non-parametric tests. Lastly, the univariate, bivariate and multivariate testings were used to analyse each of the objectives in this study. Table 3.9 shows the statistical tests involved in the study.

Table 3.9: Statistical tests involved in this study

Objectives	Type of Data	Scale of Measurements
To determine socio-demographic and socio-economic information, house condition and location, family background and dietary intake among the respondents in high and low traffic areas.	Categorical Data (Qualitative)	• Frequency and percentage (%) • Mean and Standard Deviation (SD) for parametric statistics • Median and Interquartile Range (IQR) for non-parametric statistics
To evaluate the concentrations of TRAP and IAQ parameters inside respondents' classrooms and residences in high and low traffic areas.	Numerical Data (Quantitative)	• Mean and SD for parametric statistics • Median and IQR for non-parametric statistics
To compare the reported respiratory symptoms, lung function status, histone H3 modification level and DNAm status among the respondents.	Categorical Data (Qualitative)	• Frequency and percentage (%) • Chi-Square Goodness-of-Fit Test
	Numerical Data (Quantitative)	• Independent Samples *t*-Test for parametric statistics • Mann-Whitney *U* Test for non-parametric statistics
To assess the associations between the concentrations of air pollutants and respiratory symptoms, lung function status, histone H3 level and DNAm status among the respondents.	Categorical Data (Qualitative)	• Chi-Square Test of Independence
	Numerical Data (Quantitative)	• Pearson's Correlation Test for parametric statistics • Spearman Rho's Test for non-parametric statistics

Table 3.9: Continued

Objectives	Type of Data	Scale of Measurements
To identify the factors that are significantly associated with the respiratory symptoms, lung function status, histone H3 level and DNAm status in response to TRAP-induced systemic inflammation after controlling the confounders.	Categorical Data (Qualitative)	• Multiple Logistic Regression for the categorical dependent variable
	Numerical Data (Quantitative)	• Multiple Linear Regression for the continuous dependent variable

3.4.5 Quality Control

Quality control was conducted for each study component, beginning with the study design, site selection, questionnaires, data collection and data analysis.

3.4.5.1 Site Selection

The schools and residences were particularly selected to match the criteria for HT and LT schools. A site visit was performed in each school prior to data collection to confirm their eligibility in this study.

3.4.5.2 Questionnaires

The pre-test of the questionnaire was conducted on at least 10% of the total respondents, which was among 28 respondents. This step was also performed to control information bias. The α value for the reliability test of Cronbach of 0.812 was deemed as acceptable for $\alpha \geq 0.7$. The returned questionnaires were cross-checked to avoid missing information. Any missing information was followed up through a phone conversation with the parents or guardians. Each respondent was designated with a specific ID to ensure anonymity and confidentiality. Each unique ID was also used for all biological samples from the same respondent in this study. Researcher bias was minimised by collecting information similarly from groups of respondents, which was using questionnaires adapted from ATS and ISAAC. Meanwhile, recall bias was minimised by reducing the time interval as near as possible to the time of exposure between the event and the recall period, which was mostly information within 12 months ago.

3.4.5.3 Environmental Sampling

Filter papers were pre-baked at 400°C for 4 h. The filter papers were weighed using a microbalance with an accuracy of ±0.0001 g. The filter papers were kept inside a Petri slide and sealed in an aluminium foil. The filter papers were also

conditioned in a desiccator for 24 h, before and after sampling. For the filter papers involved in BC analysis, the filter papers were kept in a desiccator in the EH Laboratory, FMHS, UPM, until it was ready to be transported to UMT. During the travel period, the filter papers were kept in an airtight container with silica gels.

Each air monitoring equipment was calibrated before performing the measurements. Measurements of each air quality parameter were performed based on the method recommended in the manual of each instrument. The equipment was kept clean, maintained and checked regularly after used. The flowrate and elapse time of MiniVol were recorded before and after sampling, whereas other instruments were calibrated to zero before each measurement. Two impactors of MiniVol were cleaned and greased on the seventh sampling.

3.4.5.4 Spirometry Test

Each respondent was provided with a specific ID. The test was performed by following ATS procedures with strict hygiene practice. The spirometer was kept clean, maintained and checked regularly after used. A test was considered valid with at least 3 acceptable trials, and both the FVC and FEV_1 were repeatable.

3.4.5.5 Biological Sampling

Each respondent was provided with a specific ID. Fresh saliva samples were kept at 2-7°C in an icebox with ice packs for up to 24 h before being transported to the EH laboratory at FMHS, UPM. Next, each respondent's extracted saliva samples were preserved in a -80°C freezer in the EH Laboratory, FMHS, UPM before carrying out ELISA and MS-PCR test. For the ELISA test of histone H3, the levels were calculated from the calibration curve. As for MS-PCR reaction, three types of control primers were used: 1) Bisulphite-converted methylated DNA control; 2) Bisulphite-converted unmethylated DNA control; 3) Unmethylated DNA control.

3.4.5.6 Data Management

Paper records will be shredded and recycled at the end of the study, whereas biological samples will be discarded in a clinical waste box for disposal.

3.4.6 Study Ethics

This study was approved by MOE Malaysia, as attached in Appendix 3. During the first meeting with the school management, researchers explained the nature

of this study mainly on the involvement of school management, teachers, children, parents and guardians. The study procedures and consent forms were approved by UPM Ethics Committee for Research Involving Human Subjects (JKEUPM), and the approval letter is attached in Appendix 4 (JKEUPM reference no.: JKEUPM-2018-278). The school management was not responsible for recruiting participants for the research.

Consent forms were given to be read and signed by parents or guardians, as attached in Appendix 5. They were written in Malay Language and simple terms that the respondents and their parents or guardians can easily understand. Only children who were given permission and submitted consent form from their parents or guardians were allowed to participate in the study. All respondents were given a choice to continue participating in the study or to pull out at any time when they choose to do so. The information about respondents and their families involved in this research remains confidential. The information obtained is used to serve this study purposes only. The personal information obtained will be destroyed at the end of the study, whereas biological samples and the data created will be archived for further research purposes. Researchers have an ethical obligation to respect respondents' rights and inform their parents or guardians of their results appropriately after peer review. However, the respondents were already informed before the research was conducted on the limitations of interpreting the research results.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characteristics of Respondents

This section outlines the results and discusses the main findings of the study pertaining to the first hypothesis. The first objective of the study was to determine sociodemographic and socioeconomic information, house condition and location, family background, and dietary intake among the respondents in high and low traffic areas.

4.1.1 Response Rate

At the beginning of the study, 646 questionnaires were delivered, but only 536 questionnaires were returned, about 83%. There were 144 respondents (27%) excluded due to not fulfilling the first-level inclusion criteria as listed in Figure 4.1. When separated according to location, 265 out of 327 (81%) questionnaires were returned among the high traffic (HT) group, whereas 271 out of 319 (85%) questionnaires were returned among the low traffic (LT) group. Further, there were 144 respondents excluded due to not fulfilling the second-level inclusion criteria. A total of 248 respondents from 392 respondents continued with biomarker collection. On the other hand, only 52 residences participated in exposure monitoring in residences.

Despite only 248 respondents from 392 respondents being involved in the biomarkers collection, the sample size is adequate because the needed total sample size for biological samples was 234 respondents from both groups. As for the residential assessment, only 52 residences out of 248 residences were successfully recruited in residential air monitoring. These were chosen from respondents who filled in the questionnaire, underwent air monitoring in schools and biological samples collection. The lack of success to get all 248 residences implies that some parents or guardians do not favour strangers to come to their residences and perform monitoring of air pollutants. Despite this limitation, the findings of this study are significant because exposure assessments were performed at both schools and residences.

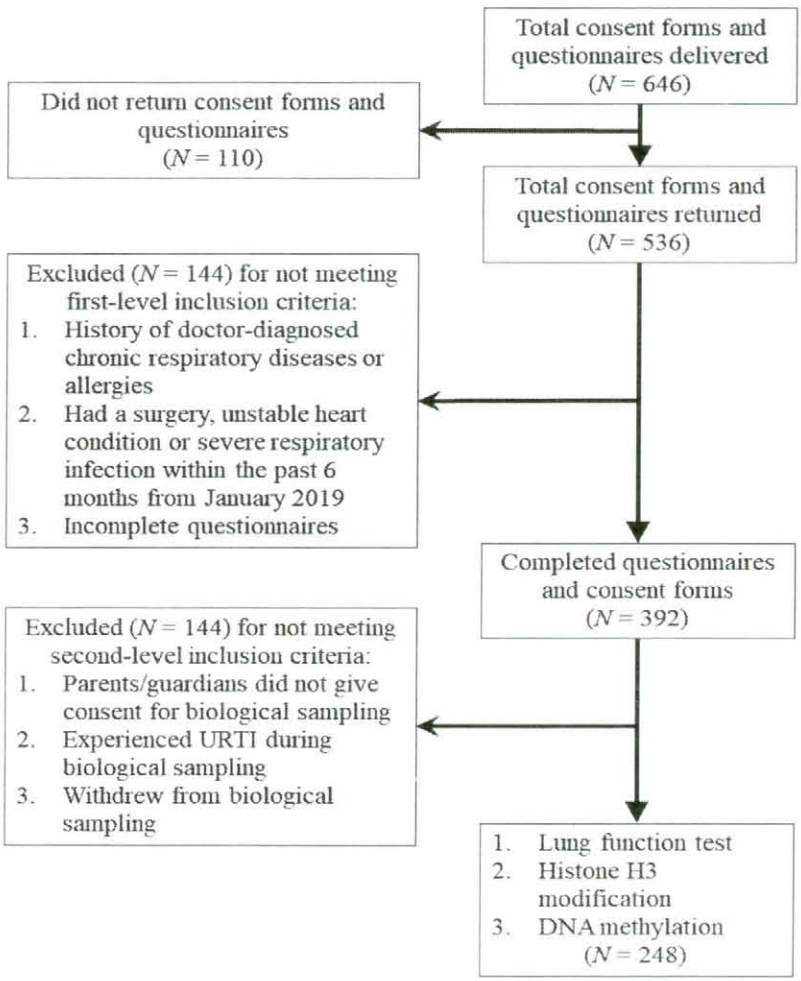


Figure 4.1: Selection of respondents

4.1.2 Sociodemographic and Socioeconomic Information

It has been established that the confounding factor is a trivial matter in research. It is almost impossible to fully eliminate confounding factors in a study that is neither *in vivo* nor *in vitro*. However, confounding factors may be reduced by controlling them effectively at study design and statistical analysis, making the studied groups as similar as possible concerning the confounding factors. Figure 4.2 shows the distribution of respondents according to gender and location. A total of 197 respondents from the HT area and 195 respondents from the LT area participated in exposure monitoring in schools by returning completed questionnaires and consent forms. Out of these, there were 107 children (54.3%) among the HT group, and 92 children (47.2%) among the LT group were boys. Meanwhile, there were 90 children (45.7%) among the HT group, and 103 children (52.8%) among the LT group were girls.

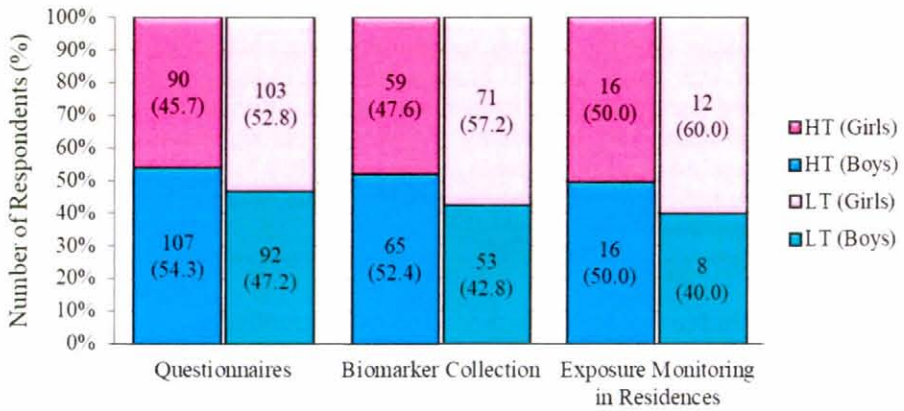


Figure 4.2: Distribution of respondents according to gender and location

Then, a total of 124 respondents from the HT area and 124 respondents from the LT area participated in biological sampling. Out of these, there were 65 children (52.4%) among the HT group, and 53 children (42.7%) among the LT group were boys. Meanwhile, 59 children (47.6%) among the HT group and 71 children (57.3%) among the LT group were girls. Nevertheless, only 52 residences from both areas successfully agreed to participate in exposure monitoring in residences. There were 16 boys (50.0%) and 16 girls (50.0%) from the HT area; also, there were 8 boys (40.0%) and 12 girls (60.0%) from the LT area who had residential monitoring. Overall, the gender distribution in both areas had no significant difference at $p<0.05$.

Table 4.1 shows the sociodemographic background of respondents from both areas. Overall, there were no significant differences between the two groups at $p>0.05$ for all sociodemographic factors. According to primary school classification in Malaysia, 7 – 9 years old are classified as lower, while 10 – 12 years old are classified as upper primary school children. Nevertheless, 12-year-old children were excluded by the school management for they needed to prepare for the Primary School Evaluation Test (UPSR).

Most of the children in the national primary schools in both areas were dominated or fully comprised of Malays. Malay formed approximately 60.7% and 46.3% of the population in Selangor and Kuala Lumpur, respectively (DOS Malaysia, 2021). Selecting one ethnicity in this study also reduced expression level variation among the respondents, which could affect the lung function (Saad, Patel, Minelli, & Burney, 2017) and concentration of biomarkers (Nédélec et al., 2016) studied in this study. According to Section 13A of the Malaysian Birth and Death Registration Act 299, a child should take after the father's race (Attorney General Chamber of Malaysia, 2013). Therefore, any genetic admixture for the respondents, their parents and grandparents were distinguished by following this classification. Malay percentage in this study refers to the percentage of Malay ethnicity in each respondent. 100% means all of the respondent's parents and grandparents are Malays. 75% means one of the respondent's grandparents is

non-Malay, making one of the respondent's parents a mix of Malay and non-Malay. 50% means that one of the respondent's parents is non-Malay. 25% means one of the respondent's grandparents is Malay, while the other grandparents are non-Malay; this makes one parent a non-Malay and the other parent a mix of Malay and non-Malay. There was no significant difference in Malay percentage among respondents in this study. It is found that at least 90% of the respondents from the HT group are 100% Malay, while at least 84% of the respondents from the LT group are 100% Malay.

Table 4.1: Sociodemographic background of respondents

Variables	HT (*N*=197)	LT (*N*=195)	χ^2	*p*
	Number (%)			
Age				
7 – 9 years	82 (41.6)	99 (50.8)	3.30	0.069
10 – 11 years	115 (58.4)	96 (49.2)		
Gender				
Boy	107 (54.3)	92 (47.2)	2.00	0.190
Girl	90 (45.7)	103 (52.8)		
Malay Percentage				
100%	179 (90.9)	165 (84.6)	6.61	0.085
75%	8 (4.1)	6 (3.1)		
50%	3 (1.5)	7 (3.6)		
25%	7 (3.6)	17 (8.7)		
Father's Education Level				
Primary Education	15 (7.6)	19 (9.7)	5.67	0.059
Secondary Education	89 (45.2)	107 (54.9)		
Tertiary Education	93 (47.2)	69 (35.4)		
Mother's Education Level				
Primary Education	17 (8.6)	25 (12.8)	5.58	0.061
Secondary Education	84 (42.6)	97 (49.7)		
Tertiary Education	96 (48.7)	73 (37.4)		

The parents' education level is divided into three categories according to parents' highest certification in education. Primary education consists of lower primary (Standard 1 – 3) and upper primary (Standard 4 – 6), while secondary education consists of lower secondary (Form 1 – 3) and upper secondary (Form 4 – 5). Tertiary education consists of any certification from tertiary education institutions such as a diploma and bachelor's degree. The parents' education background is imperative to discern the questions in the questionnaires. There was no significant difference in parents' education grade among fathers (χ^2=5.67, p=0.059) and mothers (χ^2=5.58, p=0.061) in both groups. It is found that more than half of parents from both groups finished secondary education. The observation also agrees with the results reported by Suhaimi et al. (2015) on no significant difference in parents' educational level between the two studied groups.

Table 4.2 shows the comparison of the socioeconomic background of respondents from both areas. The number of dwellers refers to the number of people living in a house. Meanwhile, Table 4.3 shows household income

categories according to national-level income thresholds for Bottom 40% (B40), Middle 40% (M40) and Top 20% (T20) (Abdul Hamid, Ho, & Ismail, 2019). Overall, there were no significant differences between the two groups at $p<0.05$ for all socioeconomic factors.

Table 4.2: Socioeconomic background of respondents

Variables	HT (*N*=197)	LT (*N*=195)	*z*	*p*
	Median (IQR)			
Total Income (RM)	3500.00 (3500.00)	3000.00 (3032.00)	-1.93	0.053
Household Income/Person (RM)	666.67 (766.67)	562.50 (523.33)	-1.94	0.052
Total Dwellers	5 (1)	6 (1)	-1.21	0.227

Table 4.3: Household income categories of respondents

Household Income Categories	HT (*N*=197)	LT (*N*=195)	χ^2	*p*
	Number (%)			
B40 (<RM3852)	108 (54.8)	123 (63.1)		
M40 (RM3852 – RM8319)	76 (38.6)	63 (32.3)	2.91	0.234
T20 (≥RM8320)	13 (6.6)	9 (4.6)		

When looking at the socioeconomic background, it is shown that the median total income of parents from the HT group was slightly higher at RM3500, compared to the LT group at RM3000.00. Besides that, total dwellers in respondents' residences show a median of 5 and 6 persons for each house among HT and LT groups, respectively. With no significant difference in the median of total income, dwellers, and a slight difference in the median of household income per person in each house for both groups, it can be said that respondents in both groups were living in almost the same sociodemographic and socioeconomic conditions.

Furthermore, the Malaysian government classified the population into three main groups based on their household income: bottom, medium, and top (Department of Statistics Malaysia, 2020). These three groups had a percentage of 40%, 40% and 20%, respectively, thus creating the terms B40, M40, and T20. Both groups had almost the same number of each category, with more than half of the respondents living in the B40. The statistical analysis proved a similar distribution in the group being compared, which can minimise the effects of confounding factors. A small-scale study by Zainudin et al. (2019) reached similar findings on no significant difference in sociodemographic and socioeconomic factors between the exposed and comparative group.

Sociodemographic and socioeconomic factors influence school children's health impacts from exposure to TRAP; however, these confounding factors are controlled at study design in this study. Moreover, only school children free from doctor -diagnosed respiratory illnesses and upper respiratory tract infections

were selected to be involved in the spirometry test and biomarker collection. This screening was done to ensure that only healthy respondents were recruited because respiratory illnesses and infections could be confounding factors, aside from those mentioned in Figure 1.3.

4.1.3 House Conditions and Locations

Although the children were chosen according to their schools' location, whether in the HT or the LT region, it is arduous to restrict their residences' location. Table 4.4 tabulates the comparison of residential background information among the respondents from both groups. Types of properties and distances of residences from main roads, highways and factories also guarantee that the difference between the two areas ruled each area's exposure. A highway is a network of federal roads, while the main road is a network of state roads as gazetted by the Malaysian Public Works Department (Public Works Department Malaysia, 2019). It is shown that there were significant differences in types of properties and distances of residences from the highway between both groups at $p<0.001$. In contrast, there was no significant difference in distance of residences from main roads and factories between both groups at $p<0.05$.

Table 4.4: Comparison of residential background information

Variables	HT (*N*=197)	LT (*N*=195)	χ^2	*p*
	Number (%)			
Types of Properties				
Landed	88 (44.7)	172 (88.2)	83.16	**<0.001***
Strata	109 (55.3)	23 (11.8)		
Distance from Highway				
< 500 m	128 (65.0)	14 (7.2)	141.70	**<0.001***
≥ 500 m	69 (35.0)	181 (92.8)		
Distance from Main Roads				
< 500 m	166 (84.3)	169 (86.7)	0.46	0.500
≥ 500 m	31 (15.7)	26 (13.3)		
Distance from Factories				
< 5 km	15 (7.6)	7 (3.6)	3.00	0.083
≥ 5 km	182 (92.4)	188 (96.4)		

***Significant at $p<0.05$**

The majority of respondents from the HT group live in strata houses, while most of the LT group respondents live in landed houses. The development within the urban vicinity has propelled more strata houses to accommodate the highly populated community. Low-cost flats are the most common residence type in the HT area, while traditional settlements are the most common type of residence in the LT area. To understand the source of outdoor pollutants exposure in residences, the location of each respondent's residence was also assessed based on information obtained from questionnaires. When economic growth and development were brought to urban areas, this has affected the surrounding

area, including establishing more road networks to transport human resources, supplies and products, and the recently built neighbourhood within the vicinity. This finding disagrees with a previous local study by Kamaruddin et al. (2015), who found a different finding whereby the location of their respondents' residences from main roads was significantly different (p=0.001) between the preschool children from the industrial and non-industrial group.

4.1.4 Dietary Intake

Dietary intake data is a useful tool for studying relations between nutrition and health because certain foods with catechol-containing polyphenols such as coffee (Lee & Zhu, 2006) and cocoa powder (Keen, Holt, Oteiza, Fraga, & Schmitz, 2005) have been shown to suppress enzyme activity and activate epigenetically silenced genes (Abdul, Yu, Chung, Jung, & Choi, 2017). Table 4.5 shows the comparison of dietary intake between both groups. There was no significant difference in all these variables between both groups at p<0.05. Hence, the contribution of dietary intake as one of the confounders in this study had been controlled. Nutrients and associated metabolites may directly alter chromatin elements, including DNA primary sequence and histone proteins at various loci, which contribute to modifications in gene expression by determining chromatin structure (Stover, James, Krook, & Garza, 2018).

Table 4.5: Comparison of dietary intake between both groups

Variables	HT (*N*=197)	LT (*N*=195)	χ^2	*p*
	Number (%)			
Eat Chicken or Meat				
Never or very rarely	13 (6.6)	13 (6.7)	0.00	0.979
At least once monthly	184 (93.4)	182 (93.3)		
Eat Fish§				
Never or very rarely	7 (3.6)	3 (1.5)	1.60	0.206
At least once monthly	190 (96.4)	192 (98.5)		
Eat Seafood				
Never or very rarely	19 (9.6)	17 (8.7)	0.10	0.751
At least once monthly	178 (90.4)	178 (91.3)		
Eat Fruits				
Never or very rarely	7 (3.6)	5 (2.6)	0.32	0.570
At least once monthly	190 (96.4)	190 (97.4)		
Eat Vegetables				
Never or very rarely	37 (18.8)	24 (12.3)	3.13	0.077
At least once monthly	160 (81.2)	171 (87.7)		
Drink Milk				
Never or very rarely	32 (16.2)	34 (17.4)	0.10	0.752
At least once monthly	165 (83.8)	161 (82.6)		
Eat Milk Products				
Never or very rarely	47 (23.9)	33 (16.9)	2.90	0.089
At least once monthly	150 (76.1)	162 (83.1)		
Eat Fast Foods§				
Never or very rarely	4 (2.0)	5 (2.6)	0.12	0.724
At least once monthly	193 (98.0)	190 (97.4)		
Drink Fruit Juices				
Never or very rarely	22 (11.2)	23 (11.8)	0.04	0.846
At least once monthly	175 (88.8)	172 (88.2)		
Drink Carbonated Drinks				
Never or very rarely	57 (28.9)	66 (33.8)	1.10	0.295
At least once monthly	140 (71.1)	129 (66.2)		
Eat Health Supplements				
Never or very rarely	92 (46.7)	110 (56.4)	3.70	0.054
At least once monthly	105 (53.3)	85 (43.6)		

***Significant at $p<0.05$;** § By χ^2 test with Yates' correction for expected value <5; very rarely = more than once monthly

From the results of this subsection, the first hypothesis in this study is true for the data; there were significant differences in house condition and location, even though there was no significant difference in sociodemographic and socioeconomic information, family background and dietary intake among the respondents in HT and LT group.

4.2 Air Pollutants

This section presents the results and discusses the main findings of the study pertaining to the second hypothesis. The second objective of the study was to evaluate the concentrations of TRAP and IAQ parameters inside respondents' classrooms and residences in high and low traffic areas.

4.2.1 Respondents' Daily Activities

The children's parents or guardians were required to complete a daily diary about their children's main indoor and outdoor activities (such as studying, eating, transportation, sleeping), indicating the start and end times for each activity using a diary of daily activities. The diaries were distributed once along with the questionnaire and collected over a week, as attached in Appendix 6. The diary method is well suited to the assessment of daily activities in children. The information on daily activities gave an insight into the respondents' mobility habits and potential sources of air pollution other than the schools they attended. Daily-activity diaries can be incorporated with the exposure prediction models to provide a detailed exposure estimation for epidemiological studies (Yarza et al., 2020). Consequently, a detailed report for each child was carried out with parents' or guardians' help to identify the activities that took up most of their time. Thus, the use of diaries reduces the likelihood of errors associated with measurement in schools and residences and enables the acquisition of valid information. Moreover, daily diary activities complement personal exposure monitoring, particularly dose-response assessments (Lei et al., 2020). Nevertheless, dose-response assessments were not included in this study.

Figure 4.3(a) shows children's daily activities on weekdays from Monday to Friday. Both groups of children were combined in the same chart. Others included locations such as playgrounds, recreational parks, and education centres. All of them (100%) spent at least 6 h (25.0%) of their time at schools and 7 h (29.2%) of their time at homes. There were 14.3% of the children who stayed back at schools in the evening for additional classes and co-curricular activities. Another 28.6% of children stayed at daycare centres in the evening while waiting for their parents to fetch them after work. In the late evening, most children had started to return home. Between 8 – 11 p.m., 17.9% of the children had tuition classes at education centres, while most of them did their homework and study revision at homes.

On the other hand, Figure 4.3(b) shows children's activities on weekends. Both groups of children were combined in the same chart. Others included locations such as sports complexes, shopping malls, relatives' houses, and tourism attractions. All of them (100%) spent at least 7 h (29.2%) of their time at home. A few children (10.7%) spent time at schools for co-curricular activities and additional classes in the morning. Some of their activities on weekends included recreational activities, helping parents with house chores, visiting relatives,

travelling, sports training, leisure activities at homes and study revision. Overall, it is clear from the findings that school children spend between 70% – 90% of their time indoors, such as in schools, homes, daycares and education centres.

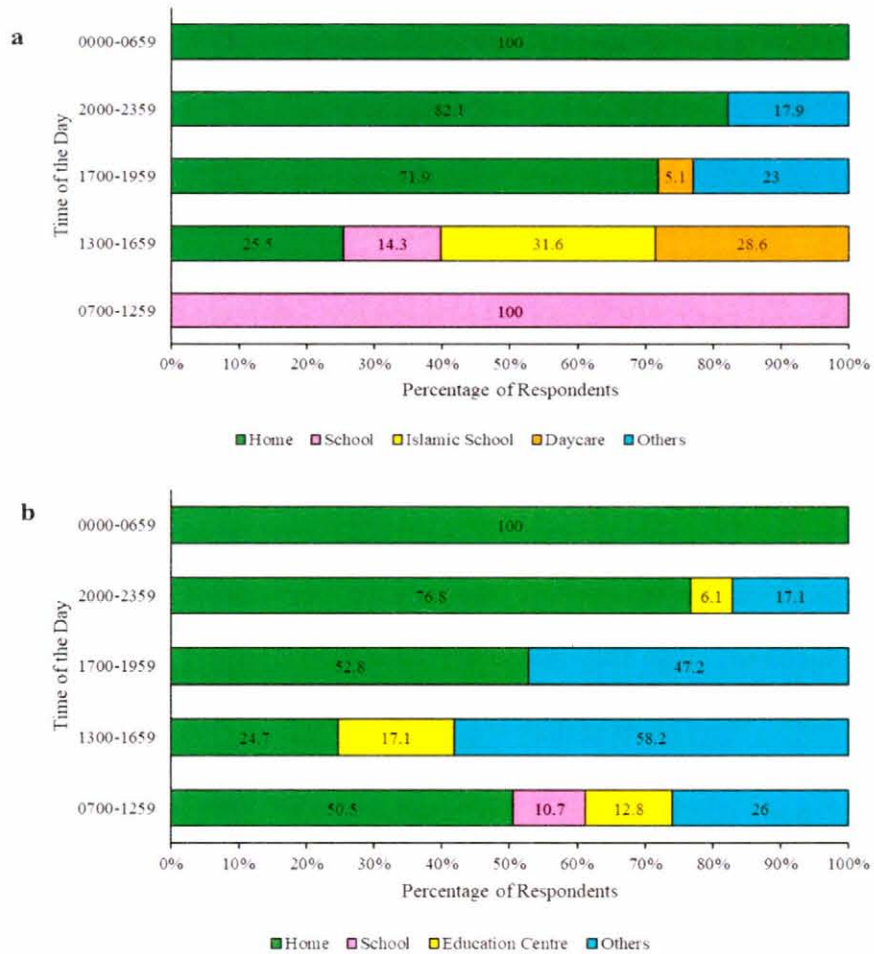


Figure 4.3: Respondents' daily activities on (a) weekdays and (b) weekends

4.2.2 Respondents' Traffic Exposure at Residences

Figure 4.4 shows respondents' mode of transportation to schools. Most children commuted to school by motorcycle; there were 86 children (43.7%) among the HT group and 99 children (50.8%) among the LT group. The second most frequent mode was car; there were 83 children (42.1%) among the HT group and 78 children (40.0%) among the LT group. Only 19 children (9.6%) among the HT group and 17 children (8.7%) among the LT group went to school by walking. Meanwhile, the least option was travelling by bus; there were 9 children (4.6%) among the HT group and 1 child (0.5%) among the LT group. There was no significant difference in transportation mode to schools between both groups at $p>0.05$.

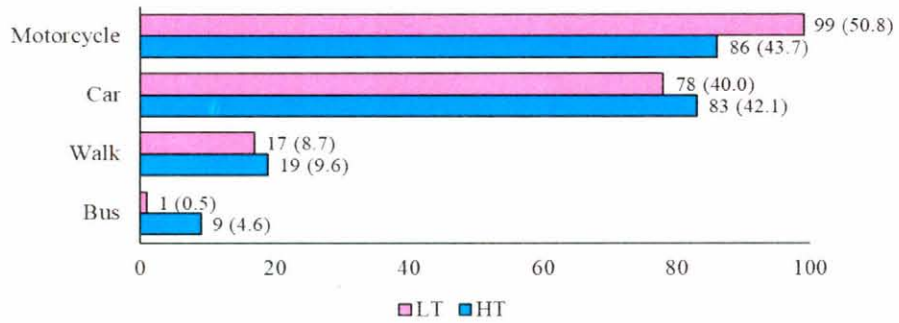


Figure 4.4: Mode of transportation to school among respondents

Figure 4.5(a) shows the number of respondents who reported <100 vehicles per day around their residences. Meanwhile, Figure 4.5(b) shows the number of respondents who reported at least 100 vehicles per day around their residences. This information was obtained from the questionnaires (Appendix **6**: G12 – G15). The percentage was calculated according to the total respondents in each HT and LT group. There were significant differences between both groups for all vehicles during weekdays and weekends at $p<0.05$, with more vehicles were reported around residential areas of the HT group. Most of the LT group respondents (≥50%) reported that the number of vehicles around their residences were less than 100 per day, either during weekdays or weekends. However, there were different patterns for the HT group. There were 118 respondents (59.9%) and 109 respondents (55.3%) who reported more than 100 vehicles per day on weekdays, for cars and motorcycles, respectively. Furthermore, cars and motorcycles were mostly reported to be <100 per day during weekends, while buses and lorries were mostly reported to be <100 per day either during weekdays or weekends.

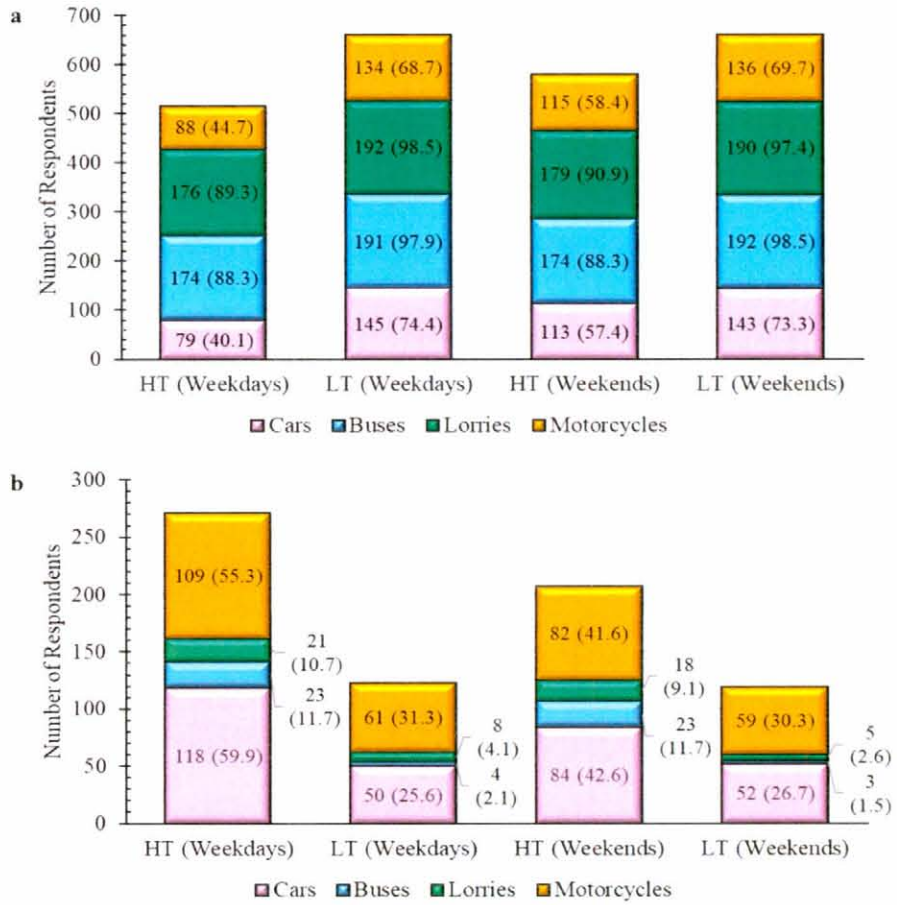


Figure 4.5: Number of respondents who reported (a) <100 vehicles per day around their residences and (b) ≥100 vehicles per day around their residences

Figure 4.6(a) shows parents' or guardians' perception among the HT group on traffic exposure in children's residences based on traffic congestion of cars, lorries and buses, black smoke, and smell of smoke from vehicles. Meanwhile, Figure 4.6(b) shows the same variables as Figure 4.6(a), but these were reported among the LT group. This information was obtained from the questionnaires (Appendix 6: G6 – G11). There were significant differences between both groups at $p<0.05$ for all variables except the smell of vehicle smoke. Most of the LT group respondents (≥50%) reported never seeing all variables assessed. However, there were different patterns for HT group respondents. Most respondents reported frequent traffic congestion of cars (42.9%), seldom congestion of lorries and buses (47.2%), never see a presence of black smoke (49.2%) and never smell smoke in vehicles (51.3%) around their residences. In this study, "seldom", "frequent", and "continuous" were defined as at least once a week, at least once a day, and at least once an hour, respectively.

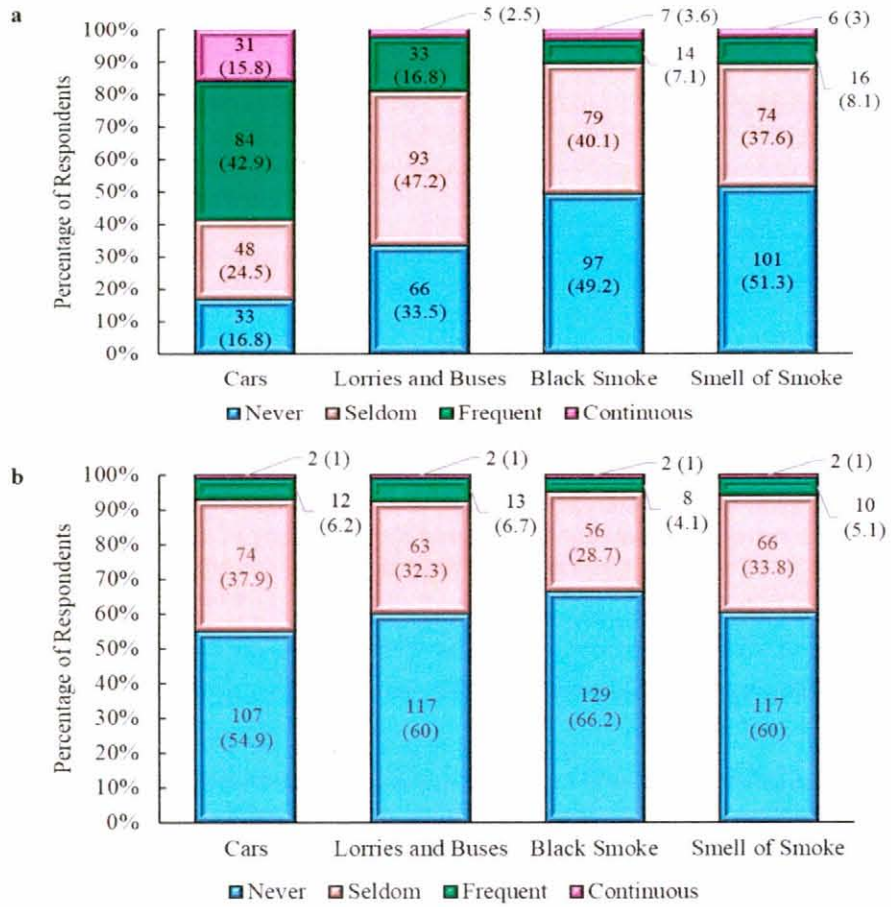


Figure 4.6: Perception of traffic exposure in respondents' residences among (a) HT group and (b) LT group

Exposure to TRAP varies in urban areas. The location and design of this study supported the assessment of the impact of TRAP in the Klang Valley region, where regional air pollution levels are relatively high (Amil et al., 2016; Noorlin Mohamad, Latif, & Khan, 2016). This particular study area enabled a better focus on variations in air quality related to localised TRAP. As proxies for TRAP exposure, proximity to the nearest major road and density of nearby roads were used to evaluate the associations between TRAP exposure and children's health impacts. Staying along busy roads is correlated with an increased risk of TRAP exposure (Urman et al., 2018). There is a fair estimation of a person's TRAP exposure; the TRAP estimate used in this analysis – the number of vehicles and traffic exposure perception. In addition, most respondents in the study do not have an air conditioning unit but are naturally ventilated by opening windows. This act has encouraged TRAP penetration indoors; mostly, those residences located close to a busy road.

Different strategies have been applied to assess the influence of residential TRAP on children. Both objective and subjective quantitative measures were applied in this study. The subjective quantitative measure used questionnaires to gather information. Self-reported traffic estimates are economically efficient and straightforward to collect but inevitably subject to different perceptions and biases. Compared to objective quantitative exposures, self-reported traffic estimations, such as estimations of traffic counts around the residences or traffic annoyances, have demonstrated that subjective evaluations are robust in homogenous population, particularly in urban areas (Hegseth et al., 2019).

While several previous research of respiratory diseases employing exposures to TRAP has been carried out, some studies have also shown associations using traffic indicators (Mohd Shafie & Mahmud, 2017; Wendt Hess, Bachler, Momin, & Sexton, 2019). These indicators help to concentrate on air emissions link explicitly to transportation. Although other studies raised possible exposure misclassification of dispersion models (Lin, Chi, & Lin, 2020; Wendt Hess et al., 2019), there have also been concerns about inconsistency in monitor-based air pollution estimates when monitoring networks are out of coverage (Zou, Zheng, Wan, Qiu, & Wilson, 2016).

4.2.3 Sources of IAQ in Residences

Various sources could lead to indoor air pollutants accumulated in residences. Table 4.6 shows the comparison of sources of IAQ in residences as obtained from the returned questionnaires. There was no significant difference in all these variables between both groups at $p<0.05$, except for ventilation systems. It is shown that there was a significant difference in the ventilation system in residences at $p<0.001$. All residences from both groups used mechanical ventilation to circulate the internal air movement. However, most of the residences had only fans, e.g. ceiling fans and table fans, while some interchanged between air-conditioning units and fans.

Even though most of the residences use only fan as their ventilation system, there was a significant difference between both groups. The number of residences using both air-conditioner and fan was doubled among the HT group than the LT group. Residences that use only fan for ventilation typically open the doors and windows to allow for more air exchange between the indoor and outdoor environment. Air is repeatedly infiltrating and departing the spots in residences through cracks in windows and others. The outdoor temperature in Malaysia is typically lower at night, so most people would embrace the cooling at night by opening the windows. Humid air can enter the building when windows are opened for ventilation at night; then, this humid air gets contained inside the residences during the day when the windows are closed (Tuck et al., 2019).

The shorter the distance of residences from main roads, the higher the concentrations of pollutants piled up from the roads. However, it is less noted

that residences located away from main roads may also hoard pollutants when the air that escaped the residences is absorbed into the residences via a slight vacuum produced by warm air in the residences. Moreover, a study discovered that household concentrations of $PM_{2.5}$ and NO_2 during window opening periods were significantly higher than during reference periods, which were 1 h before and 1 h after window-opening periods (Yen, Yang, Mena, Cheng, & Chen, 2019).

Table 4.6: Comparison of sources of residential IAQ between both groups

Variables	HT (*N*=197)	LT (*N*=195)	χ^2	*p*
	Number (%)			
Indoor Painting within the Past 12 Months				
Yes	47 (23.9)	62 (31.8)	3.08	0.079
No	150 (76.1)	133 (68.2)		
Floor Renovation within the Past 12 Months				
Yes	15 (7.6)	21 (10.8)	1.17	0.279
No	182 (92.4)	174 (89.2)		
Furry Pets				
Yes	36 (18.3)	51 (26.2)	3.52	0.060
No	161 (81.7)	144 (73.8)		
Usage of Mosquito Coils				
Yes	14 (7.1)	21 (10.8)	1.62	0.204
No	183 (92.9)	174 (89.2)		
Usage of Carpet				
Yes	96 (48.7)	86 (44.1)	0.84	0.358
No	101 (51.3)	109 (55.9)		
Usage of Cleaning Products				
Yes	49 (24.9)	47 (24.1)	0.03	0.859
No	148 (75.1)	148 (75.9)		
Usage of Moth Balls				
Yes	41 (20.8)	32 (16.4)	1.25	0.263
No	156 (79.2)	163 (83.6)		
Usage of Air Fresheners				
Yes	86 (43.7)	74 (37.9)	1.32	0.250
No	111 (56.3)	121 (62.1)		
House Cleaning Frequency Per Week				
< 6 times	99 (50.3)	96 (49.2)	0.04	0.839
≥ 6 times	98 (49.7)	99 (50.8)		
Indoor Smoking				
Yes	73 (37.4)	69 (35.6)	0.15	0.702
No	122 (62.6)	125 (64.4)		
Type of Ventilation System				
Air-Conditioning and Fan	55 (27.9)	24 (12.3)	14.84	**<0.001***
Fan Only	142 (72.1)	171 (87.7)		
Type of Cooking Stove				
Electric and Gas	41 (20.8)	39 (20.0)	0.04	0.842
Gas Only	156 (79.2)	156 (80.0)		
Daily Cooking Activity				
< 3 times	113 (57.4)	102 (52.3)	1.01	0.315
≥ 3 times	84 (42.6)	93 (47.7)		
Usage of Cooking Hood and Hob				
Yes	37 (18.8)	29 (14.9)	1.07	0.301
No	160 (81.2)	166 (85.1)		
Open Window or Door While Cooking				
Yes	190 (96.4)	186 (95.4)	0.28	0.595
No	7 (3.6)	9 (4.6)		

***Significant at $p<0.05$**

4.2.4 Traffic Counts (TC) in Schools

TC in schools is one of the traffic indicators. Figure 4.7 shows the average count of vehicles passed by the schools via the main roads nearest to the schools for 1 h in the morning (7 – 8 a.m.) and the afternoon (12.30 – 1.30 p.m.) when the children were taken to and fetched from school. All four schools in HT areas showed higher densities of medium-sized vehicles in comparison to light and heavy vehicles. In contrast, all four schools in LT areas documented more figures of light-sized vehicles as compared to medium and heavy vehicles. Light vehicles consist of motorcycle, medium for cars and taxis, while heavy category comprised of buses and good vehicles. This finding clearly shows that children in the HT group are prone to high TRAP emission than the LT group due to higher TC surveys.

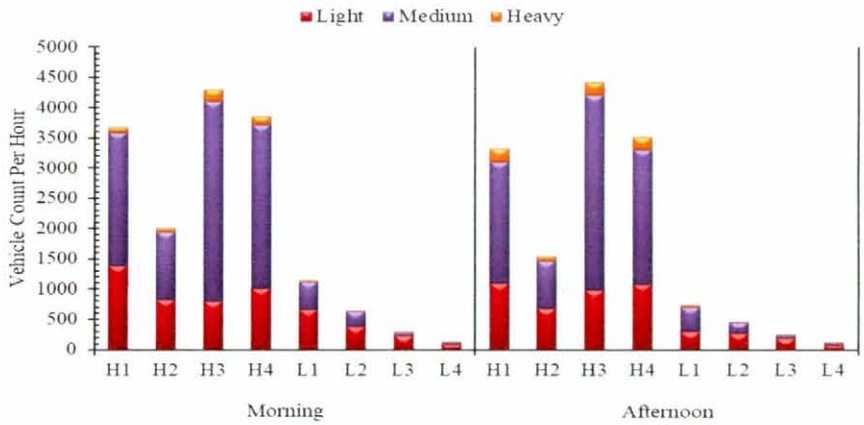


Figure 4.7: The average count of passing vehicles per hour in the morning and afternoon

The TC reflect the difference in the socioeconomic status between the community in both areas. A report on comparison of motorcycle usage in various countries found that households are more likely to own cars than to own motorcycles when the household incomes are higher (Erath, Fourie, Van Eggermond, & Ordoñez Medina, 2018). Therefore, motorcycles and cars replace each other, depending on the socioeconomic status. Moreover, a local study reported that many motorists in the urban area prefer to ride on cars than motorcycles due to the environment, such as the rainy season and road safety (Sultan et al., 2016). Apart from socioeconomic status, some of the children in this study's selected schools were observed travelling with more than one person in a car, which means that cars are a preferable mode of transportation due to the ease of car-pooling.

Idling vehicles near school areas also affect the air quality in the school environment because the running engines emit air pollutants (Barnes, Ng, Ma, & Lai, 2018). Figure 4.8 shows the average count of idling vehicles for 30 minutes in the morning (7 – 7.30 a.m.) and in the afternoon (1.00 – 1.30 p.m.) when the children were taken to and fetched from school. All schools except H4 recorded a higher number of light-sized vehicles as compared to medium-sized vehicles. H1 was the only school observed to have the children used heavy-sized vehicles, which were the school buses as their transport to school.

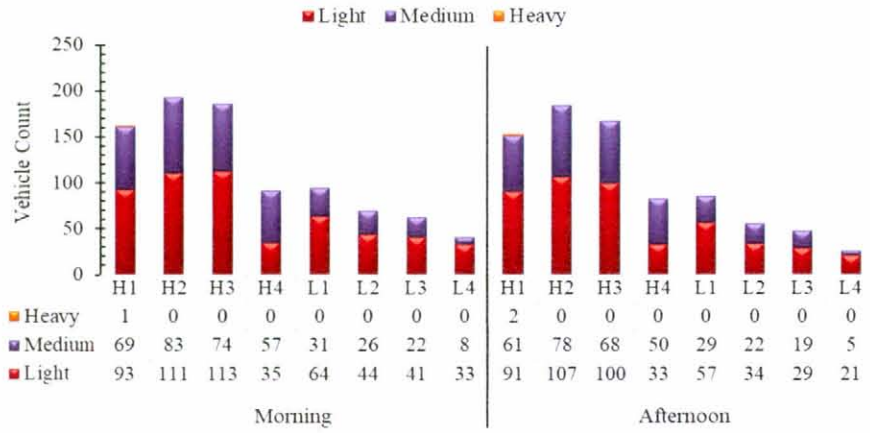


Figure 4.8: The average count of idling vehicles in the morning and afternoon

These traffic emissions could also affect the children's health when the children waited for their parents or transporters after school nearby where idling vehicles were parked (Jeong & Park, 2017). Figure 4.9 shows the average idling time of vehicles in the morning and afternoon. In the afternoon, the idling time of vehicles was nearly doubled or tripled the idling time in the morning. For example, H1 recorded an average of 165 s of idling time of medium-sized vehicles in the afternoon, which was almost doubled the idling time of medium-sized vehicles in the morning at 85 s. This finding could be due to the nature of 'drop-and-go' in the morning when parents just drop their children to school; they ensure that the children walk safely inside the school's compound, then continue their journey to work or other locations. Meanwhile, in the afternoon, the parents or transporters had to wait for the children until their school session ended for the day.

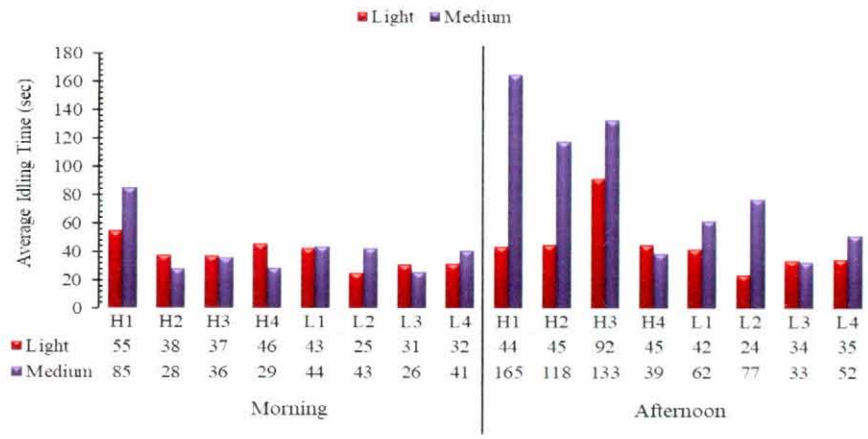


Figure 4.9: The average idling time of vehicles in the morning and afternoon

However, based on observation during the TC survey at HT and LT schools, many light-sized vehicles were observed idling nearby school compound in the afternoon. It was believed that those vehicles belong to school children's parents waiting for their children at the end of the school session. A high number of medium-sized vehicles contributed to the high emission of TRAP compared to a low number of large-sized vehicles (Kheirbek, Haney, Douglas, Ito, & Matte, 2016). Even though emissions from medium-sized vehicles have less impact than heavy-sized vehicles on air quality and health, overwhelming numbers of medium-sized vehicles contribute to congestion, which increases TRAP emissions on routes shared with heavy-sized vehicles (Jeong & Park, 2017).

Idling automobile emissions are deemed more hazardous to public health since the wake of a moving vehicle cannot disperse toxins. Pollutant accumulation around the vehicles would be high due to poor combustion conditions in the engine cylinder during idling and poor dispersion when the vehicle is not moving (Banerjee & Christian, 2017). This situation poses a threat to those waiting in the vehicles or near the vehicles. Furthermore, it is typical for parents to turn on the vehicle engine for air conditioning comfort while waiting for their children to end the school session. The locations where the school children wait to be fetched are usually near the traffic flow, including idling vehicles. Although some parents would turn off their vehicle engines and open the windows of the vehicles while waiting, they would also be at risk of exposure to TRAP from nearby idling vehicles. In a previous study of dispersion of motor vehicle pollution under idle condition, TVOC levels at idle engine were higher than that during driving (Barnes et al., 2018).

4.2.5 Concentrations of Air Pollutants in Schools

Exposure monitoring inside and outside of the classrooms was done using special equipment during school session, as mentioned in part 3.4.2 of this thesis. The physical parameters included were temperature (T) and relative humidity (RH). CO_2 was also studied as a ventilation performance indicator. Meanwhile, the parameters for air pollutants were PM_{10}, $PM_{2.5}$, PM_1, NO_2, SO_2, O_3, TVOC, CO and BC.

The indoor monitoring results are tabulated in Table 4.7, and it was revealed that the exposure to TRAP was significantly higher for the HT group compared to the LT group. There were significant differences for all TRAP and IAQ parameters between both groups at $p<0.05$. As for physical parameters in classrooms, there was no significant difference observed for T for both groups. Nevertheless, there were significant differences between the two groups at $p<0.05$ for CO_2 as a ventilation parameter and RH as a physical parameter. On the other hand, the results for outdoor monitoring are tabulated in Table 4.8, and it was found that the exposure to TRAP was significantly higher for the HT group compared to the LT group. As for meteorological parameters, there was no significant difference observed for temperature for both groups. Nevertheless, there were significant differences between CO_2 and RH between the two groups at $p<0.05$. Table 4.8 also displays various air pollutants recorded from the Batu Muda and Cheras stations at the same period when measurements of TRAP were performed at the schools, which were between 7.20 a.m. to 1.20 p.m. from Monday to Friday on the same date. As shown in **Table 3.1**, only HT schools have monitoring stations near the schools, which are Batu Muda and Cheras stations in this study. In contrast, there is no monitoring station near the LT schools. Only BC had 32 filter samples each for indoor and outdoor monitoring due to its 24-h measurement duration, whereas the other parameters were measured for 6-h each session.

The indoor parameters could not be compared directly with the ICOP for 2010 because the schools are non-commercial buildings and not equipped with mechanical ventilation and air conditioning. However, the outdoor parameters can be compared with the IT-2 of MAAQS due to good agreements between the portable and stationary instruments proven by previous studies (Lin et al., 2015; Wang et al., 2016).

Table 4.7: Indoor TRAP and physical parameters of selected schools

Variables	Locations	Min	Max	Median	IQR	*z*	*p*	ICOP
PM_{10} ($\mu g/m^3$)	HT	89.0	140.0	112.0	33.0	-17.14	**<0.001***	150
	LT	33.0	86.0	63.0	30.0			
$PM_{2.5}$ ($\mu g/m^3$)	HT	68.0	114.0	79.0	26.0	-17.15	**<0.001***	n/a
	LT	17.0	60.0	45.0	25.0			
PM_1 ($\mu g/m^3$)	HT	62.0	103.0	72.0	23.0	-17.15	**<0.001***	n/a
	LT	13.0	53.0	35.0	24.0			
NO_2 (ppb)	HT	30.0	54.0	33.0	3.0	-17.19	**<0.001***	n/a
	LT	8.0	16.0	12.0	4.0			
SO_2 (ppb)	HT	20.0	30.0	20.0	0.0	-16.55	**<0.001***	n/a
	LT	Below LOD	20.0	10.0	10.0			
O_3 (ppb)	HT	22.0	26.0	24.0	3.0	-17.24	**<0.001***	n/a
	LT	10.0	16.0	13.0	2.0			
TVOC (ppb)	HT	22.0	527.0	318.0	432.0	-17.47	**<0.001***	3,000
	LT	Below LOD	10.0	0.0	8.0			
CO (ppb)	HT	70.0	270.0	23.0	20.0	-17.41	**<0.001***	10,000
	LT	Below LOD	20.0	10.0	10.0			
BC ($\mu g/m^3$)‡	HT	25.1	71.2	35.2	16.7	-15.60	**<0.001***	n/a
	LT	3.8	37.6	9.7	13.7			
CO_2 (ppm)	HT	263.5	317.1	272.9	27.4	-13.14	**<0.001***	1000
	LT	273.2	355.9	320.6	45.4			
T (°C)	HT	28.9	30.1	29.9	0.3	-15.94	**<0.001***	23 – 26
	LT	27.0	29.4	27.9	0.7			
RH (%)	HT	65.3	76.5	67.5	4.5	-5.26	**<0.001***	40 – 70
	LT	63.5	77.1	71.7	5.3			
Vel (m/s)	HT	0.2	0.3	0.3	0.1	-0.30	0.764	0.15 – 0.50
	LT	0.2	0.4	0.3	0.1			

N=40; ***Significant at *p*<0.05; ‡ *N*=32

Table 4.8: Outdoor TRAP and meteorological factors of selected schools and nearby CAQM stations

Variables	Locations	Min	Max	Median	IQR	*z*	*p*	MAAQS
PM_{10} (µg/m^3)	HT	93.0	153.0	124.0	36.0	-16.94	**<0.001***	120 µg/m^3 for 24 h
	LT	39.0	95.0	63.0	28.0			
	Batu Muda	8.4	58.7	26.0	11.4	-2.244	**0.025***	
	Cheras	6.3	71.8	28.6	13.0			
$PM_{2.5}$ (µg/m^3)	HT	71.0	115.0	97.0	33.0	-17.15	**<0.001***	50 µg/m^3 for 24 h
	LT	23.0	69.0	40.0	24.0			
	Batu Muda	4.3	54.5	18.2	10.7	-1.518	0.129	
	Cheras	2.0	62.4	20.3	10.6			
PM_1 (µg/m^3)	HT	60.0	104.0	86.0	26.0	-17.15	**<0.001***	n/a
	LT	17.0	59.0	33.0	23.0			
NO_2 (ppb)	HT	48.0	375.0	53.0	13.0	-17.18	**<0.001***	160 ppb for 1 h
	LT	17.0	25.0	22.0	4.0			
	Batu Muda	23.0	39.9	10.0	18.0	-0.290	0.772	
	Cheras	20.8	38.2	10.9	16.9			
SO_2 (ppb)	HT	30.0	40.0	10.0	10.0	-18.18	**<0.001***	115 ppb for 1 h
	LT	10.0	20.0	0.0	0.0			
	Batu Muda	5.5	17.2	3.0	1.5	-2.851	**0.004***	
	Cheras	2.0	5.2	0.9	0.7			
O_3 (ppb)	HT	23.0	26.0	25.0	2.0	-17.27	**<0.001***	100 ppb for 1 h
	LT	10.0	18.0	14.0	5.0			
	Batu Muda	40.6	53.8	7.2	17.1	-4.016	**<0.001***	
	Cheras	63.9	86.4	16.5	33.4			
TVOC (ppb)	HT	155.0	988.0	749.0	415.0	-17.22	**<0.001***	n/a
	LT	Below LOD	98.0	29.0	72.0			
CO (ppb)	HT	310.0	380.0	350.0	20.0	-17.29	**<0.001***	30, 568 ppb for 1 hr
	LT	Below LOD	20.0	10.0	20.0			
	Batu Muda	400.0	2700.0	700.0	800.0	-3.156	**0.002***	
	Cheras	350.0	2400.0	500.0	600.0			

Table 4.8: Continued

Variables	Locations	Min	Max	Median	IQR	*z*	*p*	MAAQS
BC (μg/m^3)‡	HT	23.0	81.7	54.5	23.4	-16.61	**<0.001***	n/a
	LT	4.6	30.5	14.1	10.6			
CO_2 (ppm)	HT	272.8	354.8	280.3	59.1	-2.38	**0.017***	n/a
	LT	267.5	359.3	314.0	53.2			
AT (°C)	HT	26.0	29.2	28.3	0.9	-0.20	0.843	n/a
	LT	26.7	29.5	28.2	1.4			
	Batu Muda	23.7	35.6	29.4	6.4	-0.418	0.676	
	Cheras	23.3	34.5	29.5	5.6			
RH (%)	HT	63.4	81.7	70.4	7.7	-8.05	**<0.001***	n/a
	LT	58.4	76.4	64.4	9.3			
	Batu Muda	43.4	96.5	70.0	25.5	-0.729	0.466	
	Cheras	40.3	93.5	69.4	23.8			
WS (m/s)	HT	0.5	1.0	0.6	0.2	-11.72	**<0.001***	n/a
	LT	0.6	1.0	0.8	0.2			
	Batu Muda	0.1	8.3	1.1	1.2	-3.194	**0.001***	
	Cheras	0.2	2.3	0.9	0.5			

N=40; ***Significant at *p*<0.05**; ‡ *N*=32

For 6-h indoor measurements, the CO_2 concentrations were recorded in the sequence from the highest to the lowest concentrations of H2>H4>H3>H1>L1>L3>L4>L2 and ranged between 226.1 and 277.8 ppm with an overall average concentration of 292.1 ± 62.9 ppm. Meanwhile, for outdoors, the sequence was H2>H4>H3>H1>L1>L2>L4>L3 and ranged between 274.9 and 337.3 ppm with an overall average concentration of 310.4 ± 69.0 ppm. As determined by the median, the LT group's exposures to indoor CO_2 in schools were slightly higher than the HT group's exposures in schools with a significant difference. In addition to that, the LT group's exposures to outdoor CO_2 in schools were slightly higher than the HT group's exposures in schools with a significant difference. Higher concentrations of indoor CO_2 in the LT schools could be explained by the higher number of occupants per classroom in the LT schools than in the HT schools. Besides, a previous study suggested the effects of ventilation rate, duration of occupancy and occupants' metabolic rates; all these factors contribute to CO_2 concentrations indoors (Wargocki, Porras-Salazar, Contreras-Espinoza, & Bahnfleth, 2020). On the other hand, higher concentrations of outdoor CO_2 in the LT schools were possibly caused by open burning nearby the school area.

For 6-h indoor measurements, the temperature was recorded in the sequence from the highest to the lowest concentrations of H1>H4>H3>H2>L3>L1>L4>L2 and ranged between 25.43 and 32.49 °C with an overall average concentration of 28.75 ± 2.76 °C. Meanwhile, for outdoors, the sequence was H1>H4>H3>H2>L4>L3>L1=L2 and ranged between 23.30 and 33.30 °C with an overall average concentration of 29.13 ± 6.90 °C. As governed by the median, the HT group's exposures to indoor temperature in schools were slightly higher than the LT group's exposures in schools with a significant difference. HT group's exposures to outdoor temperature in schools had no significant difference from the LT group's exposures to the outdoor temperature.

For 6-h indoor measurements, the RH was recorded in the sequence from the highest to the lowest concentrations of L2>H2>H4>L1>L4>L3>H1>H3 and ranged between 54.87 and 84.08% with an overall average concentration of 69.91 ± 11.76%. Meanwhile, for outdoors, the sequence was H2>L1>H3>H4>H1>L2>L4>L3 and ranged between 44.75 and 91.50% with an overall average concentration of 62.28 ± 32.25%. As decided by the median, the LT group's exposures to indoor RH in schools were 1.1 times higher than the HT group's exposures in schools with a significant difference. In addition to that, the HT group's exposures to outdoor RH in schools were 1.1 times higher than the LT group's exposures in schools with a significant difference.

The temperature in indoor environments is closely related to RH. As the temperature increases, the saturation of the air to hold water vapour increases. This finding results in a lower percentage of water vapour inside, hence a lower RH value. A simple principle of convection illustrates this effect; while indoor moist air rises to space above, dry air replaces the lower space with less RH. High RH indoors may be caused by poor ventilation and is unsafe when combined with high temperature. The capacity of the body to cool itself is

disturbed, contributing to discomfort among occupants. Moreover, urban heat island could cause the temperature at HT schools to be higher than the temperature at LT schools, as confirmed in a previous local study (Zaki et al., 2020). Indoor temperature is partly contributed by the outdoor temperature due to open windows and doors that increases air moving into the classrooms. The presence of ceiling fans also helps to distribute the outdoor air in the classrooms. When temperature exceeded the recommended range, it could cause discomfort to the children in the classrooms and affect their ability to learn and function. Moreover, high RH levels reduce children's concentration and increase sleepiness (Teleszewski & Gładyszewska-Fiedoruk, 2020). In contrast to the findings of indoor RH and temperature, indoor CO_2 demonstrated adequate ventilation in the classrooms, as shown by the average concentrations below 1000 ppm.

Figure 4.10(a) shows daily variations of 24-h indoor and outdoor $PM_{2.5}$ and BC in HT schools, while Figure 4.10(b) shows daily variations of 24-h indoor and outdoor $PM_{2.5}$ and BC in LT schools. For 24-h measurements for indoors, the BC concentrations were recorded in the sequence from the highest to the lowest concentrations of H1>H2>H4>H3>L1>L4>L3>L2 and ranged between 3.8 and 71.2 $\mu g/m^3$ with an overall average concentration of 27.5 ± 18.4 $\mu g/m^3$. Meanwhile, for outdoors, the sequence was H3>H1>H2>H4>L1>L2>L3>L4 and ranged between 4.6 and 81.7 $\mu g/m^3$ with an overall average concentration of 32.5 ± 22.5 $\mu g/m^3$. As determined by the median, the HT group's exposures to indoor BC in schools were 3.6 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor BC in schools were 3.9 times higher than the LT group's exposures in schools with a significant difference.

A local study by Abdul Rahman et al. (2011) discovered that BC was one of the major components of $PM_{2.5}$ with a weight fraction of about 15.8%. Several years later, the same team of researchers conducted a similar study and reported that BC was one of the major components of $PM_{2.5}$ with a weight fraction of about 16% (Abdul Rahman et al., 2015). They conducted both of their studies at a Klang Valley site, which was within 15 km distance from all the studied schools in this study. Emissions with high BC typically represents exhaust from motor vehicles.

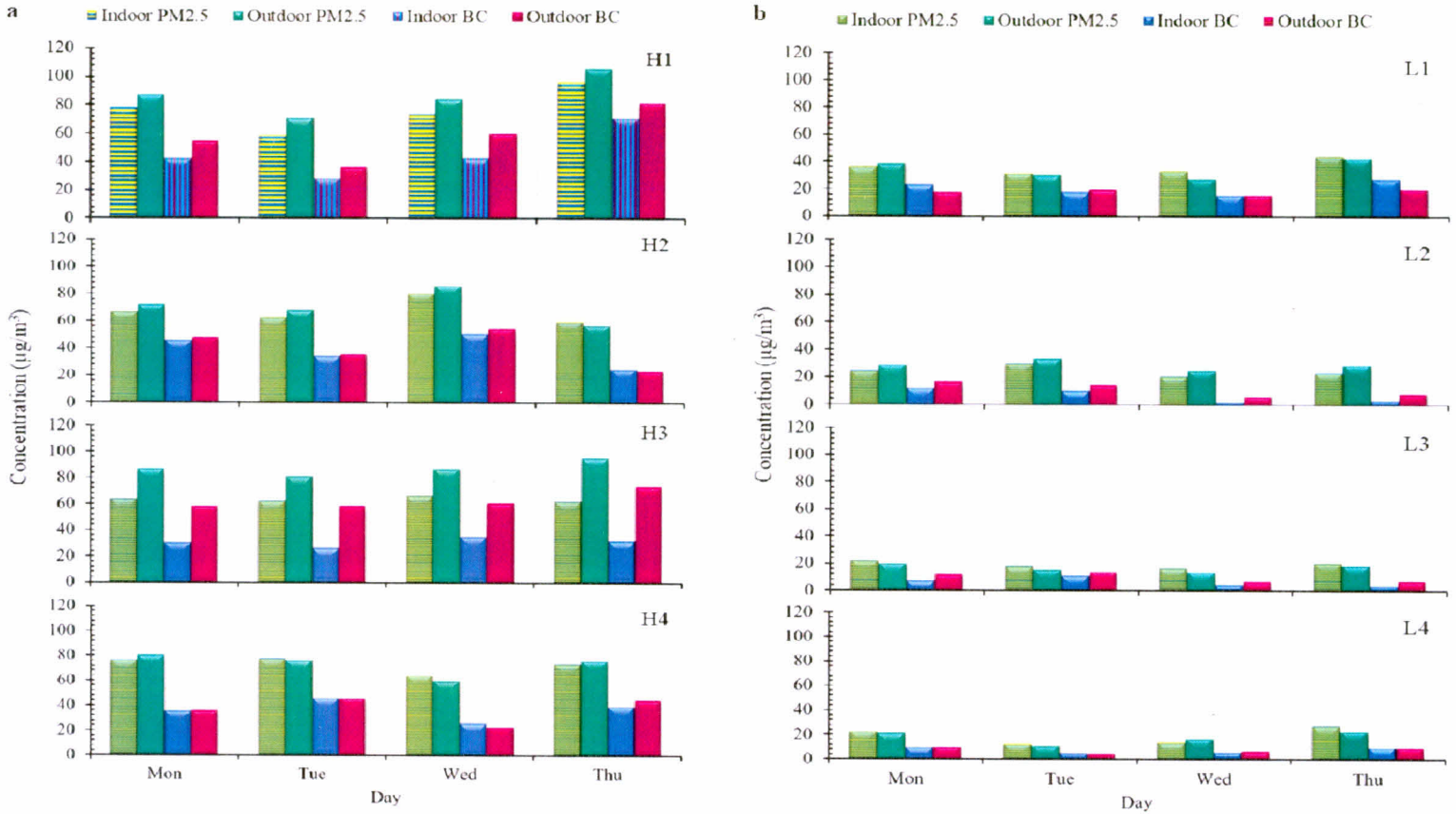


Figure 4.10: Daily variations of 24-h indoor and outdoor $PM_{2.5}$ and BC in (a) HT schools and (b) LT schools

Figure 4.11(a) shows daily variations of 6-h indoor particulate matter in HT schools, while Figure 4.11(b) shows daily variations of 6-h outdoor particulate matter in HT schools. Figure 4.12(a) shows daily variations of 6-h indoor particulate matter in LT schools, while Figure 4.12(b) shows daily variations of 6-h outdoor particulate matter in LT schools. All schools showed a similar indoor trend at the starting of the school session on Monday at 7.20 a.m. This finding could be due to the housekeeping activities performed in the classrooms after 2-days long weekends. Besides, all schools showed a similar increasing trend for outdoor particulate matter at the end of the school session every day. This trend could be due to parents or guardians who waited for the children with idling vehicles near the school compound around 12.30 p.m. onwards.

Some outdoor particulate matter sources include natural sources, which are harder to control, such as pollen, forest fires, and sea salt. However, none of the sites in this study is located within a 5 km radius from the nearest coastal areas in Selangor, hence spray of sea salt particles may not have contributed to the mass concentrations of PM_{10} and $PM_{2.5}$ in Klang Valley. Although a local study mentioned biomass burning is the second highest contributor of particulate matter in Klang Valley (Sulong et al., 2017), there was no haze occurrence during the sampling period. As for the outdoor sources from human activities, PM_{10} is commonly released from roadways, construction, and agricultural activities, whereas $PM_{2.5}$ is commonly released from industrial processes and motor vehicles. A local study reported that $PM_{2.5}$ in Klang Valley were mostly contributed by road dust and mixed secondary inorganic aerosol at about 32.4% to the $PM_{2.5}$ mass (Sulong et al., 2017). Although PM_{10} continue to suspend in the air for several days and can be dispersed by winds across long distances from the initial source, $PM_{2.5}$ may remain suspended in the air for a long time (Othman et al., 2019).

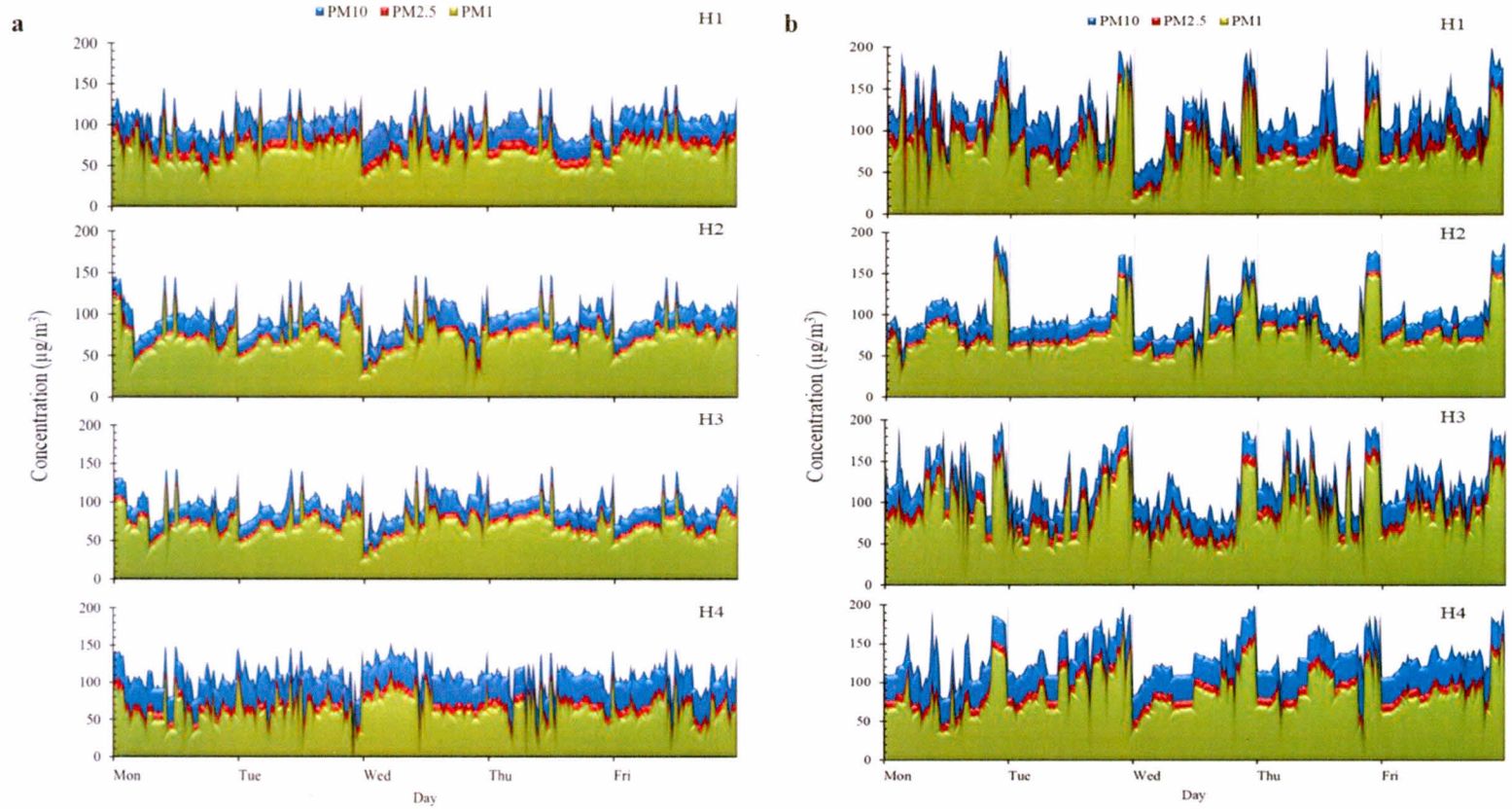


Figure 4.11: Daily variations of 6-h (a) indoor particulate matter in HT schools and (b) outdoor particulate matter in HT schools

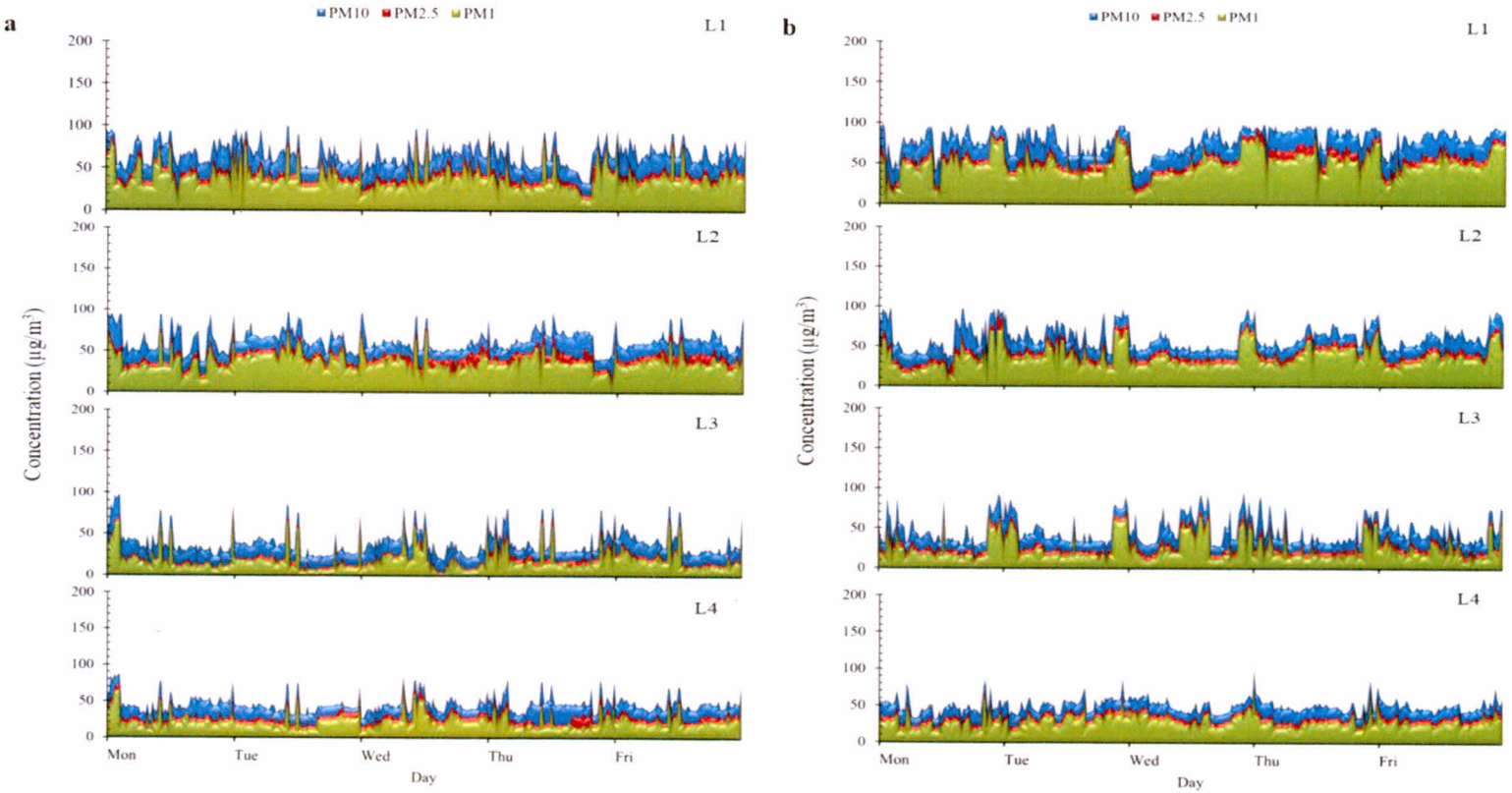


Figure 4.12: Daily variations of 6-h (a) indoor particulate matter in LT schools and (b) outdoor particulate matter in LT schools

Figure 4.13(a) shows hourly variations of 6-h indoor and outdoor PM_{10} in HT schools, while Figure 4.13(b) shows hourly variations of 6-h indoor and outdoor PM_{10} in LT schools. For 6-h measurements for indoors, the PM_{10} concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H2>H3>L1>L2>L4>L3 and ranged between 27.0 and 150.0 $\mu g/m^3$ with an overall average concentration of 84.9 ± 34.1 $\mu g/m^3$. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H3>H4>H1>H2>L1>L2>L4>L3 and ranged between 24.0 and 215.8 $\mu g/m^3$ with an overall average concentration of 90.0 ± 39.0 $\mu g/m^3$. As determined by the median, the HT group's exposures to indoor PM_{10} in schools were 1.8 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor PM_{10} in schools were 2 times higher than the LT group's exposures in schools with a significant difference.

Figure 4.14(a) shows hourly variations of 6-h indoor and outdoor $PM_{2.5}$ in HT schools, while Figure 4.14(b) shows hourly variations of 6-h indoor and outdoor $PM_{2.5}$ in LT schools. For 6-h measurements for indoors, the $PM_{2.5}$ concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H2>H3>L1>L2>L4>L3 and ranged between 12.5 and 129.8 $\mu g/m^3$ with an overall average concentration of 62.0 ± 28.2 $\mu g/m^3$. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H3>H4>H1>H2>L1>L2>L4>L3 and ranged between 12.8 and 156.0 $\mu g/m^3$ with an overall average concentration of 65.9 ± 31.6 $\mu g/m^3$. As governed by the median, the HT group's exposures to indoor $PM_{2.5}$ in schools were 1.8 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor $PM_{2.5}$ in schools were 2.4 times higher than the LT group's exposures in schools with a significant difference.

Figure 4.15(a) shows hourly variations of 6-h indoor and outdoor PM_1 in HT schools, while Figure 4.15(b) shows hourly variations of 6-h indoor and outdoor PM_1 in LT schools. For 6-h measurements for indoors, the PM_1 concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H3>H2>L1>L2>L4>L3 and ranged between 9.0 and 123.8 $\mu g/m^3$ with an overall average concentration of 54.75 ± 26.6 $\mu g/m^3$. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H3>H4>H1>H2>L1>L2>L4>L3 and ranged between 10.5 and 150 $\mu g/m^3$ with an overall average concentration of 57.5 ± 29.1 $\mu g/m^3$. As determined by the median, the HT group's exposures to indoor PM_1 in schools were 2.1 times higher than the LT group's exposures in classrooms with a significant difference. Moreover, the HT group's exposures to outdoor PM_1 in schools were 2.6 times higher than the LT group's exposures in schools with a significant difference.

When comparing the particulate matter trends, all schools showed a similar increasing trend for indoor particulate matter concentration from 9.50 a.m. to 10.25 a.m. This finding could be due to the children's movements when they left the classrooms during recess and back to the classrooms after recess. This finding agrees with a previous local study, which reported that students' movements during break time were strongly associated with the resuspension of particulate matter, thus causing a high concentration of particulate matter in the classroom (Yang Razali et al., 2015). The human-motion resuspended particles included those from clothing and shoes.

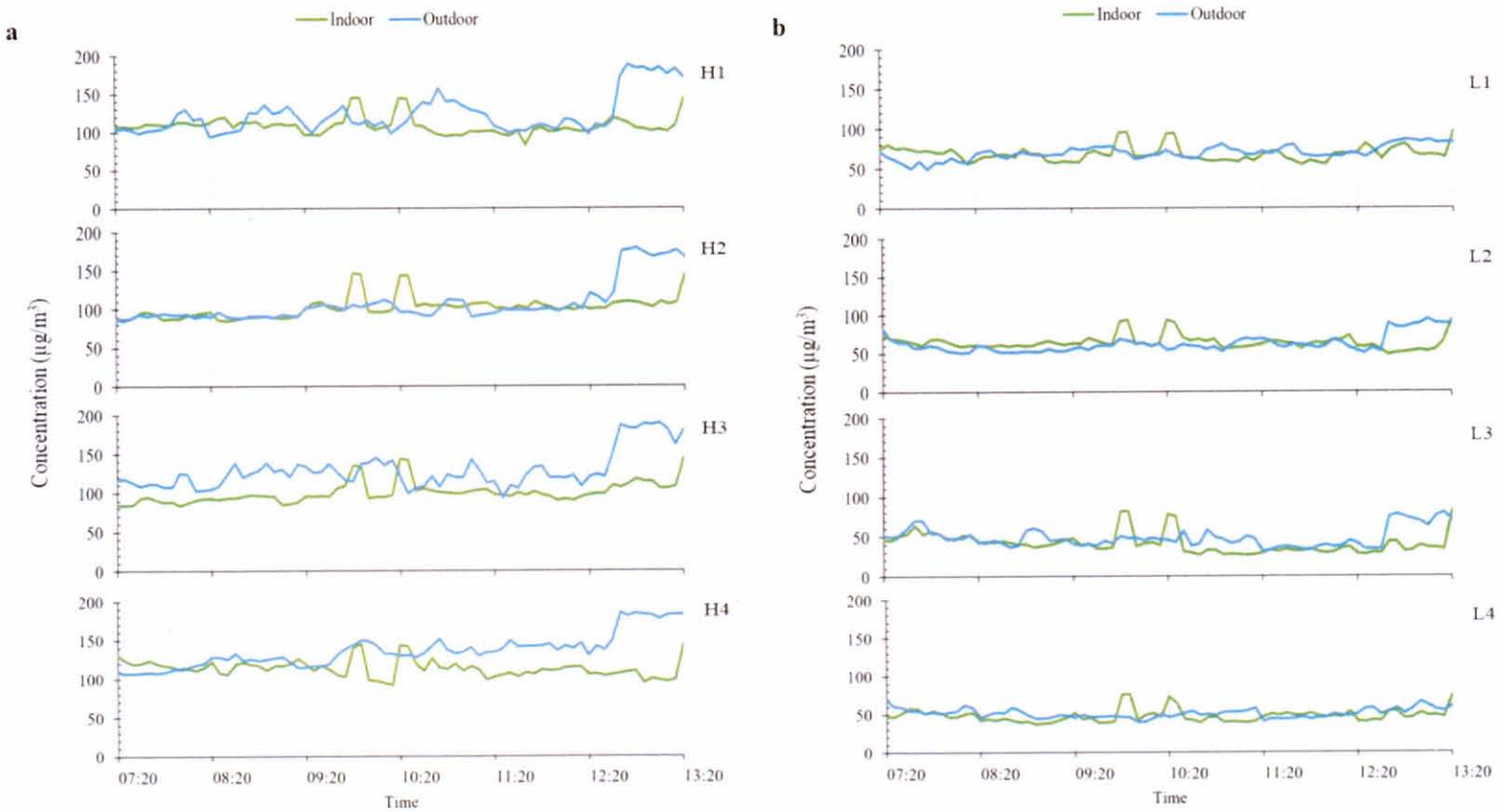


Figure 4.13: Hourly variations of 6-h indoor and outdoor PM_{10} in (a) HT schools and (b) LT schools

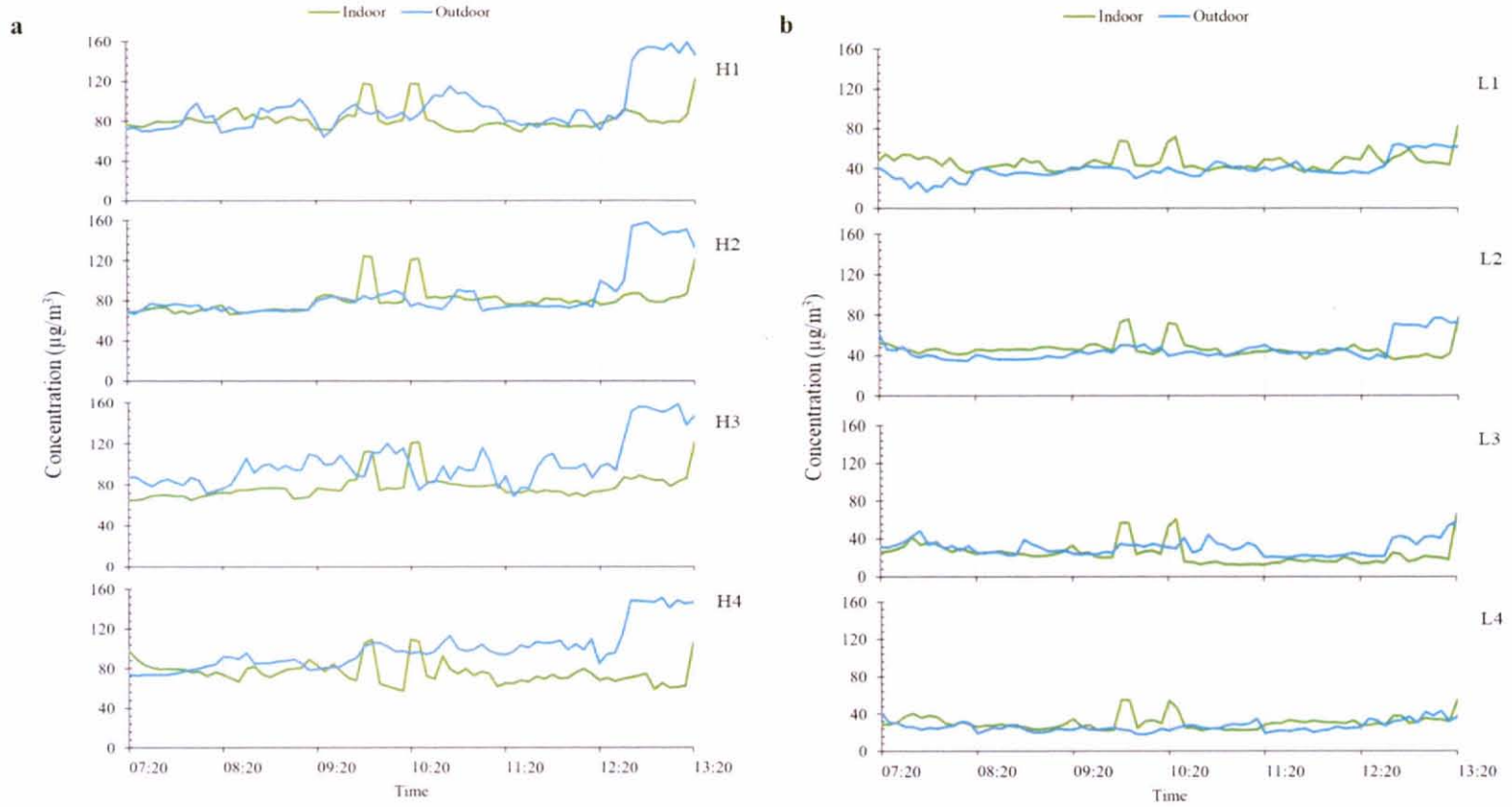


Figure 4.14: Hourly variations of 6-h indoor and outdoor $PM_{2.5}$ in (a) HT schools and (b) LT schools

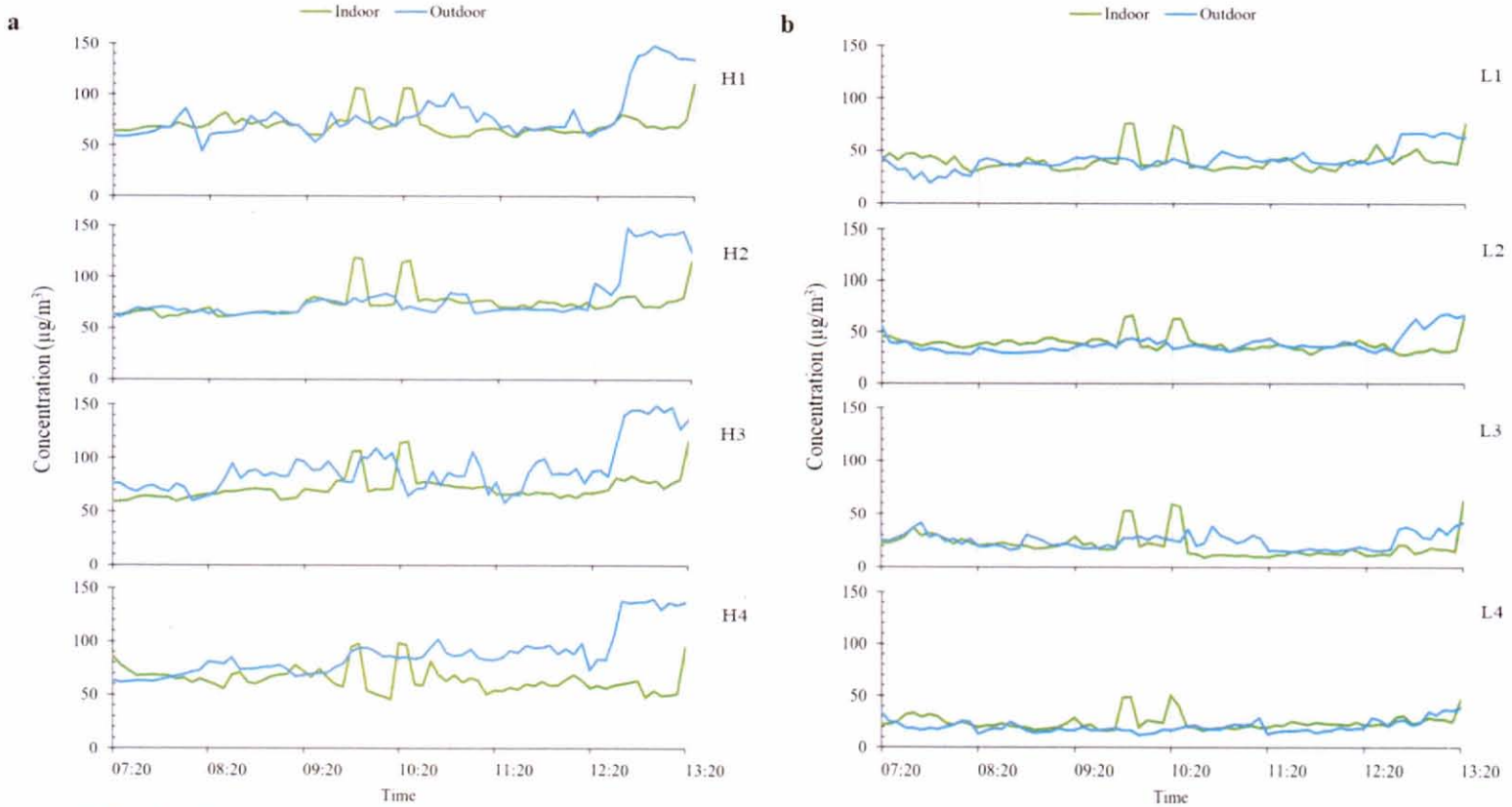


Figure 4.15: Hourly variations of 6-h indoor and outdoor PM_1 in (a) HT schools and (b) LT schools

Figure 4.16(a) shows hourly variations of 6-h indoor and outdoor NO_2 in HT schools, while Figure 4.16(b) shows hourly variations of 6-h indoor and outdoor NO_2 in LT schools. For 6-h measurements for indoors, the NO_2 concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H3>H2>L1>L2>L3>L4 and ranged between 1.0 and 79.0 ppb with an overall average concentration of 24.7 ± 15.7 ppb. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H4>H1>H2>H3>L1>L2>L3>L4 and ranged between 1.0 and 88.0 ppb with an overall average concentration of 37.4 ± 18.0 ppb. As determined by the median, the HT group's exposures to indoor NO_2 in schools were 2.8 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor NO_2 in schools were 2.4 times higher than the LT group's exposures in schools with a significant difference.

NO_2 is usually used as a proxy variable for traffic exhaust (Mohd Nadzir et al., 2020), and heavy traffic density around the schools in the HT area had contributed to the high concentrations of NO_2. Moreover, the findings on TC, as presented in part 4.2.4 of this thesis, had confirmed higher emissions of NO_2 from passing and idling vehicles in the HT area compared to the LT area.

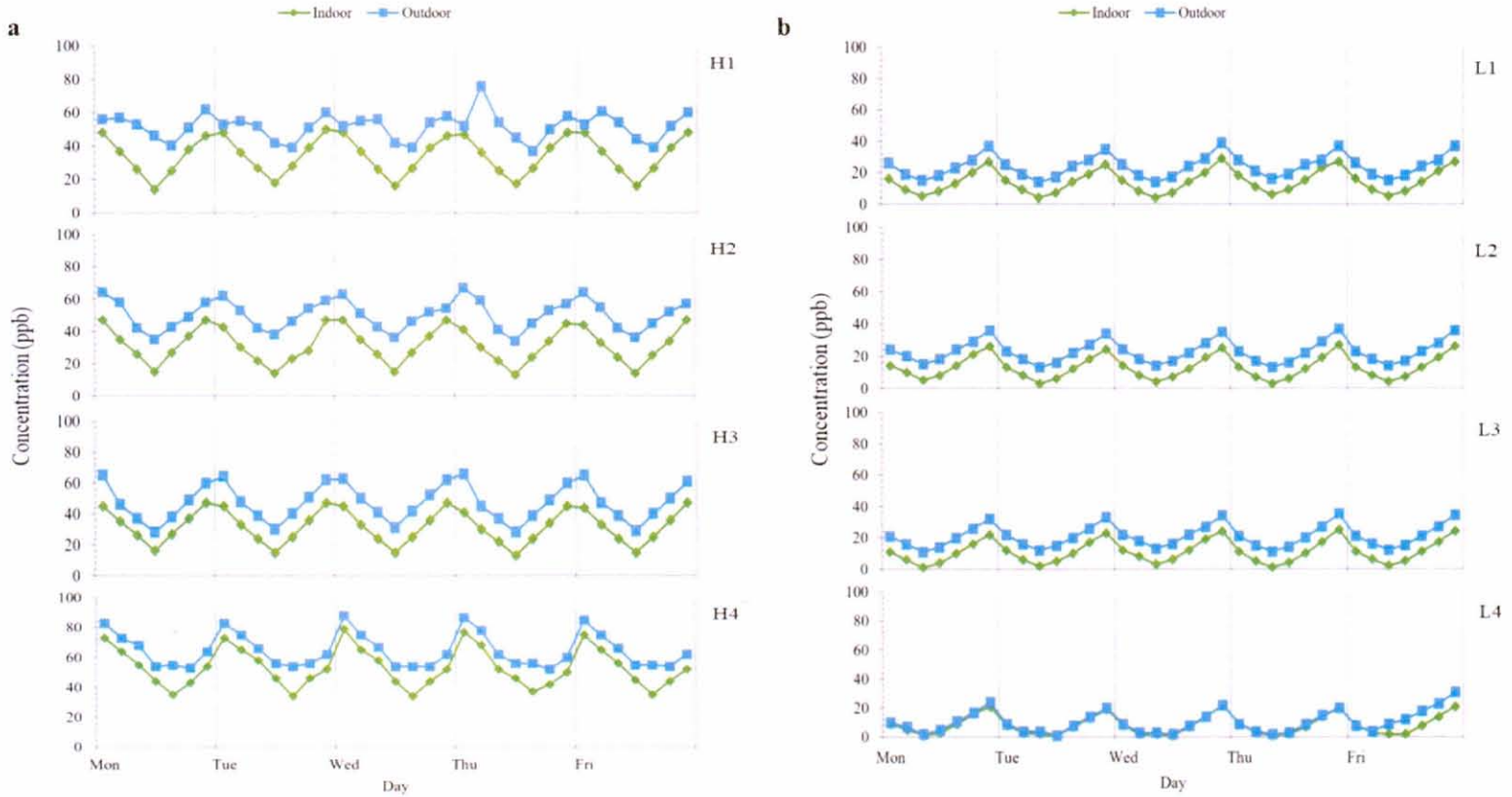


Figure 4.16: Daily variations of 6-h indoor and outdoor NO_2 in (a) HT schools and (b) LT schools

Figure 4.17(a) shows hourly variations of 6-h indoor and outdoor SO_2 in HT schools, while Figure 4.17(b) shows hourly variations of 6-h indoor and outdoor SO_2 in LT schools. For 6-h measurements for indoors, the SO_2 concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H2=H3>L1>L2>L3>L4 and ranged between 0.0 and 50.0 ppb with an overall average concentration of 14.2 ± 10.9 ppb. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H4>H1>H2>H3>L1>L2>L3>L4 and ranged between 0.0 and 50.0 ppb with an overall average concentration of 20.4 ± 13.2 ppb. As determined by the median, the HT group's exposures to indoor SO_2 in schools were 2 times higher than the LT group's exposures in classrooms with a significant difference. Moreover, the HT group's exposures to outdoor SO_2 in schools were 10 times higher than the LT group's exposures in schools with a significant difference. Some schools (L1, L2, L3 and L4) reported very low SO_2 concentration, which was below the limit of detection (LOD) at <10 ppb. This detection limit was defined by the lowest measurement detected by the instruments used in this study.

In general, SO_2 is generally emitted from industrial activities (Sopian et al., 2020). However, at these selected schools, motor vehicles and primarily diesel-engined buses and lorries are likely to be the principal SO_2 source. In a previous local study, sulphur from the combustion of engine fuel in petrol and diesel was found to add to the amount of SO_2 in the environment, particularly in urban areas and suburban regions (Othman & Latif, 2020). In contrast, fewer vehicles were travelling around the LT area compared to the HT area. Moreover, there is no major industrial area in both HT and LT areas, which removes the industrial source for SO_2.

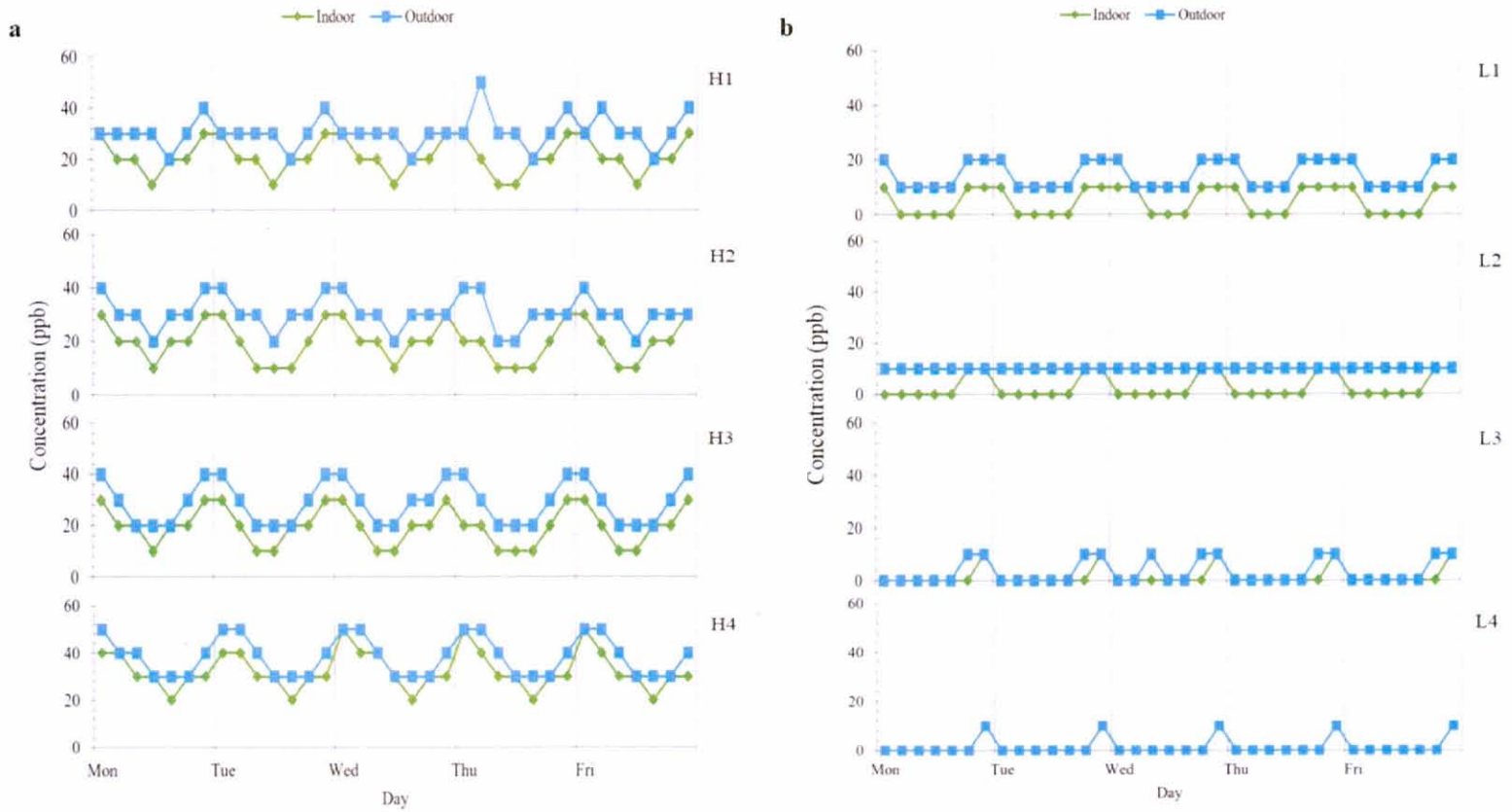


Figure 4.17: Daily variations of 6-h indoor and outdoor SO_2 in (a) HT schools and (b) LT schools

Figure 4.18(a) shows hourly variations of 6-h indoor and outdoor O_3 in HT schools, while Figure 4.18(b) shows hourly variations of 6-h indoor and outdoor O_3 in LT schools. For 6-h measurements for indoors, the O_3 concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H2>H1>H3>L1>L2>L3>L4 and ranged between 0.0 and 50.0 ppb with an overall average concentration of 18.1 ± 6.2 ppb. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H1>H4>H2>H3>L1>L2>L4>L3 and ranged between 0.0 and 50.0 ppb with an overall average concentration of 19.3 ± 6.5 ppb. As determined by the median, the HT group's exposures to indoor O_3 in schools were 1.8 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor O_3 in schools were 1.8 times higher than the LT group's exposures in schools with a significant difference.

The concentration of ground O_3 is highly dependent on the local emissions of O_3 precursors (NO_x and VOC), solar intensity and the local temperature (Hamid et al., 2020). Hence, the higher concentrations of VOC and NO_2 in HT areas can influence O_3 formation significantly, which were most likely sourced from vehicle emissions. The trend in **Figure 4.18** also shows that O_3 starts to increase during the measurement duration from 7.20 a.m. to 1.20 p.m. This finding agrees with a previous local study, which reported that vehicular and industrial emissions might release higher O_3 concentrations than the geographical proximity (Ahamad et al., 2014).

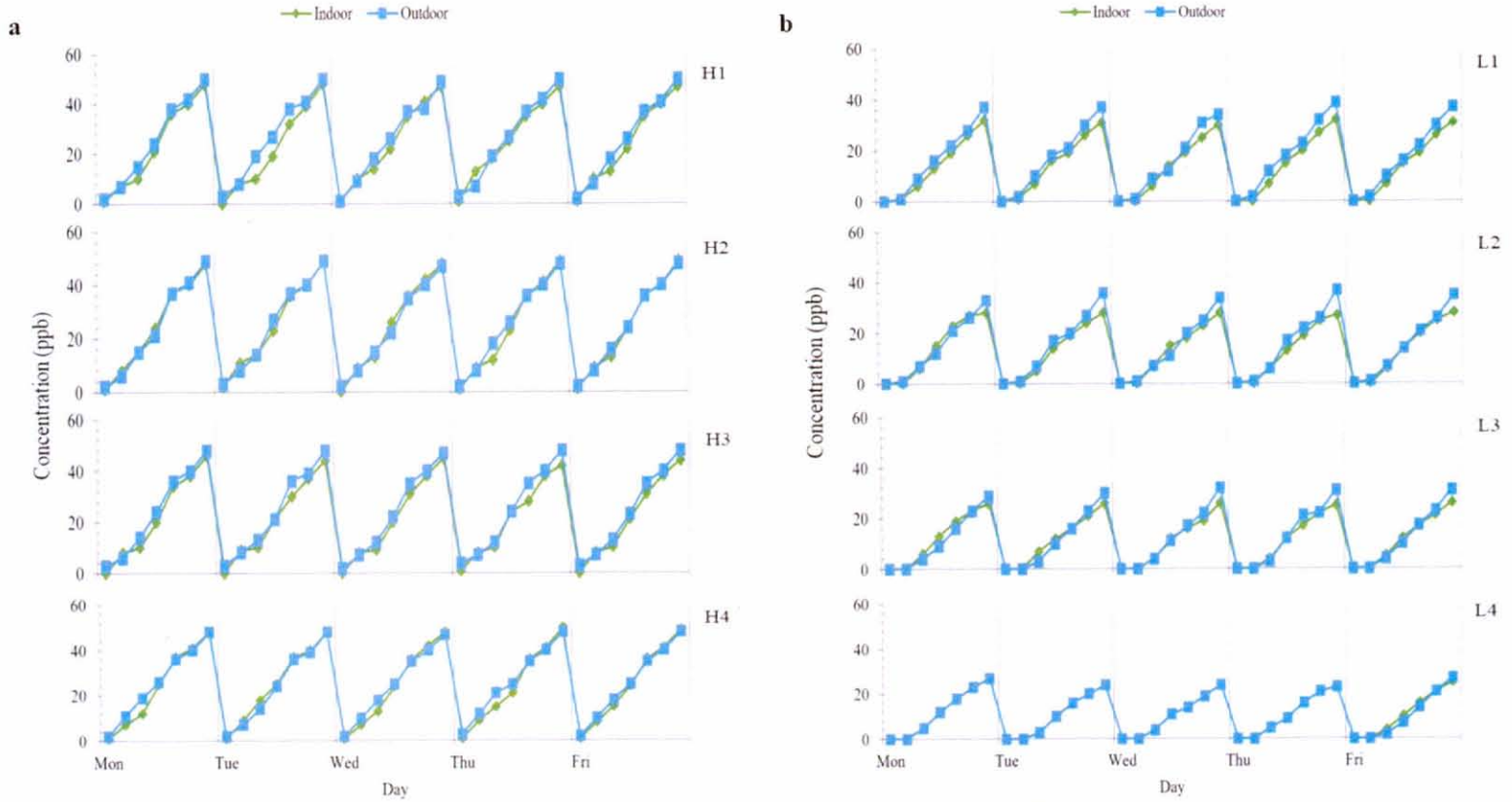


Figure 4.18: Daily variations of 6-h indoor and outdoor O_3 in (a) HT schools and (b) LT schools

Figure 4.19(a) shows hourly variations of 6-h indoor and outdoor TVOC in HT schools, while Figure 4.19(b) shows hourly variations of 6-h indoor and outdoor TVOC in LT schools. For 6-h measurements for indoors, the TVOC concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H3>H2>L1>L2=L3=L4 and ranged between 0.0 and 982.0 ppb with an overall average concentration of 121.0 ± 196.5 ppb. Meanwhile, for outdoors, the sequence from the highest to the lowest concentrations was H1>H4>H3>H2>L1>L2>L3=L4 and ranged between 0.0 and 1623.0 ppb with an overall average concentration of 325.7 ± 402.1 ppb. As determined by the median, the HT group's exposures to indoor TVOC in schools were 318 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor TVOC in schools were 25.8 times higher than the LT group's exposures in schools with a significant difference. Several schools (L1, L2, L3, L4) recorded very low TVOC concentration below LOD at <1 ppb. This detection limit was defined by the lowest measurement detected by the instruments used in this study. Meanwhile, L1 and L2 recorded TVOC concentration higher than 0 ppb between 7.20 a.m. to 9.20 a.m. due to emissions from vehicles passed by the main road connecting several villages and towns near the schools. This study was the first to compare TVOC in primary schools' environment at HT and LT regions.

Although TVOC typically records higher concentration indoors, the findings in this study did not show similar outcomes because there was a limited source of TVOC such as paints and floorings. The detected TVOC concentration was most likely contributed by traffic emissions outdoors such as Benzene, Toluene, Ethylbenzene and Xylene, as previous local studies had reported (Awang et al., 2020; Mohamad Fandi, Jalaludin, Latif, Abd Hamid, & Awang, 2020). These four aromatics compounds are more commonly known as BTEX, resulting from traffic exhaust emissions and indoor sources. The classrooms at H1 and H4 schools were close to congested main roads and highways, especially during morning rush hour. Therefore, the outdoor TVOC released from vehicular emissions could have penetrated the classrooms.

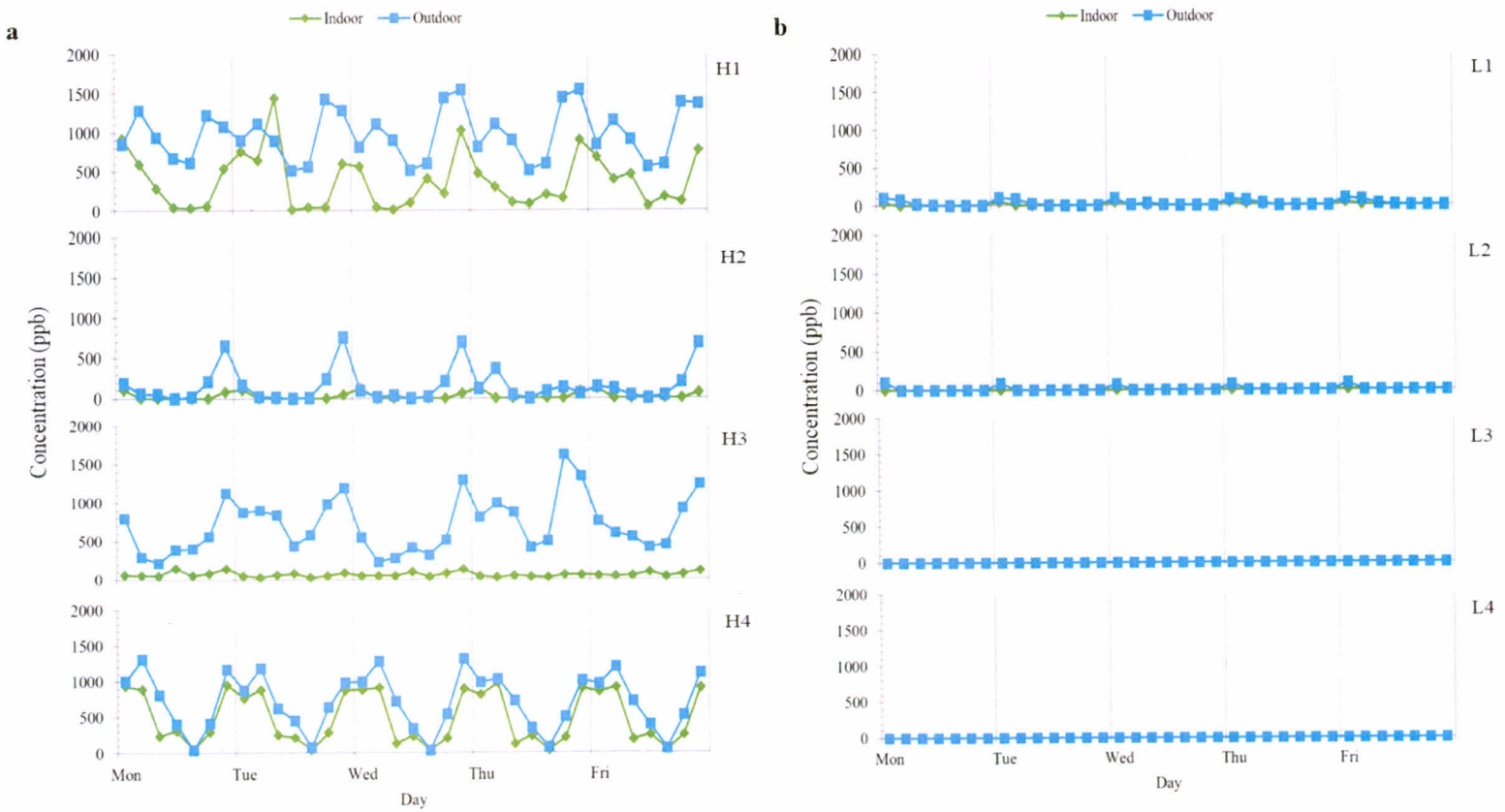


Figure 4.19: Daily variations of 6-h indoor and outdoor TVOC in (a) HT schools and (b) LT schools

Figure 4.20(a) shows hourly variations of 6-h indoor and outdoor CO in HT schools, while Figure 4.20(b) shows hourly variations of 6-h indoor and outdoor CO in LT schools. For 6-h measurements for indoors, the CO concentrations were recorded in the sequence from the highest to the lowest concentrations of H4>H1>H3>H2>L1>L2>L4>L3 and ranged between 0.0 and 1623.0 ppb with an overall average concentration of 121.9 ± 123.7 ppb. Meanwhile for outdoors, the sequence from the highest to the lowest concentrations was H4>H1>H3>H2>L1>L2>L4>L3 and ranged between 0.0 and 1623.0 ppb with an overall average concentration of 179.0 ± 181.7 ppb. As determined by the median, the HT group's exposures to indoor CO in schools were 2.3 times higher than the LT group's exposures in schools with a significant difference. Moreover, the HT group's exposures to outdoor CO in schools were 35 times higher than the LT group's exposures in schools with a significant difference. Some schools (L1, L2, L3, L4) reported very low CO concentration below LOD at <10 ppb. This detection limit was defined by the lowest measurement detected by the instruments used in this study.

The highest concentrations of CO were observed during peak hours between 7.00 a.m. to 9.00 a.m., which was due to the heavy traffic emissions at those times. These findings are comparable with a local study conducted in Kuala Lumpur, which discovered that higher CO emissions could be rooted in vehicles with higher cumulative travel mileage, engine modification, and no emission control device (Sofwan & Latif, 2021). They also found that the lowest emission factors of CO were from vehicles with mileage below 20,000 km. CO is produced mainly by incomplete combustion of vehicle fuel engines. Excessive TVOC and CO emissions are usually formed when the engine is undergoing transmission condition, such as during acceleration (Chong, Park, Kwon, & Hong, 2018). Driving around Klang Valley involves frequent accelerations and decelerations due to the congestion of vehicles on the road.

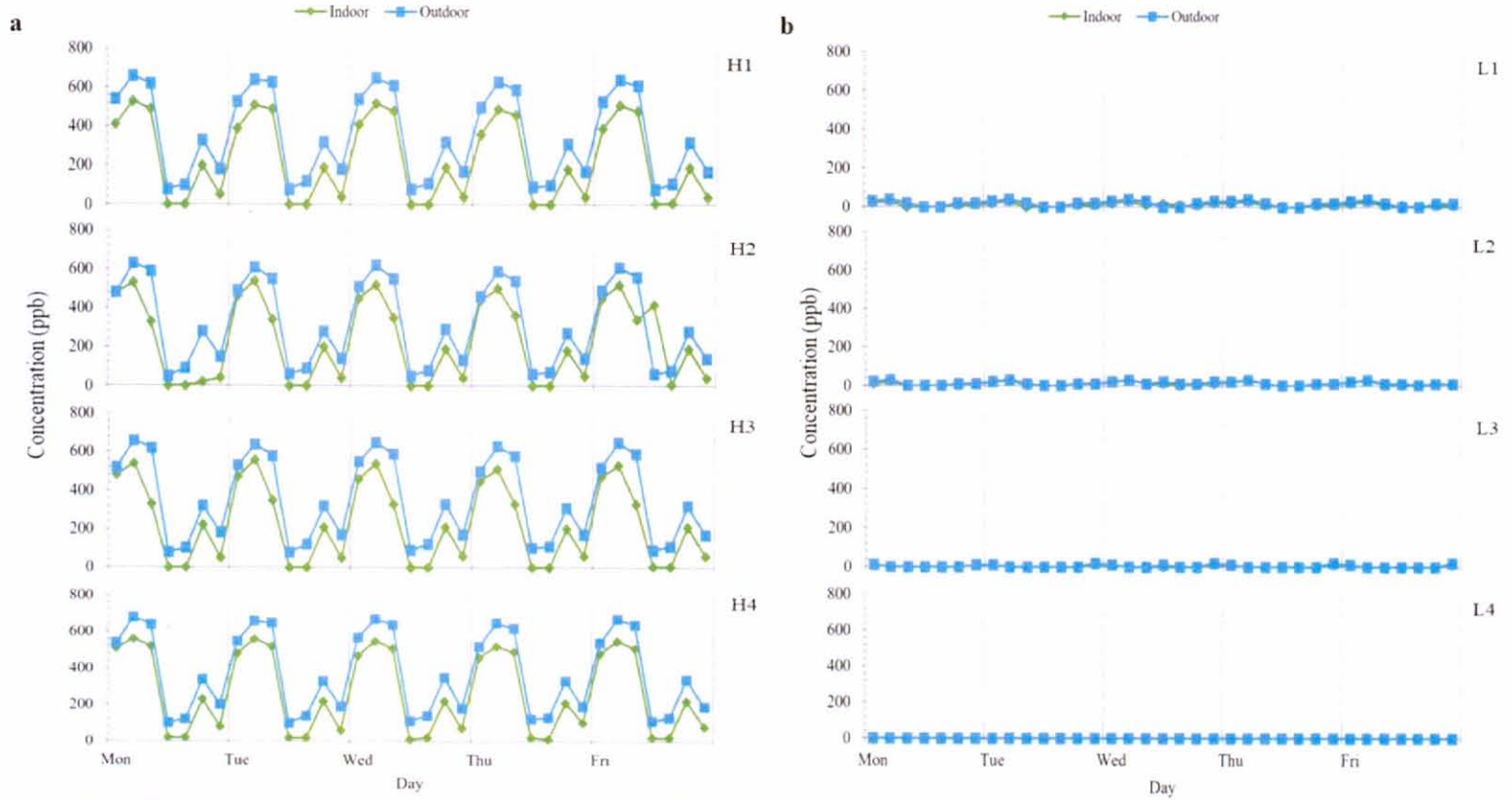


Figure 4.20: Daily variations of 6-h indoor and outdoor CO in (a) HT schools and (b) LT schools

HT schools recorded higher average concentrations of air pollutants than LT schools because, in general, the concentrations of air pollutants were significantly higher at the HT schools compared to the LT schools due to the high contribution of heavy traffic density. The building of many new housing developments, flyovers, and underpasses in Klang Valley contribute to high traffic intensity in the area (Arifuddin, Jalaludin, & Hisamuddin, 2019). School children, indoor and outdoor environment can be the causes of air emissions in the classroom; for example, movement of children inside and outside of the classroom contributes to the accumulation of particulate matter in the classroom. Moreover, both anthropogenic activities and natural sources emit particulate matter, whereas industrial activities or automobile exhaust release CO, SO_2 and NO_2 (Sopian et al., 2020).

4.2.6 Concentrations of Air Pollutants in Residences

Individual TRAP exposure was not computed using geographic information system (GIS) data or dispersion models. Instead, measurements were carried out by identifying each respondent's home address and performing air quality assessments only at residences approved by parents or guardians. Specific instruments were used to monitor air pollutants inside children's residences after school session, as mentioned in Part 3.4.2 of this thesis. The variables for air pollutants were PM_{10} and $PM_{2.5}$. The results for residential indoor monitoring are tabulated in Table 4.9, and it was found that the concentration of parameters was higher among the HT group for each pollutant than the LT group. There were significant differences for all the parameters between both groups at $p<0.05$.

Table 4.9: Indoor particulate matter concentrations in residences

Variables	Locations	Min	Max	Median	IQR	*z*	*p*
PM_{10} ($\mu g/m^3$)	HT (*N*=30)	43.1	85.2	73.9	17.1	-6.00	**<0.001***
	LT (*N*=22)	8.2	36.8	18.7	7.2		
$PM_{2.5}$ ($\mu g/m^3$)	HT (*N*=30)	19.0	67.9	50.3	13.3	-5.97	**<0.001***
	LT (*N*=22)	4.1	24.5	12.6	4.9		

***Significant at $p<0.05$**

As decided by the median, the HT group's exposures to PM_{10} in residences was 4 times higher than the LT group's exposures in residences with a significant difference. On the other hand, the HT group's exposures to $PM_{2.5}$ in residences was 4 times higher than the LT group's exposures in residences with a significant difference. Most individuals spend several hours in their homes every day. Despite low traffic on most roads at night, TRAP penetrating the house is not cleared immediately but accumulates during the day (Roldán-Henao, Hoyos, Herrera-Mejía, & Isaza, 2020). Moreover, some of the residences were observed to leave the doors and windows open during the daytime for better ventilation, which had promoted high penetration of outdoor air pollutants indoors (Sopian et al., 2020).

Distances of residences and schools from main roads and highways partly contributed to the concentrations of air pollutants indoors and outdoors. Motor vehicles emit particulate matter either from the ignition of fuels or from the usage of tyres on roads (Mohamad Fandi et al., 2020). Expansion of economic ventures in the HT area pushes the progress of road systems; hence, escalating the number of vehicles and their regularities to navigate on roads. Moreover, the vehicle age expressed in the cumulative mileage was responsible for TRAP rises (Sofwan & Latif, 2021).

4.2.7 Indoor/Outdoor (I/O) Ratios

IAQ in classrooms is often explored in terms of I/O ratios. Average I/O ratios are shown in Figure 4.21 for HT and LT schools, respectively. These findings show that IAP concentrations were not inevitably affected by outdoor air pollutants. The ratios ranged between 0.00 and 1.19. Schools with I/O ratios >1 can be justified by a higher concentration of TRAP in the classrooms compared to their outdoor concentrations. As for the physical parameters, some schools had I/O ratios of CO_2, RH and temperature (T) >1.

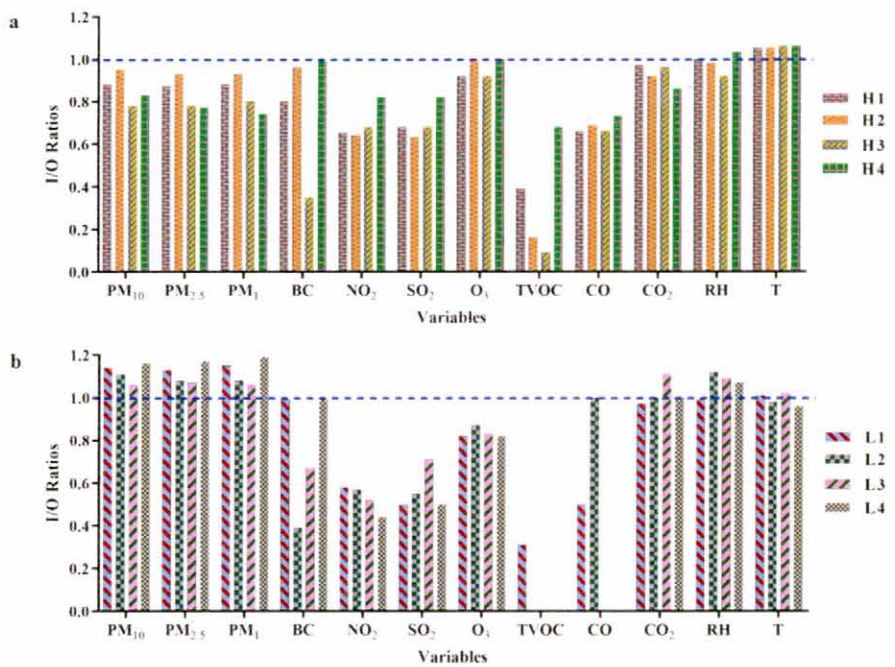


Figure 4.21: Comparison of I/O ratio in (a) HT schools and (b) LT schools

Accumulation of air pollutants in the classrooms from activities in the classrooms could generate this event, hence causing a surge in concentration in the indoor environment (Mohammadyan et al., 2017; Yang Razali et al., 2015). Only PM_{10}, $PM_{2.5}$ and PM_1 in LT schools recorded I/O ratios > 1, which suggests that

concentrations of other TRAP could be correlated with the proximity of the schools to roadways or with traffic intensity. Furthermore, $PM_{2.5}$ may be infiltrated easily due to the old age of the school building. In this study, the school buildings were at least 26 years old, considered old buildings (Jun, Hamzah, & Anua, 2017). These outcomes point that indoor particles were produced indoors, specifically from natural dust build-up, movement of occupants and classroom furniture (Othman et al., 2019). Another study supported this finding, which stated that $PM_{2.5}$ measured indoor commonly sources from the continuous suspension of vehicle emissions, soil dust, biomass burning, and Calcium(Ca)-rich particles (Carrion-Matta et al., 2019).

In addition, classroom particulate matter could be more strongly influenced by indoor activities. The poor IAQ in classrooms during teaching hours may result from outdoor urban sources and proximity to a busy road, as proven earlier (van der Zee, Strak, Dijkema, Brunekreef, & Janssen, 2017). Poor ventilation would cause a further negative impact on the indoor air environment where TRAP may easily penetrate and deposited into the indoor environment. This phenomenon could also be explained by the influence of high AT, which boosted more particle movement indoors (Othman et al., 2019).

4.2.8 Correlations between TRAP Concentrations and Traffic Counts (TC)

TRAP (PM_{10}, $PM_{2.5}$, PM_1, NO_2, SO_2, O_3, TVOC and CO) concentrations inside and outside the classrooms are affected by the TC that passed by the schools from 7 – 8 a.m. (AM) and 12.30 – 1.30 p.m. (PM). BC was excluded from the correlation test because BC was not measured using a real-time instrument. Figure 4.22 describes the correlation findings between TRAP concentrations and TC in the morning. Correlations with $p \geq 0.05$ are considered as not significant, and these Spearman's rho correlation coefficient (r) values are added with crosses. The TC in the morning were strongly correlated with SO_2 (r=0.87), NO_2 (r=0.85), TVOC (r=0.80), PM_{10} (r=0.76), $PM_{2.5}$ (r=0.75), and PM_1 (r=0.73). In contrast, the TC in the morning were moderately correlated with CO (r=0.49) and weakly correlated with O_3 (r=0.26).

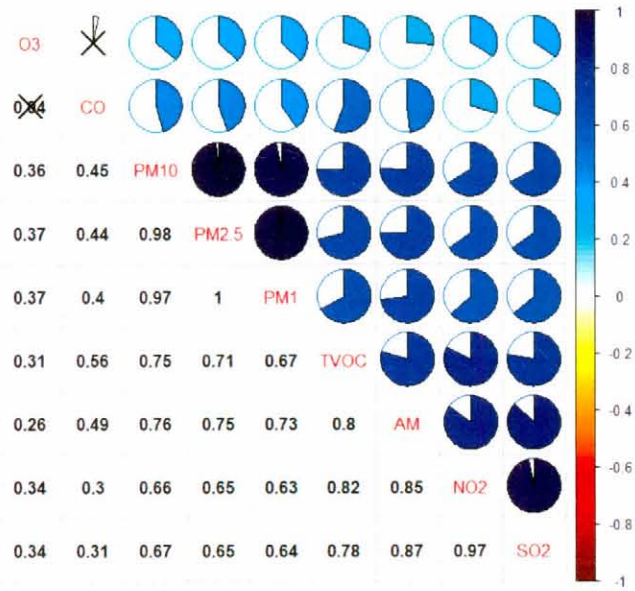


Figure 4.22: Correlations between TRAP concentrations and TC in the morning

By comparison, Figure 4.23 describes the correlation results between TRAP concentrations and TC in the afternoon. The TC in the afternoon were strongly correlated with CO (r=0.87), O_3 (r=0.84), PM_{10} (r=0.81), $PM_{2.5}$ (r=0.79), NO_2 (r=0.79), SO_2 (r=0.79) and PM_1 (r=0.78). In contrast, the TC in the afternoon were moderately correlated with TVOC (r=0.60).

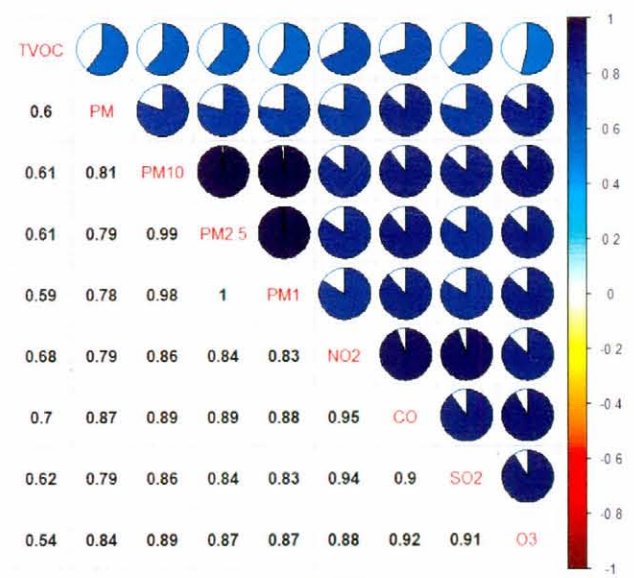


Figure 4.23: Correlations between TRAP concentrations and TC in the afternoon

These results are in line with findings from a local study in Serdang, Selangor. Their study found that PM_{10} (r=0.65) and $PM_{2.5}$ (r=0.62) concentrations were moderately correlated with TC at a busy roadside (Ezani et al., 2018). Both exhaust and non-exhaust emissions from road traffic contribute to particulate matter concentrations; hence, high particulate matter concentrations at busy roads are often associated with a high number of vehicles (Awang et al., 2019; Mohamad Jamil et al., 2020). In general, TRAP concentrations were higher during the morning and afternoon following a high number of TC at all schools. O_3 levels measured were found low in the morning and increased in the afternoon due to higher solar radiation; this finding explained why O_3 was weakly correlated with the TC in the morning but highly correlated with the TC in the afternoon.

4.2.9 Associations between Residential TRAP Concentrations and Air Pollutants Sources at Residences

TRAP concentrations inside residences regardless of HT or LT area were categorised into high or low according to their medians (PM_{10}=82 μg/m^3, $PM_{2.5}$=59 μg/m^3). Table 4.10 displays the associations between PM_{10} concentration inside children's residences and air pollutant sources at residences. There were significant associations between PM_{10} and distances of residences from highways and factories at p<0.05. The PM_{10} in residences were 4.9 times more likely to increase in the closest proximity to highways and 2.1 times more likely to increase in the closest proximity to factories. Table 4.11 displays the associations between $PM_{2.5}$ concentration inside children's residences and air pollutant sources at residences. There were significant associations between $PM_{2.5}$ and distances of residences from highways and factories at p<0.05. The $PM_{2.5}$ in residences were 14.4 times more likely to increase in the closest proximity to highways and 2.1 times more likely to increase in the closest proximity to factories.

Table 4.10: Associations between PM_{10} inside residences and air pollutant sources at residences

Variables	High	Low	χ^2	p	OR	95% CI
	Number (%)					
Indoor Painting within the Past 12 Months						
Yes	5 (41.7)	7 (58.3)	0.66	0.417	1.7	0.46 – 6.32
No	22 (55.0)	18 (45.0)				
Floor Renovation within the Past 12 Months						
Yes	1 (50.0)	1 (50.0)	0.03	0.956	1.1	0.06 – 18.30
No	26 (52.0)	24 (48.0)				
Furry Pets**						
Yes	4 (30.8)	9 (69.2)	3.11	0.078	3.2	0.85 – 12.35
No	23 (59.0)	16 (41.0)				
Usage of Mosquitoes Coils§						
Yes	2 (50.0)	2 (50.0)	0.06	0.936	1.1	0.14 – 8.36
No	25 (52.1)	23 (47.9)				
Usage of Carpet						
Yes	14 (50.0)	14 (50.0)	0.09	0.764	1.2	0.40 – 3.52
No	13 (54.2)	11 (45.8)				
Usage of Cleaning Products						
Yes	9 (64.3)	5 (35.7)	1.17	0.279	0.5	0.14 – 1.77
No	18 (47.4)	20 (52.6)				
Usage of Moth Balls§						
Yes	6 (75.0)	2 (25.0)	2.02	0.156	0.3	0.06 – 1.68
No	21 (47.7)	23 (52.3)				
Usage of Air Fresheners						
Yes	10 (45.5)	12 (54.5)	0.64	0.424	1.6	0.52 – 4.75
No	17 (56.7)	13 (43.3)				
House Cleaning Frequency Per Week						
<6 times	16 (51.6)	15 (48.4)	0.03	0.957	1.0	0.34 – 3.13
≥6 times	11 (52.4)	10 (47.6)				
Indoor Smoking						
Yes	12 (70.6)	5 (29.4)	3.53	0.060	3.2	0.93 – 11.05
No	15 (42.9)	20 (57.1)				
Type of Ventilation System						
Air-Conditioner and Fan	10 (62.5)	6 (37.5)	1.04	0.309	1.9	0.56 – 6.22
Fan	17 (47.2)	19 (52.8)				
Type of Cooking Stove						
Electric and Gas	10 (62.5)	6 (37.5)	1.04	0.309	1.9	0.56 – 6.22
Gas Only	17 (47.2)	19 (52.8)				
Daily Cooking Activity						
<3 times	14 (41.2)	20 (58.8)	4.54	0.303	0.3	0.08 – 0.93
≥3 times	13 (72.2)	5 (27.8)				
Usage of Cooking Hood and Hob§						
Yes	3 (42.9)	4 (57.1)	0.27	0.606	0.7	0.13 – 3.28
No	24 (53.3)	21 (46.7)				
Open Window or Door While Cooking§						
Yes	25 (50.0)	25 (50.0)	1.93	0.165	0.5	0.38 – 0.66
No	2 (100.0)	0 (0.0)				
Distance from Main Roads§						
<500 m	23 (50.0)	23 (50.0)	0.59	0.442	0.5	0.08 – 3.00
≥500 m	4 (66.7)	2 (33.3)				
Distance from Highways§						
<500 m	13 (76.5)	4 (23.5)	6.10	**0.014***	4.9	1.32 – 18.05
≥500 m	14 (40.0)	21 (60.0)				
Distance from Factories§						
<5 km	4 (100.0)	0 (0.0)	4.01	**0.045***	2.1	1.55 – 2.80
≥5 km	23 (47.9)	25 (52.0)				

N=52, ***Significant at p<0.05**; § By χ^2 test with Yates' correction for expected value <5

Table 4.11: Associations between $PM_{2.5}$ inside residences and air pollutant sources at residences

Variables	High	Low	χ^2	p	OR	95% CI
	Number (%)					
Indoor Painting within the Past 12 Months						
Yes	6 (50.0)	6 (50.0)	0.02	0.879	1.1	0.30 – 4.02
No	21 (52.5)	19 (47.5)				
Floor Renovation within the Past 12 Months§						
Yes	1 (50.0)	1 (50.0)	0.03	0.956	1.1	0.06 – 18.30
No	26 (52.0)	24 (48.0)				
Furry Pets						
Yes	5 (38.5)	8 (61.5)	1.26	0.262	2.1	0.57 – 7.48
No	22 (56.4)	17 (43.6)				
Usage of Mosquitoes Coils§						
Yes	1 (25.0)	3 (75.0)	1.26	0.262	3.6	0.34 – 36.56
No	26 (54.2)	22 (45.8)				
Usage of Carpet						
Yes	14 (50.0)	14 (50.0)	0.09	0.764	1.2	0.40 – 3.52
No	13 (54.2)	11 (45.8)				
Usage of Cleaning Products						
Yes	8 (57.1)	6 (42.9)	0.21	0.647	0.8	0.22 – 2.58
No	19 (50.0)	19 (50.0)				
Usage of Moth Balls §						
Yes	6 (75.0)	2 (25.0)	2.02	0.156	0.3	0.06 – 1.68
No	21 (47.7)	23 (52.3)				
Usage of Air Fresheners						
Yes	11 (50.0)	11 (52.0)	0.06	0.812	1.1	0.38 – 3.44
No	16 (53.3)	14 (46.7)				
House Cleaning Frequency Per Week						
<6 times	15 (48.4)	16 (51.6)	0.38	0.535	1.4	0.47 – 4.34
≥6 times	12 (57.1)	9 (42.9)				
Indoor Smoking						
Yes	11 (64.7)	6 (35.3)	1.65	0.199	2.2	0.66 – 7.20
No	16 (45.7)	19 (54.3)				
Type of Ventilation System						
Air-Conditioner and Fan	10 (62.5)	6 (37.5)	1.04	0.309	1.9	0.56 – 6.22
Fan	17 (47.2)	19 (52.8)				
Type of Cooking Stove						
Electric and Gas	10 (62.5)	6 (37.5)	1.04	0.309	1.9	0.56 – 6.22
Gas Only	17 (47.2)	19 (52.8)				
Daily Cooking Activity						
<3 times	16 (59.3)	18 (72.0)	0.93	0.335	0.6	1.78 – 1.81
≥3 times	11 (61.1)	7 (38.9)				
Usage of Cooking Hood and Hob§						
Yes	4 (57.1)	3 (42.9)	0.09	0.766	1.2	0.26 – 6.36
No	23 (51.1)	22 (48.9)				
Open Window or Door While Cooking§						
Yes	25 (50.0)	25 (50.0)	1.93	0.165	0.5	0.38 – 0.66
No	2 (100.0)	0 (0.0)				
Distance from Main Roads§						
<500 m	23 (50.0)	23 (50.0)	0.59	0.442	0.5	0.08 – 3.00
≥500 m	4 (66.7)	2 (33.3)				
Distance from Highways§						
<500 m	15 (88.2)	2 (11.8)	13.34	**<0.001***	14.4	2.81 – 73.53
≥500 m	12 (34.3)	23 (65.7)				
Distance from Factories§						
<5 km	4 (100.0)	0 (0.0)	4.01	**0.045***	2.1	1.55 – 2.80
≥5 km	23 (47.9)	25 (52.1)				

N=52, ***Significant at p<0.05**; § By χ^2 test with Yates' correction for expected value <5

Indoor exposure to PM_{10} and $PM_{2.5}$ can be originated from either outdoor or indoor sources. In this study, indoor sources of PM_{10} and $PM_{2.5}$ inside residences were mostly from traffic and industrial emissions. When there is less air movement from indoors to outdoors, the indoor pollutants tend to remain indoors and build up within the contained spaces. Since the measurements of PM_{10} and $PM_{2.5}$ in residences were done on 24 h, it is not easy to outsource the particle emissions. There were 13 residences (48.1%) with high PM_{10}, which were located <500 m from highways, compared to 4 residences (16.0%) with low PM_{10}, which were located <500 m from highways. Meanwhile, there were 4 residences (14.7%) with high PM_{10}, which were located <5 km from factories, compared to 0 residence (0.0%) with low PM_{10}, which were located <5 km from factories. As for $PM_{2.5}$, There were 15 residences (55.6%) with high $PM_{2.5}$, which were located <500 m from highways, compared to 2 residences (8.0%) with low $PM_{2.5}$ which were located <500 m from highways. Meanwhile, there were 4 residences (14.7%) with high $PM_{2.5}$, which were located <5 km from factories, compared to 0 residence (0.0%) with low $PM_{2.5}$, which were located <5 km from factories.

The findings in this study are backed up by the evidence in a study by Srithawirat et al. (2016), who found that PM_{10} concentrations in residential buildings were positively associated with highway intensity, traffic congestion and industrial emissions in Phitsanulok, Thailand. Their sampling sites had I/O ratios <1, which suggests that particulate matter was generated by vehicle emissions and penetrated indoors by infiltration and ventilation. Besides, previous local studies testified that distances of residences from traffic sources and industrial complexes contribute to influencing air pollutants in residences (Fadzir & Jalaludin, 2013; Suhaimi et al., 2015).

4.2.10 The Trend of Air Pollutants in Nearby Continuous Air Quality Monitoring (CAQM) Stations

Batu Muda and Cheras stations were the nearest CAQM stations from the selected HT schools and located in the city centre of Kuala Lumpur. As mentioned earlier, in Table 3.1 of this thesis, there was no CAQM station near the selected LT schools. Therefore, comparisons could only be made between primary data measured in HT schools and secondary data obtained from nearby CAQM stations. However, comparison can only be made in term of trend because there were differences in the type of instrument deployed between both measurements; hence, there were different flow rates.

Both stations show that PM_{10} and $PM_{2.5}$ concentrations tend to peak around 9 a.m. For NO_2, there is a very pronounced increase in concentrations during the peak morning rush hour. O_3 shows very different behaviour because O_3 reacts with NO_2. There is a very pronounced increase in O_3 concentrations in the afternoon between 2 to 3 p.m. CO and SO_2 had similar trends daily. Just like NO_2, CO showed a very pronounced increase in concentrations during the peak

morning rush hour. These trends indicate very similar source origins, which are most likely traffic emissions.

Factors that impact air pollution distribution are wind speed (WS), wind direction (WD), ambient temperature (AT), relative humidity (RH), and solar radiation (SR). Meteorological conditions determine outflow strength and depend on megacity geographic location and the season for the period of emission (Rani, Azid, Khalit, Juahir, & Samsudin, 2018). Pollution Rose by RStudio is useful for considering pollutant concentrations by WD, or more specifically, the percentage time the concentration is in a particular range. This type of approach can be very informative for air pollutant species. Figure 4.24 – Figure 4.26 show the dominance of north-easterly winds controlling the overall mean of PM_{10}, $PM_{2.5}$, NO_2, SO_2, O_3 and CO concentrations at Batu Muda station. Meanwhile, most of the higher PM_{10}, $PM_{2.5}$, NO_2, SO_2 and CO concentrations at Cheras station are also associated with the easterly winds, as shown in Figure 4.27 – Figure 4.29. Southern winds mostly influenced only O_3 concentrations at Cheras station. These data presented the measurements at both CAQM stations throughout the year 2019.

Referring to Batu Muda station, WD is predominantly from the east and relatively low WS (0 – 2 m/s) throughout the year. WS are the highest in March (1.31 m/s) and the lowest in November (0.69 m/s). Both higher and lower concentrations of PM_{10} (Figure 4.24a), $PM_{2.5}$ (Figure 4.24b), NO_2 (Figure 4.25a), SO_2 (Figure 4.25b) and CO (Figure 4.26b) at Batu Muda station are generally associated with northeast wind sectors. However, O_3 concentrations at Batu Muda station recorded non-similar findings; higher concentrations of O_3 are generally associated with southeast wind sectors, while lower concentrations of O_3 are generally associated with northeast wind sectors (Figure 4.26a).

Referring to Cheras station, WD is also predominantly from the east and relatively low WS (0 – 2 m/s) throughout the year. WS are the highest in August (1.11 m/s) and the lowest in February (0.88 m/s). Both higher and lower concentrations of PM_{10} (Figure 4.27a), $PM_{2.5}$ (Figure 4.27b), NO_2 (Figure 4.28a), SO_2 (Figure 4.28b) and CO (Figure 4.29b) at Cheras station are generally associated with easterly wind sectors. However, O_3 concentrations at Cheras station recorded non-similar findings; higher concentrations of O_3 are generally associated with southerly wind sectors, while lower concentrations of O_3 are generally associated with easterly wind sectors (Figure 4.29a).

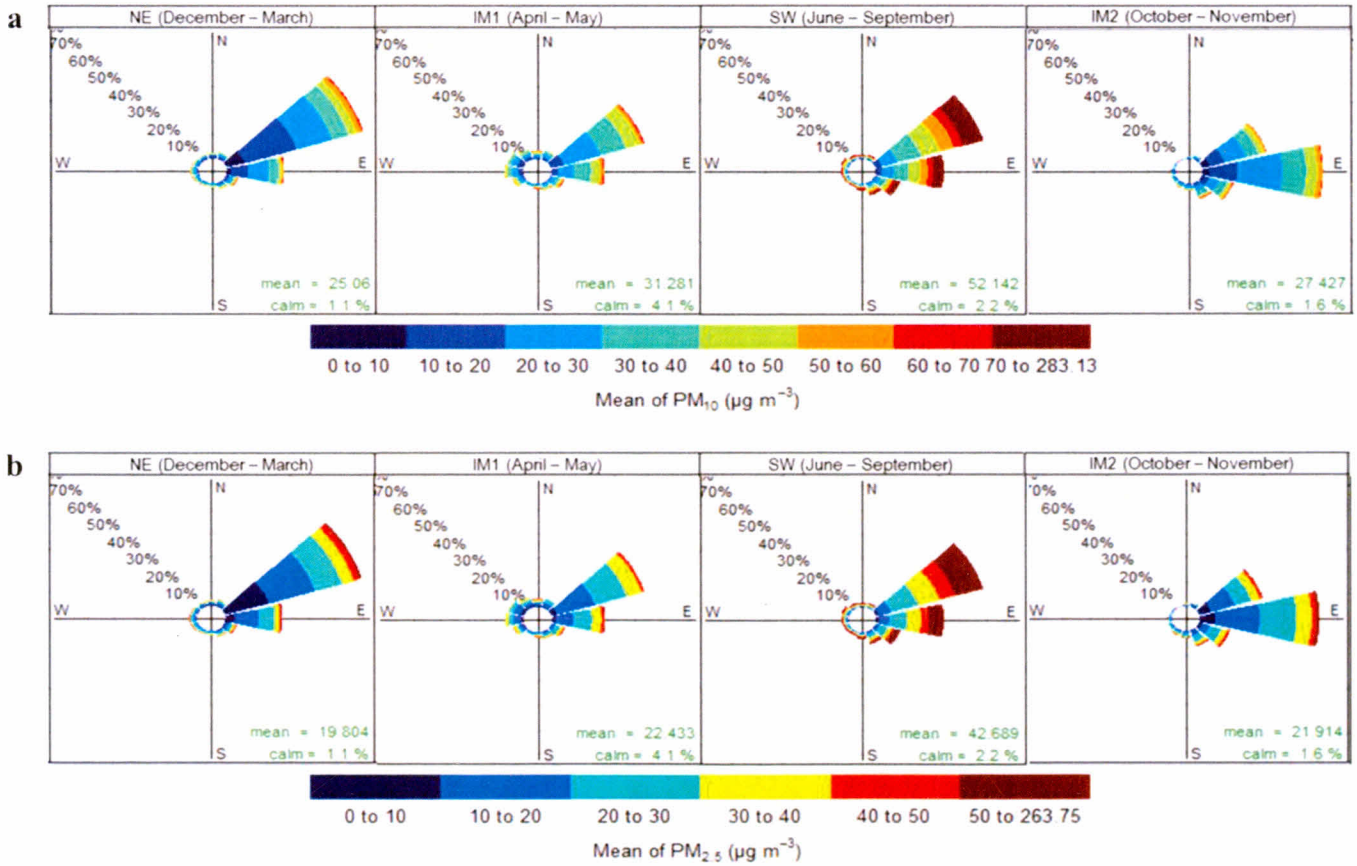


Figure 4.24: Pollution rose of (a) PM_{10} and (b) $PM_{2.5}$ in Batu Muda station in 2019

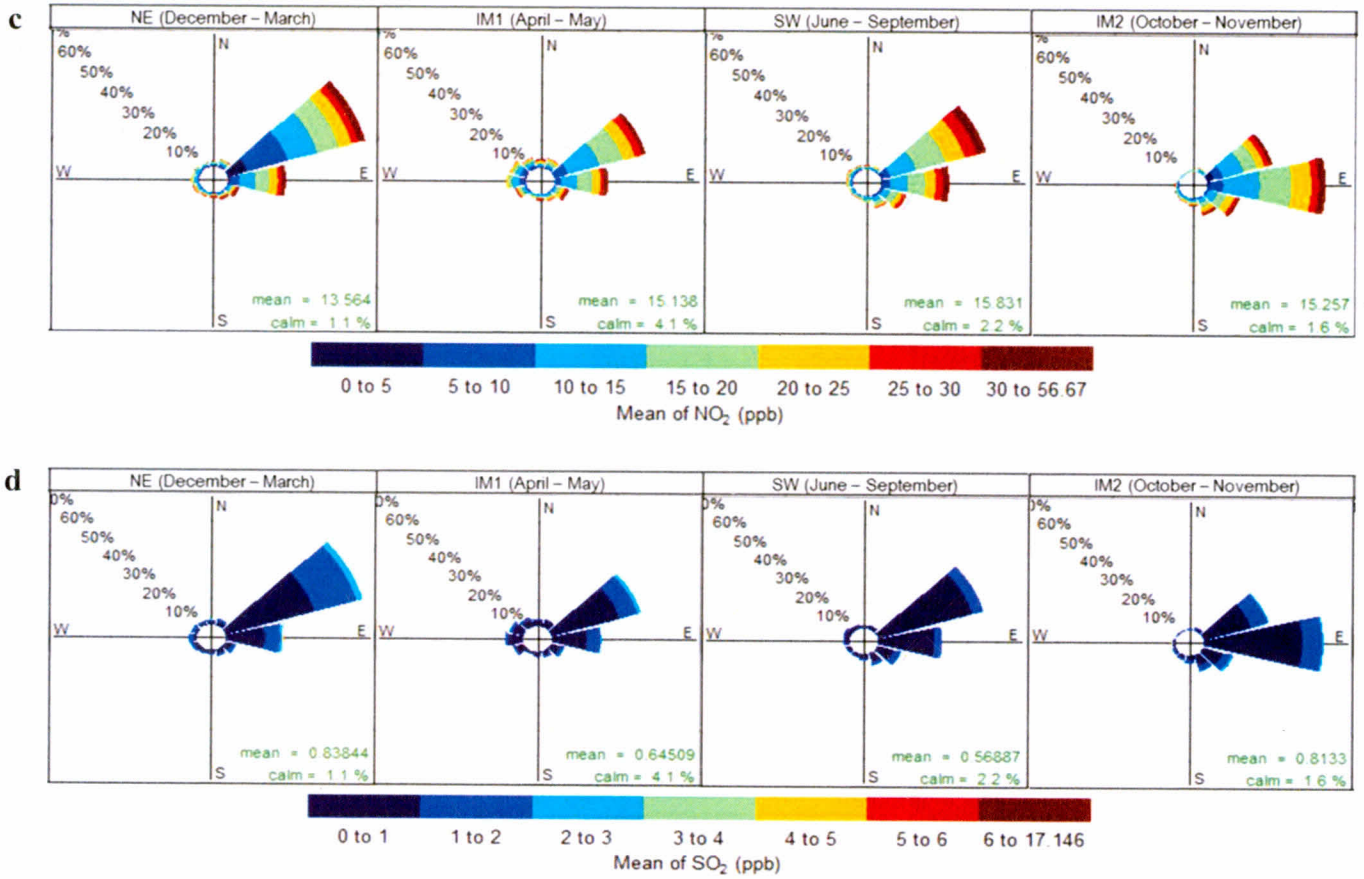


Figure 4.25: Pollution rose of (a) NO_2 and (b) SO_2 in Batu Muda station in 2019

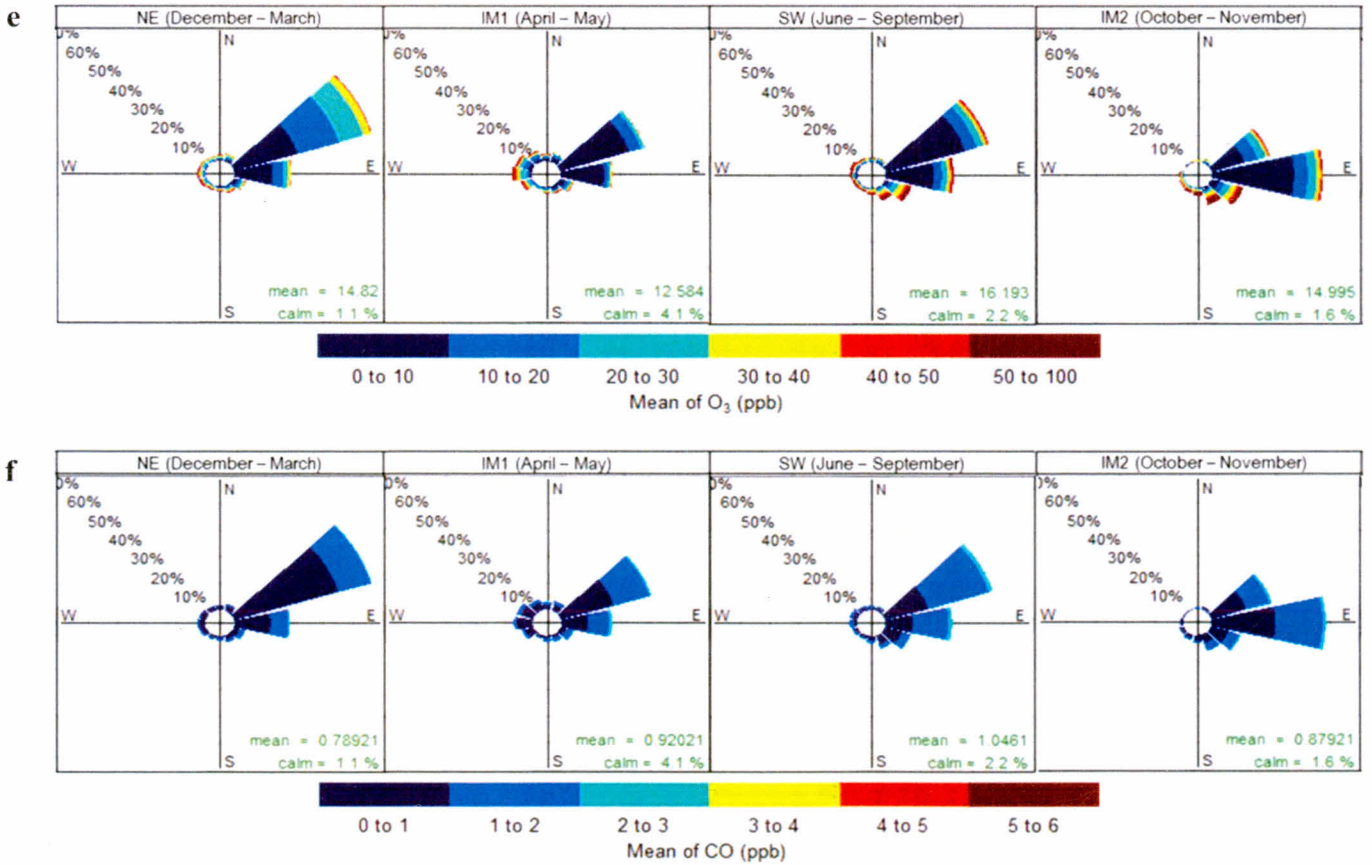


Figure 4.26: Pollution rose of (a) O_3 and (b) CO in Batu Muda station in 2019

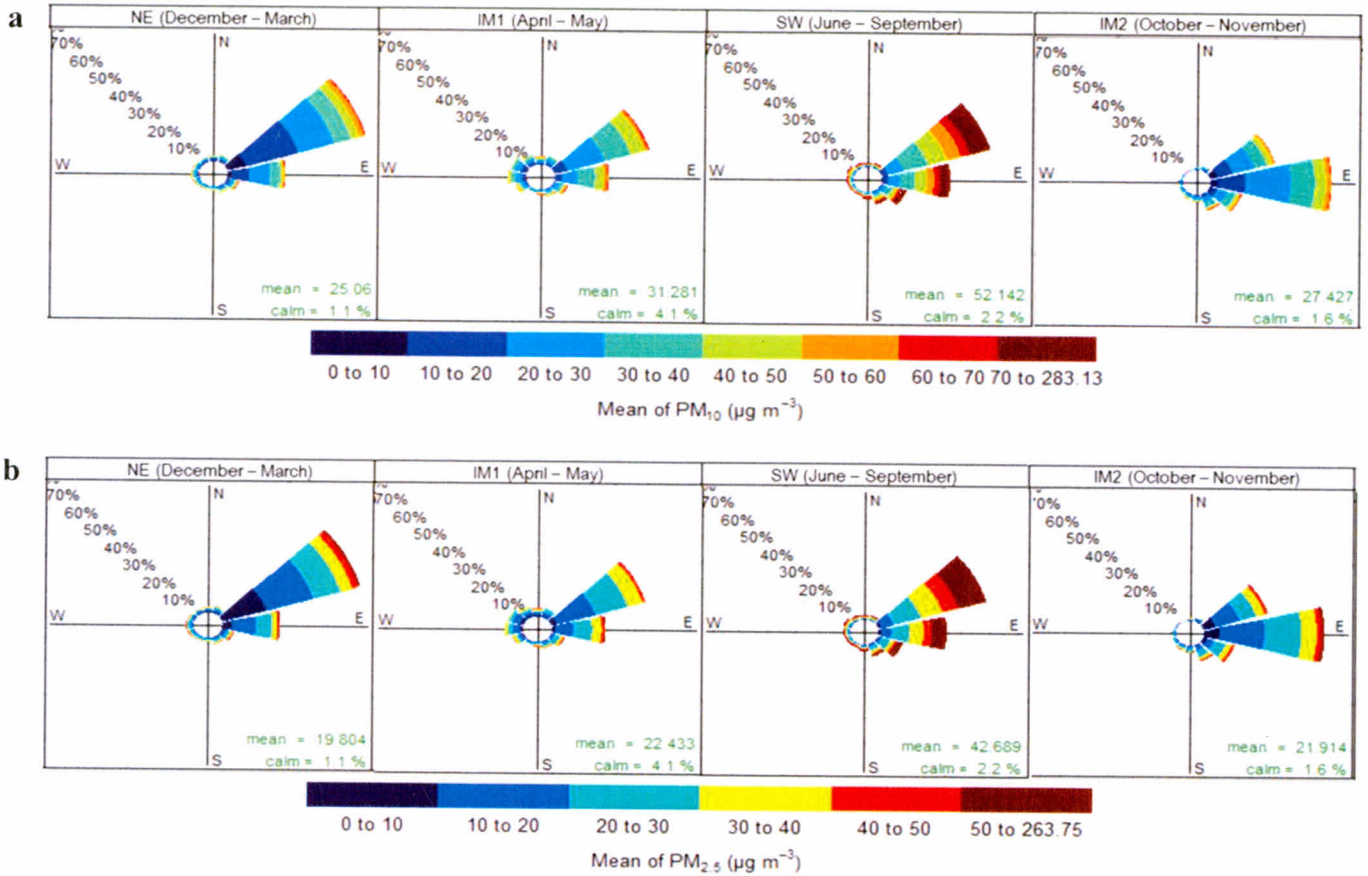


Figure 4.27: Pollution rose of (a) PM_{10} and (b) $PM_{2.5}$ in Cheras station in 2019

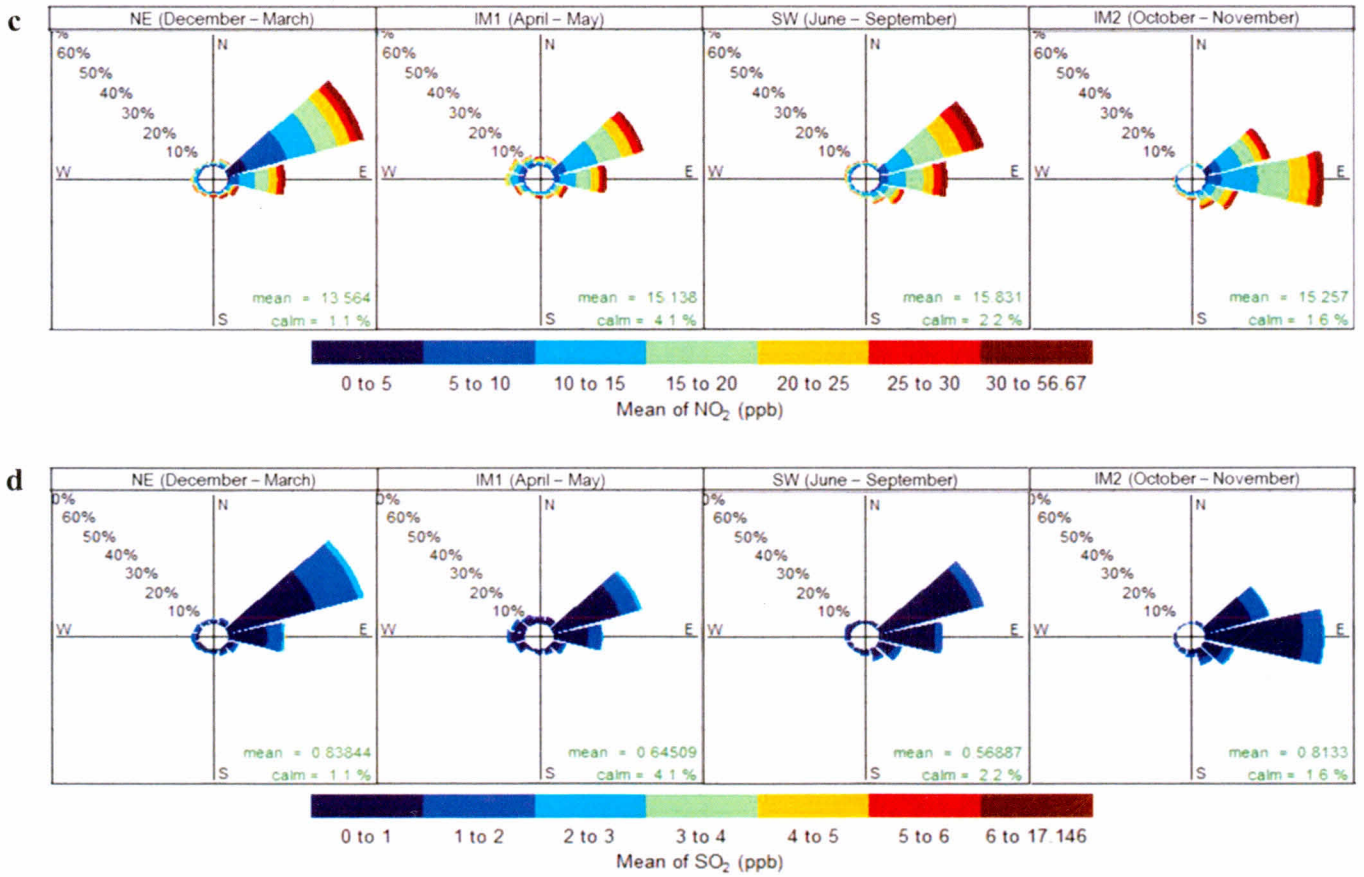


Figure 4.28: Pollution rose of (a) NO_2 and (b) SO_2 in Cheras station in 2019

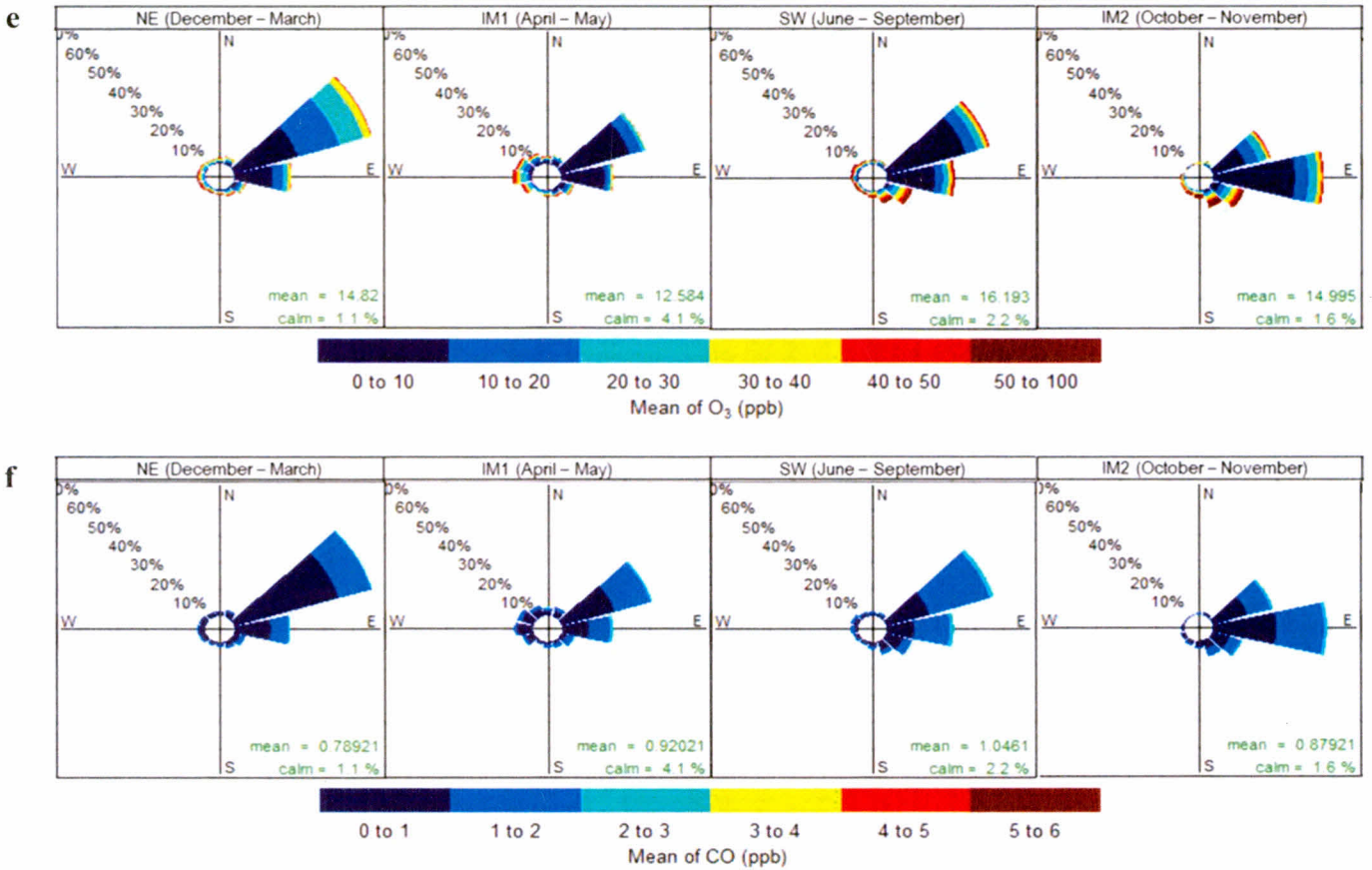


Figure 4.29: Pollution rose of (a) O_3 and (b) CO in Cheras station in 2019

All selected HT schools and both stations are in Klang Valley. The topography and climate of the Klang Valley have the potential to trap air pollutants (Abdullah, 2020). Due to the valley topography and insufficient flushing along the valley that runs from the hilly terrain to the north, south-east, and to the Malacca Straits in the west, pollutants are restricted (Abdul Rahman et al., 2011). It is experiencing worse air pollution problems due to increased traffic volume, urbanisations, and industrial activities. Malaysia experiences four seasons, which are the northeast monsoon (NE) from December to March, inter-monsoon 1 (IM1) from April to May, southwest monsoon (SW) from June to September, and inter-monsoon 2 (IM2) from October to November (Sulong et al., 2019). On average, these monsoon seasons yield more rainfall than hot seasons with higher RH, lower SR, and lower AT. Klang Valley encountered the SW and IM1 during the study period (January – May).

H1, H2, H3 and H4 are located within 2.3 km (163° SE), 5.6 km (152° SE), 8.7 km (100° SE) and 5.9 km (168° SE) from the Batu Muda station. However, the wind that comes from these directions were <10% of the time during the study period except for H3, which had ~25%. Most higher concentrations of PM_{10}, $PM_{2.5}$, NO_2, SO_2, O_3 and CO concentrations are associated with the NE. The dominance of north-easterly winds (46 – 75° WD) controlling the overall mean of air pollutants at Batu Muda station (Figure 4.24 – Figure 4.26) is associated with the heavily populated outflow and massive regional traffic flow in North-eastern Kuala Lumpur such as Wangsa Maju and Setiawangsa. The winds were calm most of the time throughout the study period, between 1.1 to 2.2% despite monsoon seasons. It may get a little chilly after heavy rain, but Klang Valley is one of the least affected areas by monsoon winds coming either from the east or west (Sulong et al., 2019).

H1, H2, H3 and H4 are located within 10.0 km (342° NW), 7.0 km (350° NW), 11.4 km (25° NE) and 6.5 km (337° NW) from the Cheras station. Nevertheless, the wind that comes from these directions were <5% of the time throughout the study period. The dominance of easterly winds (166 – 105° WD) controlling the overall mean of air pollutants at Cheras station during NE monsoon, while the dominance of north-easterly winds (46 – 75° WD) controlling the overall mean of air pollutants at Cheras station during IM1 (Figure 4.27 – Figure 4.29). These higher concentrations of air pollutants are associated with the outflow from the heavily populated and massive regional traffic flow in Eastern Selangor such as Cheras and Ampang. The winds were calm most of the time throughout the study period, between 0 to 0.5% despite monsoon seasons.

4.2.11 Principal Component Analysis (PCA) of Air Pollutants

PCA has been applied as a multivariate statistical tool to identify the major sources of air pollutants. The data sets of air pollutants obtained from selected schools and CAQM nearby the schools were normalised using a procedure explained earlier (Hosaini et al., 2017) before further statistical analysis and subjected to PCA varimax rotation. Kaiser-Meyer-Olkin (KMO) measure of

sampling adequacy and Bartlett's Test of Sphericity indicate the suitability of data for structure detection. High values of KMO, which is close to 1.0, generally indicate that factor analysis may be useful with the data. In contrast, the value of <0.50 indicates the results of the factor analysis probably will not be very useful. The probability related to the Bartlett test is <0.05, which fulfils this prerequisite. Both requirements were met in this study; hence, PCA could be performed for the data set. Table 4.12 shows the KMO and Bartlett's Test values for selected schools, while Table 4.13 shows the values for nearby CAQM stations. The values of KMO and Bartlett's Test satisfy the requirement for PCA.

Table 4.12: The Kaiser-Meyer-Olkin and Bartlett's test result of air monitoring in selected schools

		HT	LT
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.669	0.809
Bartlett's Test of Sphericity	Approx. Chi-Square	347.96	371.25
	df	36	36
	p	<0.001	<0.001

Table 4.13: The Kaiser-Meyer-Olkin and Bartlett's test result of nearby CAQM stations

		Batu Muda	Cheras
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.670	0.668
Bartlett's Test of Sphericity	Approx. Chi-Square	8001.93	8359.87
	df	15	15
	p	<0.001	<0.001

The eigenvalue is a measure of how much of the variance of the observed variables a factor explains. Any factor with an eigenvalue >1 explains more variance than a single observed variable. Meanwhile, the rotated component matrix table represents how the variables are weighted for each factor and the correlation between the variables and the factor. The measured value is called factor loading, where the higher the absolute value of the loading, the more the factor contributes to the variable. Rotated matrix rotation utilising varimax with Kaiser Normalisation has appeared in Table 4.14 for air monitoring in schools based on nine air pollutants, whereas Table 4.15 for air monitoring in nearby CAQM stations based on six air pollutants. Two main Principal Component (PC) were generated for all sites. Contingent upon the indication of the relating PC coefficient, the commitment of a variable to a PC can be either positive or negative. The loading factor of ≥0.7, which demonstrate a strong significance within a factor, was selected to interpret the sources for each factor (Tang, Lung, Chang, Tu, & Chang, 2019). The retained factors were the ones that explained at least 70% of the cumulative total percentage of variance.

Table 4.14: Factor loadings after PCA-varimax rotation of selected schools

	HT		LT	
	Component			
	1	2	1	2
PM_{10}	0.257	**0.934**	**0.947**	0.214
$PM_{2.5}$	0.123	**0.980**	**0.961**	0.182
PM_1		**0.977**	**0.962**	0.187
BC	0.588	-0.119	**0.718**	0.218
NO_2	**0.792**	0.447	0.150	**0.921**
SO_2	0.590	0.159	0.576	0.611
O_3	**0.787**		0.676	0.497
CO	**0.883**	0.264	0.657	0.579
TVOC	**0.762**	0.418	0.158	**0.748**
Eigenvalue	4.845	2.107	5.949	1.252
Variability (%)	40.870	36.372	51.937	28.074
Cumulative (%)	40.870	77.243	51.937	80.011

Extraction Method: Principal Component Analysis
Rotation Method: Varimax with Kaiser Normalisation[a]

a. Rotation converged in 3 iterations

a. Rotation converged in 3 iterations

Values in bold indicate the variables that mostly influence the associated principal component

Table 4.15: Factor loadings after PCA-varimax rotation of nearby CAQM stations

	Batu Muda		Cheras	
	Component			
	1	2	1	2
PM_{10}	**0.926**	0.207	**0.896**	0.277
$PM_{2.5}$	**0.927**	0.200	**0.891**	0.295
NO_2	**0.834**	-0.109	**0.757**	-0.355
SO_2	0.293	-0.145	0.390	0.421
CO	**0.728**	-0.368	**0.750**	-0.430
O_3		**0.946**		**0.862**
Eigenvalue	3.030	1.146	2.893	1.395
Variability (%)	50.505	19.094	37.157	34.320
Cumulative (%)	50.505	69.599	37.157	71.477

Extraction Method: Principal Component Analysis
Rotation Method: Varimax with Kaiser Normalisation[a]

a. Rotation converged in 3 iterations

a. Rotation converged in 3 iterations

Values in bold indicate the variables that mostly influence the associated principal component

The total percentage variance was approximately 77.24% for HT and is explained by 2 factors. Factor 1 had a strong loading for NO_2, O_3, CO and TVOC, whereas factor 2 revealed a strong loading for PM_{10}, $PM_{2.5}$ and PM_1. On the other hand, the total percentage variance was approximately 80.01% for LT and is

explained by 2 factors. Factor 1 had a strong loading for PM_{10}, $PM_{2.5}$, PM_1 and BC, whereas factor 2 revealed a strong loading for NO_2 and TVOC. As for the CAQM stations, the total percentage variance was approximately 69.60% for Batu Muda and is explained by 2 factors. Meanwhile, the total percentage variance was approximately 71.48% for Cheras and is explained by 2 factors. For both sites, factor 1 had a strong loading for PM_{10}, $PM_{2.5}$, NO_2 and CO, whereas factor 2 revealed a strong loading for O_3. SO_2 was the only pollutant that did not have a strong contribution to HT, LT and both CAQM stations.

Afterwards, multiple linear regression (MLR) modelling was performed to identify the behaviour of variables. MLR is widely used in atmospheric modelling (Dominick, Juahir, Latif, Zain, & Aris, 2012). This study was used to justify the relationship between air quality parameters and total API data. The source apportionment of air pollutant variables was used to identify the potential API. Only data from CAQM stations had API values. Therefore, two models were developed using the API value as a dependent variable, while the independent variables were based on the air pollutant variables taken from Batu Muda (4 variables) and Cheras (4 variables). The value for R^2, Adjusted R^2 and Root Mean Square Error (RSME) for Batu Muda are 0.347, 0.341 and 6.499, respectively; and 0.116, 0.106, and 6.951 for Cheras. The proposed equations of the coefficient of determination (R^2), adjusted coefficient of determination (adjusted R^2) and RMSE are shown in Equation 4.1 and Equation 4.2:

Batu Muda (4 variables)

$$Total\ API = 23.51 - 0.79(PM_{10}) + 1.09(PM_{2.5}) + 0.12(NO_2) + 5.54(CO) \quad \textbf{(Equation 4.1)}$$

(R^2=0.654, Adjusted R^2=0.530, RMSE=5.624)

Cheras (4 variables)

$$Total\ API = 31.84 - 0.52(PM_{10}) + 0.66(PM_{2.5}) + 0.15(NO_2) + 4.59(CO) \quad \textbf{(Equation 4.2)}$$

(R^2=0.353, Adjusted R^2=0.121, RMSE=7.064)

Measured results of the above equations show that the highest R^2 came from Batu Muda with 0.654 for $PM_{2.5}$, NO_2 and CO, but negative for PM_{10}; followed by Cheras with R^2=0.353 for $PM_{2.5}$, NO_2 and CO, as well as negative for PM_{10}. From the finding, Batu Muda has been determined as the best model compared to Cheras due to the closest R^2 value to 1 and the smallest RMSE (Ang, 2018; Azid et al., 2015).

Figure 4.30 shows the analysis of residual and prediction of total API using Batu Muda and Cheras. The results indicated that the deficiency of the model contained some differences in the range of -1.6 to 1.9 for Batu Muda and -1.8 to 1.4 for Cheras. Meanwhile, the standard predicted values for Batu Muda and Cheras ranged between -2.1 and 2.0, -1.4 and 2.0, respectively. The main objective of the scatter plot chart was to testify that the Batu Muda model is

appropriate to be used for total API prediction. The model showed a greater difference between the predicted API and the calculated API compared to another model.

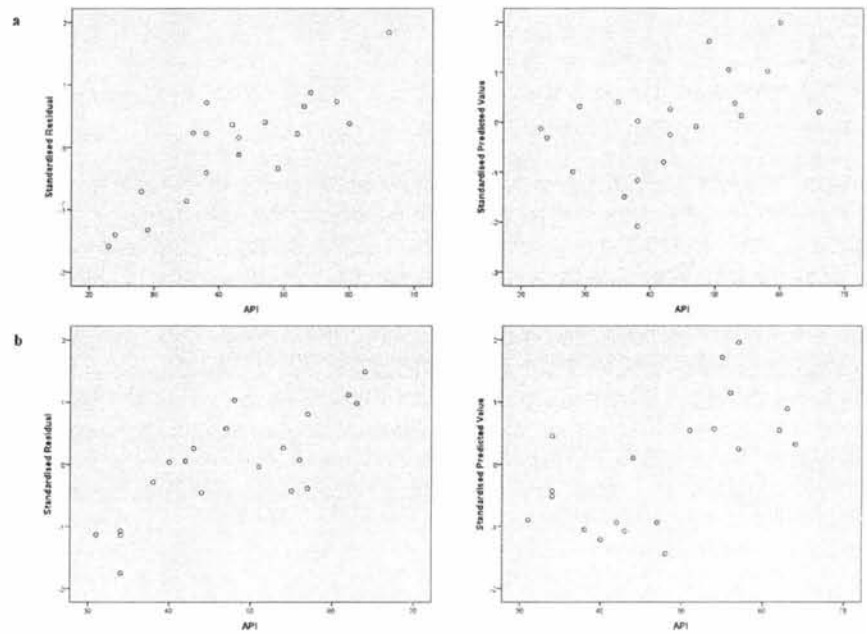


Figure 4.30: Scatter plot diagram of standardised residuals and standard predicted value for (a) Batu Muda and (b) Cheras

This outcome clearly shows that the air quality in the HT region has been affected by the increasing number of vehicles (Othman et al., 2019; Sulong et al., 2019) since NO_2, CO, O_3 and TVOC were included in the factor 1 group influenced by the traffic conditions. This finding is also supported by PCA results of CAQM stations nearby selected HT schools. Internal combustion engines produce several indicators of air pollutants from vehicle combustion such as NO_2, CO, VOC, PM_{10}, $PM_{2.5}$ and its components, including BC (Winkler et al., 2018). Fuel combustion at high temperatures generates NO_2, while incomplete combustion of fuel engines produces CO. O_3 is not directly released from vehicle emission. Instead, a mixture of NO_2 and VOC in the presence of sunlight creates ground-level O_3. In this study, only SO_2 is not contributing to any of the study areas – either selected schools or CAQM stations nearby selected HT schools. SO_2 is only produced at a low percentage from vehicle emissions and a high percentage from industrial emissions such as coal combustion to produce electricity (Mohamad, Ash'aari, & Othman, 2015); hence, this finding confirms the low contribution of industrial emissions in the selected locations and high contribution of vehicle emissions.

As for the PM_{10}, a local study confirmed that the highest contribution of PM_{10} in both urban and suburban r egion was from the resuspension of road dust with

36% and 55%, respectively (Mohamad et al., 2016). Non-combustion vehicle emissions and indoor activities contributed 9% of PM_{10} in the urban region, whereas vehicle emissions, construction and building materials contributed 12% of PM_{10} in the sub-urban region (Mohamad et al., 2016). Moreover, the construction of a new highway named East Klang Valley Expressway (EKVE) within a 10 km radius from the selected schools in the LT region at the time of the study duration could have contributed to the high concentrations of PM_{10}, $PM_{2.5}$, PM_1 and BC. Another contributor was suspected to be small-scale burning activities by villagers to get rid of dry leaves from their residential compounds and protect themselves from mosquito bites that can transmit dengue fever.

From the results of this subsection, the second hypothesis in this study is true for the data; there were significant differences between the concentrations of air pollutants inside respondents' classrooms and residences in the high and low traffic areas.

4.3 Health Impacts

This section presents the results and discusses the main findings of the study pertaining to the third hypothesis. The third objective of the study was to compare the reported respiratory symptoms, lung function status, histone H3 level and DNA methylation (DNAm) status among the respondents.

4.3.1 Reported Respiratory Symptoms

Respiratory symptoms in this study were disclosed by parents or guardians using the standardised questionnaire adapted from ATS and ISAAC. The variables for respiratory symptoms were cough, phlegm, wheezing and chest tightness. Figure 4.31 shows the reported respiratory symptoms, and it was found that the prevalence of respiratory symptoms was higher among the HT group for each symptom than the LT group. There were significant differences in reported cough (p<0.001, OR=3.0, 95% CI=1.70-5.23), phlegm (p=0.023, OR=2.3, 95% CI=1.10-4.88) and wheezing (p=0.029, OR=2.3, 95% CI=1.07-5.05) for both groups. Chest tightness had no significant difference between both groups. The HT respondents were 3 times more likely to have a cough and 2 times more likely to have phlegm and wheezing compared to the LT children. Meanwhile, Table 4.16 shows the reported respiratory symptoms among children in each school. Cough was mostly reported among children in H1>H3>H2>H4>L1>L2>L3>L4. Phlegm was mostly reported among children in H2>H4>H1>H3>L3>L2>L4>L1. Wheezing was mostly reported among children in H4>H1>H3>H2>L4>L2>L1>L3. Chest tightness was mostly reported among children in H3>H1>H2>H4>L1>L2>L3=L4.

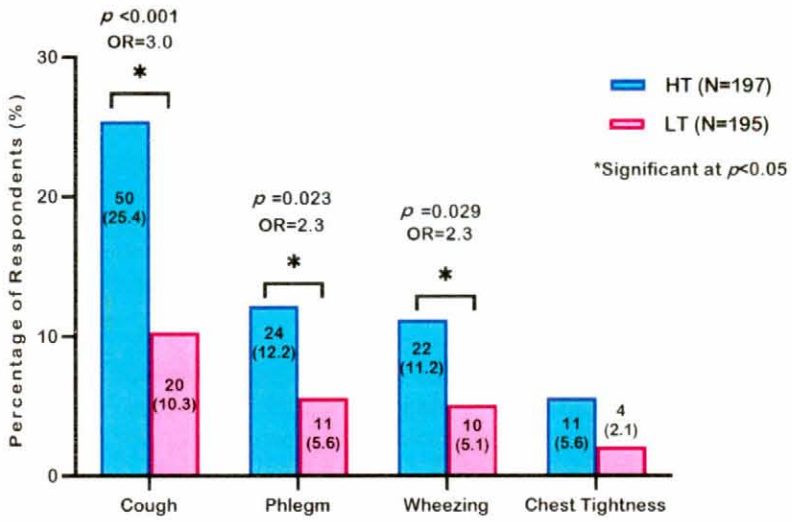


Figure 4.31: Reported respiratory symptoms

Table 4.16: Reported respiratory symptoms among children in each school

School	Number of Respondents (%)							
	Cough		Phlegm		Wheezing		Chest Tightness	
	Yes	No	Yes	No	Yes	No	Yes	No
H1 (*N*=75)	22 (29.3)	53 (70.7)	8 (10.7)	67 (89.3)	10 (13.3)	65 (86.7)	5 (6.7)	70 (93.3)
H2 (*N*=43)	10 (23.3)	33 (76.7)	7 (16.3)	36 (83.7)	2 (4.7)	41 (95.3)	2 (4.7)	41 (95.3)
H3 (*N*=43)	11 (25.6)	32 (74.4)	4 (9.3)	39 (90.7)	4 (9.3)	39 (90.7)	3 (7.0)	40 (93.0)
H4 (*N*=36)	7 (19.4)	29 (80.6)	5 (13.9)	31 (86.1)	6 (16.7)	30 (83.3)	1 (2.8)	35 (97.2)
L1 (*N*=63)	9 (14.3)	54 (85.7)	3 (4.8)	60 (95.2)	2 (3.2)	61 (96.8)	3 (4.8)	60 (95.2)
L2 (*N*=51)	6 (11.8)	45 (88.2)	3 (5.9)	48 (94.1)	2 (3.9)	49 (96.1)	1 (2.0)	50 (98.0)
L3 (*N*=42)	3 (7.1)	39 (92.9)	3 (7.1)	39 (92.9)	1 (2.4)	41 (97.6)	0 (0.0)	42 (100.0)
L4 (*N*=39)	2 (5.1)	37 (94.9)	2 (5.1)	37 (94.9)	5 (12.8)	34 (87.2)	0 (0.0)	39 (100.0)

Children in the HT group with a higher concentration of air pollutants are more likely to acquire respiratory symptoms than those in the LT group. However, all symptoms were not common among the children, as shown by the percentage of reported symptoms, which were all <50%. Chest tightness was the most rarely occurring, with 5.6% among the HT group and 2.1% among the LT group. High exposure towards TRAP in schools and residences have contributed to a high prevalence of cough, phlegm and wheezing among school children in the HT group. These findings are in line with results reported by other works in the literature, which had shown a higher prevalence of cough (Sopian et al., 2020), phlegm (Muhamad Daud et al., 2018), and wheezing (Zainudin et al., 2019)

among children who were exposed to higher concentrations of air pollutants than the children who had less exposure to air pollutants.

4.3.2 Lung Function Status

A spirometry test was performed to determine the lung function status of the respondents. This test was done concurrently with exposure monitoring during the school session before recess time. Height, weight and Body Mass Index (BMI) affect lung function status (Bhatti, Laghari, & Syed, 2019; Sadiq, Rizvi, Soleja, & Abbasi, 2019); hence, anthropometry data was also collected. The results are tabulated in Table 4.17. The mean height was 1.32 (0.08) m in the HT group, which was not significantly higher than that in the LT group at 1.30 (0.11) m (t=1.84, p=0.067). The median weight was 27.50 (9.00) kg in the HT group, which was not significantly higher than that in the LT group at 26.10 (7.35) kg (z= -1.88, p=0.060). The median BMI was 15.71 (3.21) kg/m^2 in the HT group, which was not significantly higher than that in the LT group at 15.43 (2.13) kg/m^2 (z= -0.57, p=0.567). The median BMI for both groups were within the normal range for children aged 7 to 11 years old in Malaysia. Hence, the contribution of obesity as one of the confounders in this study had been controlled. Obesity often substantially impairs the respiratory system by reducing lung capacity, especially the expiratory reserve volume, leading to mechanical restraints on the muscles responsible for breathing (Mafort, Rufino, Costa, & Lopes, 2016).

Table 4.17: Comparison of anthropometry data between two groups of respondents

Var	Locations	Min	Max	Mean	SD	Median	IQR	z/t	p
Height[b] (m)	HT (N=124)	1.15	1.54	1.32	0.08	1.32	0.10	1.84	0.067
	LT (N=124)	1.04	1.59	1.30	0.11	1.29	0.15		
Weight[a] (kg)	HT (N=124)	16.20	47.10	28.00	6.27	27.50	9.00	-1.88	0.060
	LT (N=124)	15.00	48.80	26.64	6.31	26.10	7.35		
BMI[a] (kg/m^2)	HT (N=124)	11.49	21.75	16.02	2.18	15.71	3.21	-0.57	0.567
	LT (N=124)	11.37	21.99	15.61	1.97	15.43	2.13		

Var: Variables; a: Mann-Whitney U Test; b: t-Test

Total lung capacity (TLC) is defined as the total air volume in the lungs after a maximal inspiration (Hind, 2013). It was found that there was no significant difference for both FVC and FEV_1 between both groups at p<0.05, as shown in Table 4.18. The mean FVC value was 1.32 (0.31) L in the HT group, which was not significantly lower than that in the LT group at 1.36 (0.35) L (t= -0.98, p=0.330). The mean FEV_1 value was 1.19 (0.28) L in the HT group, which was not significantly lower than that in the LT group at 1.24 (0.33) L (t= -1.22, p=0.223). TLC among individuals is affected by the depth of respiration, age, gender, body composition, and ethnicity (Lutfi, 2017). Still, the differences

between these anthropometry factors between the two groups were not significant, as displayed in Table 4.17.

Based on Table 4.18, it was also found that there were significant differences for both FVC% and FEV_1%, but not for FEV_1/FVC% between both groups at $p<0.05$. The median FVC% was 84.30 (16.34)% in the HT group, which was significantly lower than that in the LT group at 90.74 (12.27)% (z= -5.23, p=0.002). The median FEV_1% value was 82.30 (16.94)% in the HT group, which was significantly lower than that in the LT group at 89.22 (12.97)% (z= -5.01, $p<0.001$). The median FEV_1/FVC% value was 99.31 (7.39)% in the HT group, which was not significantly lower than that in the LT group at 99.52 (7.13)% (z= -0.16, p=0.871).

Table 4.18: Comparison of lung function status between two groups of respondents

Variables	HT (*N*=124)	LT (*N*=124)	*z/t*	*p*
	Median ± IQR / Mean ± SD			
FVC (Litre)[b]	1.32 ± 0.31	1.36 ± 0.35	-0.98	0.330
FEV_1 (Litre)[b]	1.19 ± 0.28	1.24 ± 0.33	-1.22	0.223
FVC %[a]	84.30 ± 16.34	90.74 ± 12.27	-5.23	**<0.001***
FEV_1 %[a]	82.30 ± 16.94	89.22 ± 12.97	-5.01	**<0.001***
FEV_1/FVC %[a]	99.31 ± 7.39	99.52 ± 7.13	-0.16	0.871

a: Mann-Whitney *U* Test; b: *t*-Test; ***Significant at $p<0.05$**

Table 4.19 shows the comparison of lung function abnormalities between two groups of respondents. The lung function abnormalities were determined based on the reference values according to ATS, as shown in Table 3.6. When comparing FVC%, there were 45 children (36.3%) among the HT group compared to 11 children (8.9%) among the LT group who had FVC% abnormality ($p<0.001$, OR=1.2, 95% CI=1.08-1.35). When comparing FEV_1%, there were 50 children (40.3%) among the HT group compared to 13 children (10.5%) among the LT group who had FEV_1% abnormality ($p<0.001$, OR=1.2, 95% CI=1.09-1.34). When comparing FEV_1/FVC%, there were 9 children (7.3%) among the HT group compared to 3 children (2.4%) among the LT group who had FEV_1/FVC% abnormality, but this comparison was not significant (p=0.076). Meanwhile, Table 4.20 displays the distribution of lung function abnormality among children in each school. Abnormality of FVC% was mostly reported among children in H1>H2>H4>H3>L3>L2>L4>L1. Abnormality of FEV_1% was mostly reported among children in H1>H2>H4>H3>L3>L2>L1=L4. Abnormality of FEV_1/FVC% was mostly reported among children in H1=H2>H3>H4=L1=L2=L4>L3.

Table 4.19: Comparison of lung function abnormality between two groups of respondents

Var	HT (*N*=124)		LT (*N*=124)		χ^2	*p*	OR	95% CI
	Normal (%)	Abnormal (%)	Normal (%)	Abnormal (%)				
FVC%	79 (63.7)	45 (36.3)	113 (91.1)	11 (8.9)	26.66	**<0.001***	1.2	1.08 – 1.35
FEV_1 %	74 (59.7)	50 (40.3)	111 (89.5)	13 (10.5)	29.13	**<0.001***	1.2	1.09 – 1.34
FEV_1/ FVC%	115 (92.7)	9 (7.3)	121 (97.6)	3 (2.4)	3.15	0.076	0.3	0.08 – 1.20

Var: Variables; ***Significant at *p*<0.05**

Table 4.20: Lung function abnormality among children in each school

School	Number of Respondents (%)					
	FVC%		FEV_1%		FEV_1/FVC%	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
H1 (*N*=55)	31 (56.4)	24 (43.6)	30 (54.5)	25 (45.5)	52 (94.5)	3 (5.5)
H2 (*N*=35)	22 (62.9)	13 (37.1)	20 (57.1)	15 (42.9)	32 (91.4)	3 (8.6)
H3 (*N*=15)	12 (80.0)	3 (20.0)	12 (80.0)	3 (20.0)	13 (86.7)	2 (13.3)
H4 (*N*=19)	14 (73.7)	5 (26.3)	12 (63.2)	7 (36.8)	18 (94.7)	1 (5.3)
L1 (*N*=38)	37 (97.4)	1 (2.6)	36 (94.7)	2 (5.3)	37 (97.4)	1 (2.6)
L2 (*N*=23)	20 (87.0)	3 (13.0)	19 (82.6)	4 (17.4)	22 (95.7)	1 (4.3)
L3 (*N*=32)	27 (84.4)	5 (15.6)	27 (84.4)	5 (15.6)	32 (100.0)	0 (0.0)
L4 (*N*=31)	29 (93.5)	2 (6.5)	29 (93.5)	2 (6.5)	30 (96.8)	1 (3.2)

The present study revealed that children in the HT area were more prone to have lung function impairment than the children in the LT area. This finding is supported by a previous study which reported a reduction in FVC% and FEV_1% among children residing in the urban area compared to the rural area in Egypt (Al-Qerem & Ling, 2018). The present findings also agree with previous local studies showing that the location of the school primarily in the urban or industrial area significantly contributed to lowering lung function among the children (Ab Jamil et al., 2015; Arifuddin et al., 2019). While obstructions in small airways can decline FEV_1%, increased airway resistance and decreased lung tissue elasticity are likely to reduce FVC% and FEV_1% (Das, Verstraete, Topalovic, Aerts, & Janssens, 2019). Moreover, reduced FEV_1 might also reflect reduced lung growth, but FEV_1/FVC had no significant difference between both groups in this study.

4.3.3 Level of Histone H3 Modification

Circulating histones serve as mediators for organ damage, such as lungs, by interacting with membrane phospholipids (Abrams et al., 2013). Circulating histone H3 ELISA kit specific for humans was used to determine the concentration of histone H3 in saliva samples obtained from the respondents. This research is the first human study conducted in Malaysia to study exposure to air pollutants by associating them with histone H3 modification among respondents, so there is no normal value for histone H3. Therefore, the median value was used as a cut off point. Non-parametric analysis disclosed a significantly different level of histone H3 between the two groups. The median level was 885.10 (620.04) ng/mL in HT group, which was significantly higher than that in LT group at 623.41 (305.10) ng/mL (z = -5.13, p<0.001).

A previous study reported that 76.5% of patients with severe blunt trauma with circulating histone levels ≥50 µg/ml suffered a respiratory failure compared to 18.8% who had histone levels below this limit (Abrams et al., 2013). In another study, 75% of septic patients with histone levels ≥75 µg/mL had left ventricular dysfunction compared with 8.3% of patients with histone levels <75 µg/mL (Alhamdi et al., 2015). Both studies used median data, which show that a high level of circulating histones in respondents may be an early biomarker for lung injury and multiple organ dysfunction. In comparison to these two studies, the threshold histone H3 level obtained in the current study among healthy children from HT and LT groups was lower than the threshold level obtained in the previous studies. Figure 4.32 shows the expression profile of histone H3 level among both groups. Meanwhile, Table 4.21 shows the distribution of histone H3 level among children in each school. High histone H3 level was mostly found among children in H3>H4>H2>H1>L3>L2>L1>L4.

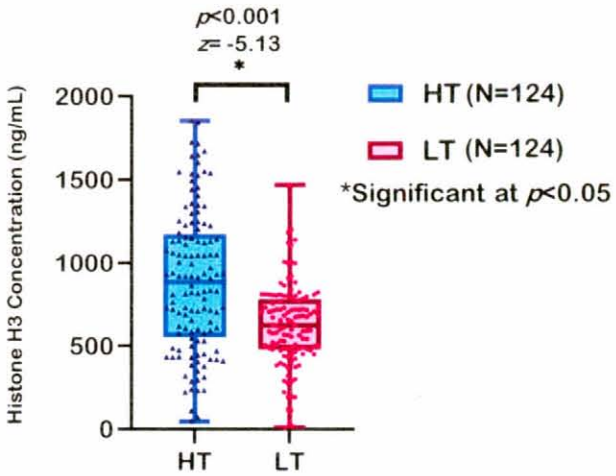


Figure 4.32: Expression profile of histone H3 modification between two groups of respondents

Table 4.21: Histone H3 level among children in each school

School	Number of Respondents (%)	
	Histone H3 Level	
	High	Low
H1 (*N*=55)	32 (58.2)	23 (41.8)
H2 (*N*=35)	21 (60.0)	14 (40.0)
H3 (*N*=15)	12 (80.0)	3 (20.0)
H4 (*N*=19)	14 (73.7)	5 (26.3)
L1 (*N*=38)	10 (26.3)	28 (73.7)
L2 (*N*=23)	11 (47.8)	12 (52.2)
L3 (*N*=32)	16 (50.0)	16 (50.0)
L4 (*N*=31)	8 (25.8)	23 (74.2)

Histones are particularly sensitive to oxidative stress, altering the molecular conformation of these molecules and their natural functional attributes (Bhargava et al., 2018). However, these histone modifications can either be caused directly or indirectly (Kietzmann, Petry, Shvetsova, Gerhold, & Görlach, 2017). The high air pollution levels in the HT area could induce oxidative stress in the children and affect their histone H3 levels, as suggested by a previous study among newborns (Vrijens et al., 2020). Air pollutants, particularly those that could freely access the alveoli, can increase ROS production and affect the modification of histone H3 (Zheng et al., 2017).

4.3.4 DNA Methylation (DNAm) Status

DNAm in the promoter regions of genes contributes to gene expression regulation, along with histone modifications (Shukla et al., 2019), and act as molecular signatures for the association between TRAP exposure and respiratory health among children in this study. DNAm status was determined based on the presence of gel bands after gel electrophoresis following MS-PCR. The studied genes were *CYP1A1* and *TNFα*. *CYP1A1* plays a vital role in carcinogenesis via the metabolism of PAH, one of the pollutants that form TRAP (Janssen et al., 2017). Meanwhile, *TNFα* has been implicated as an essential cytokine in many inflammatory lung diseases (Kumar et al., 2017).

Figure 4.33 illustrates two of the gels run for determination of *TNFα* DNAm status following MS-PCR. The methylation status was divided into methylated (M) or unmethylated (U). A homozygous methylated band and a heterozygous methylated and unmethylated bands were considered as methylation, while a homozygous unmethylated band was considered as unmethylation. The expected product size of M and U were both the same at 120 bp. Meanwhile, Figure 4.34 displays two of the gels run for determination of *CYP1A1* DNAm status following MS-PCR. The expected product size of M and U were both the same at 194 bp. Therefore, this experiment to get the targeted products was successful and as expected. These results are reliable as in the experiment included in the control by Cordero et al., (2011) and He et al., (2015).

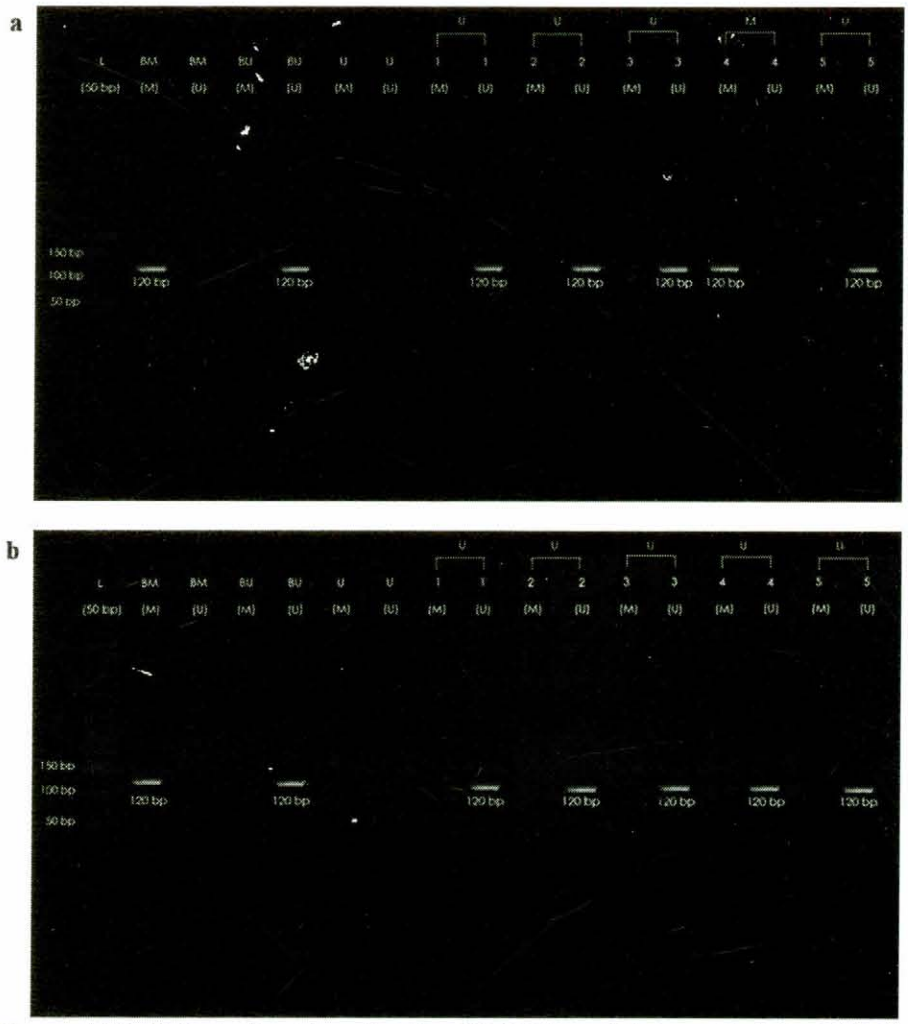


Figure 4.33: Detected gel bands after MS-PCR on *TNFα* methylated (120 bp) and unmethylated (120 bp) primers among children from the (a) HT group and (b) LT group

BM: Bisulphite-converted methylated DNA control; BU: Bisulphite-converted unmethylated DNA control; U: Unmethylated DNA control; M: Methylated primer; U: Unmethylated primer.

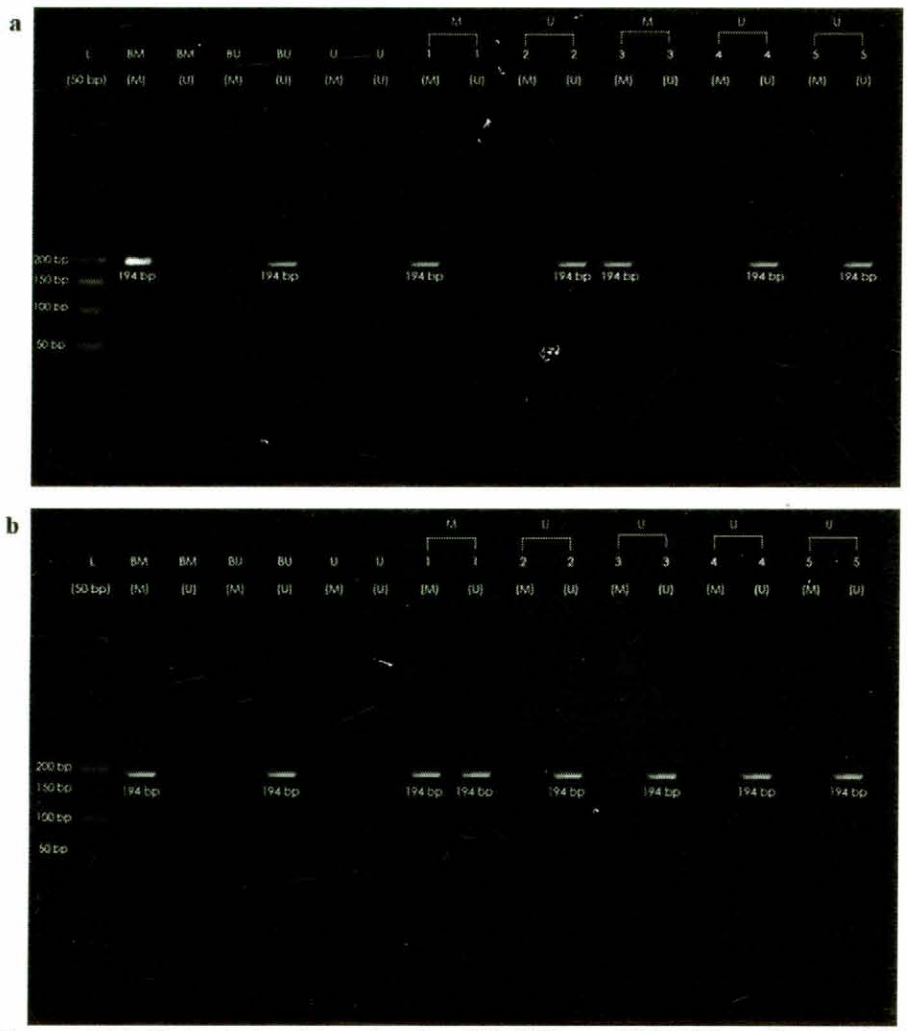


Figure 4.34: Detected gel bands after MS-PCR on *CYP1A1* methylated (194 bp) and unmethylated (194 bp) primers among children from the (a) HT group and (b) LT group

Table 4.22 shows the comparison of DNAm status between the two groups, and it was found that there were significant differences in *TNFα* (p=0.007, OR=2.0) and *CYP1A1* (p=0.030, OR=1.7) for both groups. There were 67 children (54.0%) from the HT group than 46 children (37.1%) from the LT group with methylated *TNFα*. Meanwhile, there were 64 children (51.6%) from the HT group compared to 47 children (37.9%) who had methylated *CYP1A1* from the LT group. The HT children were 2 times more likely to have methylated *TNFα* and almost 2 times more likely to have methylated *CYP1A1* than the LT children. When comparing the detection of both genes among the respondents, 47 children (37.9%) from the HT group and 34 children (27.4%) from the LT group had methylated *TNFα* and *CYP1A1*. There were 40 children (32.3%) from the HT group and 65 children (52.4%) from the LT group who had unmethylated

TNFα and *CYP1A1*. Meanwhile, 37 children (29.8%) from the HT group and 25 children (20.2%) from the LT group had a combination of either methylated *TNFα* and unmethylated *CYP1A1* or vice versa. Notably, the current findings also revealed that children in the HT area manifested a higher risk of methylated CpG islands of *TNFα* and *CYP1A1* than those in the LT area. Meanwhile, Table 4.23 shows the distribution of DNAm status among children in each school. Methylated *TNFα* was mostly reported among children in H4>H2>H3>H1>L3>L2>L4>L1. Methylated *CYP1A1* was mostly reported among children in H3>H2>H4>H1>L3>L2>L1>L4.

Table 4.22: Comparison of DNAm status between the two groups

Variables	HT (*N*=124)	LT (*N*=124)	χ^2	*p*	OR	95% CI
	Number (%)					
TNFα						
Methylated	67 (54.0)	46 (37.1)	7.17	**0.007***	2.0	1.20 – 3.31
Unmethylated	57 (46.0)	78 (62.9)				
CYP1A1						
Methylated	64 (51.6)	47 (37.9)	4.71	**0.030***	1.7	1.05 – 2.90
Unmethylated	60 (48.4)	77 (62.1)				
TNFα* and *CYP1A1						
Both Methylated	47 (37.9)	34 (27.4)				
Both Unmethylated	40 (32.3)	65 (52.4)	10.36	**0.006***	n/a	n/a
Combination	37 (29.8)	25 (20.2)				

***Significant at *p*<0.05**; n/a: Data could not be calculated

Table 4.23: DNAm status among children in each school

School	Number of Respondents (%)			
	TNFα		*CYP1A1*	
	Methylated	Unmethylated	Methylated	Unmethylated
H1 (*N*=55)	28 (50.9)	27 (49.1)	28 (50.9)	27 (49.1)
H2 (*N*=35)	20 (57.1)	15 (42.9)	19 (54.3)	16 (45.7)
H3 (*N*=15)	8 (53.3)	7 (46.7)	9 (60.0)	6 (40.0)
H4 (*N*=19)	11 (57.9)	8 (42.1)	10 (52.6)	9 (47.4)
L1 (*N*=38)	11 (28.9)	27 (71.1)	12 (31.6)	26 (68.4)
L2 (*N*=23)	10 (43.5)	13 (56.5)	10 (43.5)	13 (56.5)
L3 (*N*=32)	15 (46.9)	17 (53.1)	14 (43.8)	18 (56.3)
L4 (*N*=31)	10 (32.3)	21 (67.7)	9 (29.0)	22 (71.0)

There are not many available previous studies on DNAm as the outcomes of exposure to air pollution during childhood (Ferrari et al., 2019). However, no similar gene-specific DNAm study concerning air pollution exposure among children has been performed using MS-PCR. Previous studies in this field utilised quantitative measures of DNAm, such as pyrosequencing (Alvarado-Cruz et al., 2017; Prunicki et al., 2018). A previous study between two longitudinal birth cohorts by Langie et al. (2018) proved that salivary epigenetic DNAm changes are plausible underlying molecular mechanisms by confirming DNAm signatures can differentiate individuals with respiratory allergy from healthy subjects.

Not all genes are active at all times, and DNAm is an epigenetic mechanism that cells use to regulate gene expression. DNAm may revise the control of genes

engaged in airways development or immune-mediated inflammatory routes; for example, Fractional Exhaled Nitric Oxide (FeNO) levels are affected by DNAm of allergic asthma genes in a previous study by Jung et al. (2017). The mechanisms included in the relationship between TRAP and DNAm could involve oxidative effects of TRAP or activation of ROS following TRAP exposure. The oxidative stress mediates epigenetic changes and triggers downstream inflammatory pathways, prompting stimulation of immune cells, elevated cytokine expression, and eventually inflammation (Rider & Carlsten, 2019). DNAm is regulated by the program of DNA methyltransferases (DNMT) (Gowher & Jeltsch, 2018; Tirado-Magallanes, Rebbani, Lim, Pradhan, & Benoukraf, 2017). The methyl groups are moved from S-adenosyl methionine (SAMe), which is produced by constituents of the methionine adenosyltransferase (MAT) enzyme group as an element of the one-carbon cycle (Moen et al., 2015; Tirado-Magallanes et al., 2017). On the other hand, DNA demethylation happens passively through inadequate support during cell division or by the work of enzymes, including family members of ten-eleven translocation methylcytosine dioxygenase (TET). TETs switch 5-mC to 5-hydroxymethylcytosine (5-hmC), followed by 5-formylcytosine (5-fC) and subsequently 5- carboxycyotosine (5-caC), along with other pertinent alterations (Moen et al., 2015; Tirado-Magallanes et al., 2017).

From the results of this subsection, the third hypothesis in this study is true for the data; there were significant differences between the reported respiratory symptoms, lung function status, histone H3 level and DNAm status among the respondents in high and low traffic areas.

4.4 Relationships between Air Pollutants and Health Impacts

This section presents the results and discusses the main findings of the study pertaining to the fourth hypothesis. The fourth objective of the study was to assess the association between the concentrations of air pollutants and respiratory symptoms, lung function status, histone H3 level, and DNAm status among the respondents.

4.4.1 Relationships between Respondents' Daily Activities and Health Impacts

Household income is one factor that determines how and where the children would spend their time outside school hours (Krishnaswamy, Koon, & Annamalai, 2019). Besides, the availability of centres for child care, private academic lessons, supplementary non-academic classes, recreational parks and shopping malls are different according to residential locations. With the median household income of RM3500 and RM3000 among the HT group and LT group in this study, respectively, it is no surprise that more children may have no option but to resort to stay at home outside school hours. As shown in Table 4.24, a higher number of children from both groups spent ≥14 h/day at home on

either weekdays or weekends compared to those who spent <14 h/day at home on either weekdays or weekends. Meanwhile, a higher number of children from both groups spent ≥14 h/day at home on weekends compared to weekdays. When comparing the HT and LT group, a higher number of children from the LT group spent ≥14 h/day at home on either weekdays or weekends.

Table 4.24: Hours spent at home according to group

Group	Number of Respondents (%)			
	≥14 H/Day at Home on Weekdays	<14 H/Day at Home on Weekdays	≥14 H/Day at Home on Weekends	<14 H/Day at Home on Weekends
	Weekdays		Weekends	
HT (*N*=124)	64 (51.6)	60 (48.4)	77 (62.1)	47 (37.9)
LT (*N*=124)	85 (68.5)	39 (31.5)	91 (73.4)	33 (26.6)

Regardless of the respondent's group, associations between respondent's daily activities on weekdays and health impacts (reported respiratory symptoms, lung function status, histone H3 level and DNAm status) are reported in Table 4.25. When comparing the percentage of children, all studied health impacts except chest tightness were reported higher among those children who spent <14 h/day at home on weekdays. There were significant differences in reported cough (p<0.001, OR=1.2, 95% CI=1.11-1.51), abnormality of FVC% (p<0.001, OR=6.1, 95% CI=3.11-11.81), abnormality of FEV_1% (p<0.001, OR=4.8, 95% CI=2.56-8.81), histone H3 level (p<0.001, OR=1.3, 95% CI=1.20-1.58), methylated *TNFα* (p<0.001, OR=1.3, 95% CI=1.20-1.57) and methylated *CYP1A1* (p=0.023, OR=1.6, 95% CI=1.33-1.93) for both groups of activities on weekdays.

Table 4.25: Associations between hours spent at home on weekdays and health impacts

Health Impacts	≥14 H/Day at Home on Weekdays (*N*=149)	<14 H/Day at Home on Weekdays (*N*=99)	OR (95% CI)
Respiratory Symptoms, *N* (%)			
Cough	11 (7.4)	25 (25.3)	**1.2 (1.11 – 1.51)***
Phlegm	9 (6.0)	9 (9.1)	1.6 (1.25 – 2.68)
Wheezing	9 (6.0)	6 (6.1)	1.0 (0.34 – 2.89)
Chest Tightness	5 (3.4)	2 (2.0)	1.7 (0.32 – 8.86)§
Abnormal Lung Function Status, *N* (%)			
FVC%	15 (10.1)	40 (40.4)	**6.1 (3.11 – 11.81)***
FEV_1%	20 (13.4)	42 (42.4)	**4.8 (2.56 – 8.81)***
FEV_1/FVC%	6 (4.0)	5 (5.1)	1.3 (0.38 – 4.27)
High Histone H3 Level, *N* (%)			
Histone H3	56 (37.6)	63 (63.6)	**1.3 (1.20 – 1.58)***
Methylated DNA, *N* (%)			
TNFα	52 (34.9)	61 (61.6)	**1.3 (1.20 – 1.57)***
CYP1A1	58 (38.9)	53 (53.5)	**1.6 (1.33 – 1.93)***

***Significant at p<0.05**; § By χ^2 test with Yates' correction for expected value <5

Table 4.26 shows associations between the respondent's daily activities on weekends and health impacts (reported respiratory symptoms, lung function status, histone H3 level, and DNAm status). When comparing the percentage of children, all studied health impacts except chest tightness were reported higher among those children who spent <14 h/day at home on weekends. There were significant differences in abnormality of FVC% (p<0.001, OR=5.2, 95% CI=2.74-9.78), abnormality of FEV_1% (p<0.001, OR=6.6, 95% CI=3.55-12.42), abnormality of FEV_1/FVC% (p<0.001, OR=7.5, 95% CI=2.22-19.93), histone H3 level (p=0.004, OR=1.5, 95% CI=1.26-1.78), methylated *TNFα* (p=0.009, OR=1.5, 95% CI=1.29-1.84) and methylated *CYP1A1* (p=0.012, OR=1.5, 95% CI=1.29-1.86) for both groups of activities on weekends.

No similar study among children has been reported earlier. However, it is known that when children have activities outside their residences, they could be exposed to environmental hazards such as TRAP while travelling and when they are at the locations outside residences. Therefore, they are at an increased risk for adverse health effects compared to when they stay at home. Recently published studies had testified a reduction in emergency visits for asthma attacks (Kenyon, Hill, Henrickson, Bryant-Stephens, & Zorc, 2020), infection-related hospital admissions (Kadambari, Abo, Phuong, Osowicki, & Bryant, 2020) and premature deaths due to air pollution (Wang, Liu, & Zheng, 2020) during the Coronavirus Disease 2019 (COVID-19) pandemic. These findings were partly contributed by the movement restrictions with varying degrees imposed by each country's government to prevent the spread of COVID-19. The noticeable drop in air pollution and improved air quality throughout the world had reduced the risk of health impacts (Gupta, Bush, & Nagakumar, 2020).

Table 4.26: Associations between hours spent at home on weekends and health impacts

Health Impacts	≥14 H/Day at Home on Weekends (*N*=168)	<14 H/Day at Home on Weekends (*N*=80)	OR (95% CI)
Respiratory Symptoms, *N* (%)			
Cough	21 (12.5)	15 (18.8)	1.6 (1.30 – 2.28)
Phlegm	10 (6.0)	8 (10.0)	1.6 (1.22 – 2.50)
Wheezing	7 (4.2)	8 (10.0)	1.4 (1.14 – 2.12)
Chest Tightness	5 (3.0)	2 (2.5)	1.2 (0.23 – 6.30)
Abnormal Lung Function Status, *N* (%)			
FVC%	21 (12.5)	34 (42.5)	**5.2 (2.74 – 9.78)***
FEV_1%	22 (13.1)	40 (50.0)	**6.6 (3.55 – 12.42)***
FEV_1/FVC%	2 (1.2)	9 (11.3)	**7.5 (2.22 – 19.93)*§**
High Histone H3 Level, *N* (%)			
Histone H3	70 (41.7)	49 (61.3)	**1.5 (1.26 – 1.78)***
Methylated DNA, *N* (%)			
TNFα	67 (39.9)	46 (57.5)	**1.5 (1.29 – 1.84)***
CYP1A1	66 (39.3)	45 (56.3)	**1.5 (1.29 – 1.86)***

***Significant at p<0.05**; § By χ^2 test with Yates' correction for expected value <5

4.4.2 Relationships between Children's Mode of Transport to Schools and Health Impacts

Traffic emissions lead to the accumulation of air pollutants in the atmosphere. Moreover, studies have proven high concentrations of air pollutants at pedestrian crossings, traffic intersections and junctions (Azeez, Pradhan, & Shafri, 2018; Sofwan & Latif, 2021). Regardless of the school location, comparisons between children's transport mode to schools and health impacts (reported respiratory symptoms, lung function status, histone H3 level and DNAm status) are reported in Table 4.27. Cough was the most reported symptom for all children who travelled to schools by walking, motorcycles and cars. In contrast, cough and chest tightness were equally reported among bus travellers. When comparing abnormality of lung function and histone H3 level, there was not much difference between those who travelled by motorcycles and cars. In contrast, a higher number of children who travelled by motorcycles were found to have methylated *TNFα* and *CYP1A1* genes.

Table 4.27: Comparisons between children's mode of transport to schools and health impacts

Health Impacts	Total (*N*=248)	Car (*N*=94)	Bus (*N*=8)	Walk (*N*=22)	Motorcycle (*N*=124)
Reported Symptoms, *N* (%)					
Cough	36 (14.5)	11 (11.7)	2 (25.0)	5 (22.7)	18 (14.5)
Phlegm	18 (7.3)	4 (4.3)	0 (0.0)	3 (13.6)	11 (8.9)
Wheezing	15 (6.1)	7 (7.4)	0 (0.0)	0 (0.0)	8 (6.5)
Chest Tightness	7 (2.8)	3 (3.2)	2 (25.0)	1 (4.5)	1 (0.8)
Abnormal Lung Function Status, *N* (%)					
FVC%	55 (22.2)	26 (27.7)	1 (12.5)	3 (13.6)	25 (20.2)
FEV_1%	62 (25.0)	29 (30.9)	2 (25.0)	4 (18.2)	27 (21.8)
FEV_1/FVC%	11 (10.4)	3 (3.2)	1 (12.5)	2 (9.1)	5 (4.0)
High Histone H3 Level, *N* (%)					
Histone H3	119 (48.0)	48 (51.1)	6 (75.0)	15 (68.2)	50 (40.3)
Methylated DNA, *N* (%)					
TNFα	113 (45.6)	38 (40.4)	6 (75.0)	14 (63.6)	55 (44.4)
CYP1A1	111 (44.8)	40 (42.6)	5 (62.5)	10 (45.5)	56 (45.2)

These vehicles were further classified into "open vehicle" or "closed vehicle"; walk and motorcycle were categorised into "open vehicle", whereas car and bus were categorised into "closed vehicle". Table 4.28 displays the associations between children's mode of transport to schools and health impacts. There was no significant association between children's mode of transport to school and any of the respiratory symptom, any of the abnormality of lung function, histone H3 level and any of the DNAm statuses at $p<0.05$. These findings could be due to the round-trip duration of time taken from home to school, which varies according to the type of vehicles, as shown in Table 4.29. Children who went to school by bus spent the most time on the road, followed by those who travelled by car, motorcycle and walk.

Table 4.28: Associations between children's mode of transport to schools and health impacts

Health Impacts	Open (*N*=146)	Closed (*N*=102)	OR	95% CI
Reported Symptoms, *N* (%)				
Cough	23 (15.8)	13 (12.7)	0.78	0.38 – 1.63
Phlegm	14 (9.6)	4 (3.9)	0.39	0.12 – 1.21
Wheezing	8 (5.5)	7 (6.9)	1.27	0.45 – 3.62
Chest Tightness	2 (1.4)	5 (4.9)	3.71§	0.71 – 19.52
Abnormal Lung Function Status, *N* (%)				
FVC%	28 (19.2)	27 (26.5)	0.66	0.36 – 1.20
FEV_1%	31 (21.2)	31 (30.4)	0.62	0.35 – 1.10
FEV_1/FVC%	7 (4.8)	4 (3.9)	1.23§	0.35 – 4.33
High Histone H3 Level, *N* (%)				
Histone H3	54 (52.9)	65 (44.5)	1.40	0.84 – 2.33
Methylated DNA, *N* (%)				
TNFα	69 (47.3)	44 (43.1)	0.85	0.51 – 1.41
CYP1A1	66 (45.2)	45 (44.1)	0.96	0.58 – 1.60

§ By χ^2 test with Yates' correction for expected value <5

Table 4.29: Travel period from children's residences to schools

Duration (min)	Car (*N*=94)	Bus (*N*=8)	Walk (*N*=22)	Motorcycle (*N*=124)
Min	2	5	2	1
Max	60	30	10	30
Mean	14.7	15.9	6.9	10.0
SD	12.7	8.3	3.4	6.1

Those who walked or rode motorcycles to schools were deemed to have higher exposure to TRAP during the round-trip travel period from home to school because they were exposed to inhaling pollutants from vehicle emissions. A previous study in Indonesia had testified that the time spent in and near vehicles had enormously contributed to children's daily exposure to TRAP (Both, Westerdahl, Fruin, Haryanto, & Marshall, 2013). Moreover, most roads in Malaysia are choked with different vehicle types in the same lane (Abdul Manan, Várhelyi, Çelik, & Hashim, 2018). Nonetheless, some of the users in closed vehicles tend to open the windows of the vehicles. Based on the findings in this study, 4 children (50.0%) who travelled by bus and 67 children (71.3%) who travelled by car to school reported to open windows at least once during the round-trip travel period from home to school.

No similar study among children has been reported earlier. However, a study by Kelkar et al. (2019) among young adults testified that more prolonged periods of exposure to TRAP were connected to reduced FEV_1/FVC. Those who rode open transport vehicles such as motorcycles or non-air-conditioned buses for travelling from home to college had significantly lower values of FVC and FEV_1 in comparison to those who rode closed transport vehicles such as cars or air-conditioned buses. In the same vein, a study by Arphorn et al. (2018) revealed that motorcycle taxi riders had more lung function reduction than car taxi drivers.

The results suggest that exposure to high ambient PM_{10} concentration is a likely cause of lung function decline in motorcycle taxi drivers. These findings could be explained by higher exposures to air pollution experienced by those who used open transport vehicles than those who used closed transport vehicles. Besides, the health effects are worse when the same concentrations of air pollutants exposed to adults are also exposed to children.

4.4.3 Correlations between TRAP Concentrations, Lung Function Status and Histone H3 Levels

Figure 4.35 describes the correlation results between TRAP, lung function percentage and histone H3 level. Correlations with $p \geq 0.05$ are considered as not significant, and these r values are added with crosses. FVC% were negatively and weakly correlated with PM_1 (r= -0.14), $PM_{2.5}$ (r= -0.15), PM_{10} (r= -0.16), NO_2 (r= -0.16), TVOC (r= -0.16), CO (r= -0.19), SO_2 (r= -0.19),O_3 (r= -0.22) and BC (r= -0.22). FEV_1% were negatively and weakly correlated with concentrations of TVOC (r= -0.19), PM_1 (r= -0.20), $PM_{2.5}$ (r= -0.20), PM_{10} (r= -0.22), NO_2 (r= -0.24), CO (r= -0.26), O_3 (r= -0.27), SO_2 (r= -0.27) and BC (r= -0.29). FEV_1/FVC% were negatively and weakly correlated with concentrations of CO (r= -0.14), SO_2 (r= -0.17), and NO_2 (r= -0.17). By contrast, histone H3 level were positively and weakly correlated with concentrations of NO_2 (r=0.37), CO (r=0.36), PM_1 (r=0.35), $PM_{2.5}$ (r=0.34), SO_2 (r=0.34), PM_{10} (r=0.33), O_3 (r=0.33), TVOC (r=0.25) and BC (r=0.19).

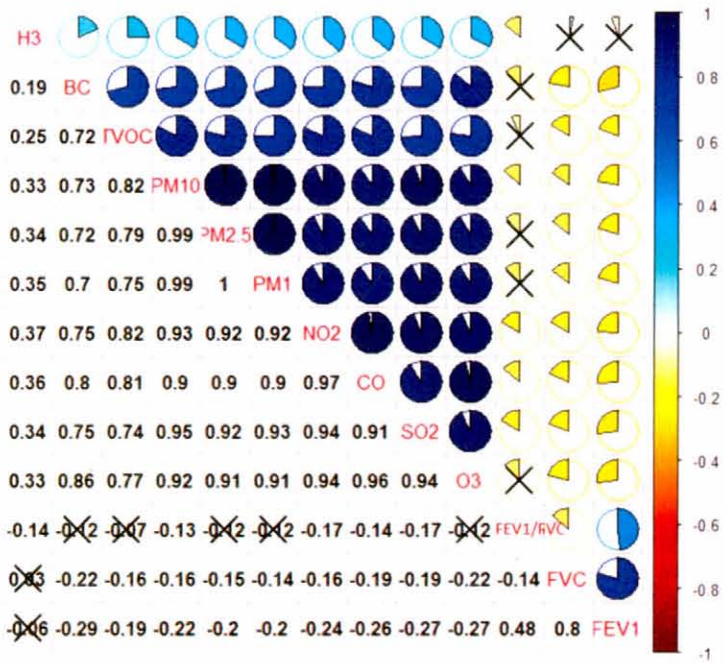


Figure 4.35: Correlations between TRAP concentrations, lung function percentage and histone H3 level

Early life and school-age exposure to TRAP has a negative impact on lung function development. These results agree with a local study among children by Hussin & Jalaludin (2016), who found that higher $PM_{2.5}$ concentrations was inversely correlated with lower FVC% (r= -0.35, p=0.023). However, they found no significant correlation between concentrations of $PM_{2.5}$ with FEV_1% (r=0.03, p=0.894) and FEV_1/FVC% of the studied group, (r= -0.021, p=0.267). In another local study among children by Ab Jamil et al. (2015), they reported negative correlations between FVC% and concentrations of PM_{10} (r=-0.34, p<0.001), VOC (r= -0.16, p<0.001), NO_2 (r= -0.64, p<0.001). They also testified negative correlations between FEV_1% and concentrations of PM_{10} (r= -0.36, p<0.001), VOC (r= -0.19, p<0.001), NO_2 (p= -0.88, p<0.001).

Epigenetics has been proposed as one of the links between exposure to air pollution and respiratory health outcomes, for example, histone H3 modification in this study. Circulating histones function as the mediators of remote organ injury, inducing cellular calcium infusion by contact with membrane phospholipids (Abrams et al., 2013). No similar histone H3 level study in relation to air pollution exposure among children has been performed using ELISA. However, the results in this study agree with a cohort study by Vrijens et al. (2020), who investigated the adverse effects of gestational air pollution exposure on circulating levels of histones among newborns. Circulating histone H3 were positively associated with gestational $PM_{2.5}$ and BC exposure, with a 40.2% increment (95% CI, 24.1% to 58.3%, p<0.001) and a 38.4% increment (95% CI, 6.2% to 80.3%, p=0.003).

4.4.4 Relationships between TRAP Concentrations, Reported Respiratory Symptoms and DNAm Status

4.4.4.1 Associations between TRAP Concentrations and Reported Respiratory Symptoms

TRAP concentrations in schools were categorised into high or low concentration according to their medians (PM_{10}=89 μg/m^3, $PM_{2.5}$=68 μg/m^3, PM_1=60 μg/m^3, BC=25.9 μg/m^3, NO_2=30 ppb, SO_2=20 ppb, O_3=22 ppb, TVOC=22 ppb, CO=150 ppb). Meanwhile, TRAP concentrations inside residences were categorised into high or low according to their medians (PM_{10}=82 μg/m^3, $PM_{2.5}$=59 μg/m^3). Any concentration below the median was considered a low concentration.

Table 4.30 shows the relationship between TRAP concentrations in schools with the reported respiratory symptoms (cough, phlegm, wheezing and chest tightness) among the children. It is shown that there were significant associations between reported cough and TRAP at p<0.05, except for SO_2 and CO. Besides, there were significant associations between reported phlegm and TRAP at p<0.05, except for NO_2, SO_2, CO and TVOC. There were also significant associations between reported wheezing and TRAP at p<0.05, except for SO_2 and CO. On the contrary, there was no significant association between reported

chest tightness and TRAP at $p<0.05$. There was also no significant association between any reported respiratory symptoms and TRAP inside residences at $p<0.05$.

Table 4.30. Associations of TRAP concentrations in schools with reported respiratory symptoms

TRAP Variables	Respiratory Symptoms			
	Cough OR (95% CI)	Phlegm OR (95% CI)	Wheezing OR (95% CI)	Chest Tightness OR (95% CI)
	Schools			
PM_{10} ($\mu g/m^3$)	**3.0 (1.70 – 5.23)***	**2.3 (1.10 – 4.88)***	**2.3 (1.07 – 5.05)***	2.8 (0.88 – 9.03)
$PM_{2.5}$ ($\mu g/m^3$)	**3.0 (1.70 – 5.23)***	**2.3 (1.10 – 4.88)***	**2.3 (1.07 – 5.05)***	2.8 (0.88 – 9.03)
PM_1 ($\mu g/m^3$)	**3.0 (1.70 – 5.23)***	**2.3 (1.10 – 4.88)***	**2.3 (1.07 – 5.05)***	2.8 (0.88 – 9.03)
BC ($\mu g/m^3$)	**2.6 (1.48 – 4.49)***	**2.2 (1.04 – 4.61)***	**2.2 (1.01 – 4.78)***	2.7 (0.84 – 8.56)
NO_2 (ppb)	**2.2 (1.29 – 3.69)***	1.7 (0.78 – 3.14)	**3.0 (1.39 – 6.34)***	2.9 (1.02 – 9.28)
SO_2 (ppb)	1.6 (0.90 – 2.78)	1.9 (0.90 – 3.85)	0.8 (0.34 – 1.95)	1.4 (0.75 – 7.62)
O_3 (ppb)	**2.5 (1.50 – 4.28)***	**3.0 (1.46 0 6.00)***	**2.1 (1.03 – 4.41)***	2.5 (0.98 – 9.23)
CO (ppb)	1.7 (0.98 – 2.78)	1.1 (0.56 – 2.31)	1.5 (0.75 – 3.19)	1.8 (0.88 – 9.03)
TVOC (ppb)	**2.1 (1.21 – 3.54)***	1.6 (0.76 – 3.22)	**2.8 (1.34 – 5.80)***	2.7 (0.74 – 9.63)
	Residences			
PM_{10} ($\mu g/m^3$)‡	0.4 (0.67 – 2.18)	2.0 (1.52 – 2.64)	2.2 (1.61 – 2.94)	2.0 (1.50 – 2.57)
$PM_{2.5}$ ($\mu g/m^3$)‡	0.5 (0.09 – 2.86)	1.8 (1.40 – 2.28)	1.8 (1.40 – 2.28)	1.8 (1.39 – 2.23)

N=392; ***Significant at $p<0.05$**; ‡ *N*=52

There were significant associations between particulate matter concentrations at schools with cough, phlegm and wheezing in this study, which was coherent with a local study by Hussin & Jalaludin (2016). They found that children exposed to higher PM_{10} concentrations were 3 times (p=0.032, 95% CI=1.07-10.07) and 5 times (p=0.032, 95% CI=1.03-26.45) more likely to get cough and wheezing, respectively. Besides, Kamaruddin et al. (2016) revealed that children exposed to higher $PM_{2.5}$ concentrations were 2 times (p=0.008, 95% CI=1.25-4.76) more likely to get cough. PM concentrations in this study also did not show significant associations with chest tightness, similar to previous findings (Hussin & Jalaludin, 2016; Kamaruddin et al., 2016). Coarse particles are more likely deposited at the trachea entrance on the upper respiratory tract, whereas finer particles can be deposited in the deep lung (Deng, Deng, Miao, Guo, & Li, 2019).

NO_2 concentrations in this study were significantly associated with cough and wheezing, which corroborate with a local study by Kamaruddin et al. (2016). They reported that children exposed to higher NO_2 concentrations were 2 times more likely to get cough (p=0.010, 95% CI=1.21-4.49) and wheezing (p=0.005, 95% CI=1.47-14.16). Moreover, NO_2 concentrations in this study also did not

show significant associations with phlegm and chest tightness, similar to previous local findings (Ab Jamil et al., 2015; Suhaimi et al., 2015). SO_2 concentrations in this study were not significantly associated with all respiratory symptoms, which disagreed with a local study by Ab Jamil et al. (2015). They reported that children exposed to higher SO_2 concentrations were 2 times (p=0.002, 95% CI=1.12-4.66) more likely to get phlegm. Exposure to NO_2 triggers an inflammatory response, and it is more likely to reach the lower airways due to its lower water solubility than SO_2 (Liu et al., 2016).

CO concentrations in this study were not significantly associated with all respiratory symptoms, which was contrasted with a study by Van Vliet et al. (2019). They reported significant associations between CO concentrations with cough (OR=1.1, 95% CI=0.83-1.32), phlegm (OR=1.2, 95% CI=0.98-1.35) and wheezing (OR=1.3, 95% CI=1.00-1.63) among pregnant women cooking with biomass fuels. Meanwhile, TVOC concentrations in this study were significantly associated with cough and wheezing, which were not in line with a local study by Jalaludin & Jasme (2015). They did not find any significant association between TVOC and any of the respiratory symptom. Gases like CO and VOC may irritate the eyes, nose and throat, causing headaches and fatigue (Schulze et al., 2017).

Increased exposure to air pollutants from road traffic and indoor sources may contribute to respiratory symptoms among children. In general, exposure to higher concentrations of TRAP have significantly contributed to a higher prevalence of respiratory symptoms in this study, as supported by findings from previous studies (Arifuddin et al., 2019; Awang et al., 2020).

4.4.4.2 Associations between TRAP Concentrations and DNAm Status

Table 4.31 shows the relationship between TRAP concentrations in schools with DNAm of *TNFα* and *CYP1A1* among the children. It is shown that there were significant associations between methylated *TNFα* and TRAP in schools at $p<0.05$, except for SO_2. There was no significant association between methylated *CYP1A1* and TRAP in schools at $p<0.05$, except for PM_{10}, $PM_{2.5}$, PM_1 and TVOC. On the contrary, there was no significant association between DNAm of *TNFα* and *CYP1A1* and TRAP inside residences at $p<0.05$.

Table 4.31: Associations of TRAP concentrations in schools with DNAm of *TNFα* and *CYP1A1*

TRAP Variables	DNAm Status	
	TNFα OR (95% CI)	*CYP1A1* OR (95% CI)
	Schools	
PM_{10} (μg/m^3)	**2.0 (1.20 – 3.31)***	**1.8 (1.05 – 2.90)***
$PM_{2.5}$ (μg/m^3)	**2.0 (1.20 – 3.31)***	**1.8 (1.05 – 2.90)***
PM_1 (μg/m^3)	**2.0 (1.20 – 3.31)***	**1.8 (1.05 – 2.90)***
BC (μg/m^3)	**1.7 (1.03 – 2.82)***	1.6 (0.97 – 2.65)
NO_2 (ppb)	**1.8 (1.07 – 3.17)***	1.6 (0.95 – 2.79)
SO_2 (ppb)	1.4 (0.86 – 2.39)	1.4 (0.82 – 2.26)
O_3 (ppb)	**1.8 (1.09 – 3.03)***	1.5 (0.90 – 2.49)
CO (ppb)	**1.7 (1.00 – 2.77)***	1.5 (0.93 – 2.56)
TVOC (ppb)	**1.9 (1.12 – 3.12)***	**1.9 (1.13 – 3.11)***
	Residences	
PM_{10} (μg/m^3)‡	0.3 (0.10 – 1.01)	0.9 (0.28 – 2.52)
$PM_{2.5}$ (μg/m^3)‡	0.2 (0.07 – 0.74)	0.9 (0.28 – 2.52)

N=248; ***Significant at *p*<0.05;** ‡ *N*=52

No similar gene-specific DNAm study concerning air pollution exposure among children has been performed using MS-PCR; hence, relationships between exposure to TRAP and DNAm in this study are compared with previous limited studies that addressed instruments relevant to air pollutant exposures and DNAm among children. *TNFα*, *IFNγ* and *IL4* are known to activate nuclear factor kappa B cells (NF-κB), a key regulator of inducible gene expression in the immune system (Liu, Zhang, Joo, & Sun, 2017). In contrast, the NF-κB signalling pathway plays an essential role in factor forkhead box P3 (*FOXP3*) expression (Long, Park, Strickland, Hayden, & Ghosh, 2009) and *CYP1A1* expression (Zordoky & El-Kadi, 2009). These results suggest that minor variations in the κB binding region and adjacent bases can have significant consequences upon the resulting ability of activated NF-κB to trigger gene expression (Leung, Hoffmann, & Baltimore, 2004).

Li et al. (2018) revealed a positive correlation between PM_{10} exposure and methylation level in *IFNγ*. In the case of $PM_{2.5}$ exposure, Li et al. (2018) demonstrated that $PM_{2.5}$ exposure and methylation level in *IFNγ* were positively correlated, while Prunicki et al. (2018) found a positive relationship between exposure to a higher concentration of $PM_{2.5}$ and higher methylation in *FOXP3*. Jung et al. (2017) demonstrated a connection between 24-h averaged BC with hypomethylation of DNA at the *IL4* promoter, albeit after the earlier methylation levels were adjusted. Prunicki et al. (2018) exhibited that exposure to a higher concentration of NO_2 was positively associated with higher methylation in *FOXP3,* but O_3 exposure was negatively associated with *FOXP3* methylation. Furthermore, previous research points to evidence that increased exposure to diesel exhaust particle were associated with increased *FOXP3*, which was linked to a higher risk of asthma development in children (Brunst et al., 2013; Jiang, Jones, Sava, Kobor, & Carlsten, 2014). As childhood years are the crucial moment for lung development, lowering TRAP exposure lowers the risk of chronic respiratory diseases in the future (Checkley et al., 2019).

4.4.5 Relationships between Air Pollutant Sources at Residences and Health Impacts

4.4.5.1 Associations between Air Pollutant Sources at Residences and Reported Respiratory Symptoms

Table 4.32 displays the associations between reported respiratory symptoms (cough, phlegm, wheezing and chest tightness) and air pollutant sources at residences. There was no significant association between reported respiratory symptoms and air pollutant sources at residences at $p<0.05$ except for the distance of residences from highways. Cough ($p<0.001$, 95% CI=1.58-4.53) was 2.7 times more likely to increase when the residences were near highways. Phlegm ($p<0.001$, 95% CI=1.63-6.87) and wheezing ($p<0.001$, 95% CI=1.54-6.87) were 3.3 times more likely to increase when the residences were located near highways. Chest tightness ($p=0.012$, 95% CI=1.24-11.09) was 3.7 times more likely to increase when the residences were near highways.

The self-reporting of the distance of residences from highways may have introduced bias in this study. Therefore, the reported residential addresses were confirmed by using Google Maps. There were ≥70% of respondents who live <500 m from highways, and ≥80% of respondents who live ≥500 m from highways reported no respiratory symptom. The current findings are generally in line with previous studies linking residential TRAP exposure to increased risk for respiratory symptoms in persons living closer to heavy-traffic roads such as highways (McConnell et al., 2006; Morgenstern et al., 2008). In a nested study among communities living along highways by Hazenkamp-Von Arx et al. (2011), there was a significant association between residential exposure to highways and chronic cough (OR=2.88, $p=0.048$, 95% CI=1.17-7.05). In contrast, another study by Kim et al. (2008) reported that associations were elevated but not significant using distance to highways on a linear scale. Those living downwind and within 300 m of highways were at increased risk of both outcomes, but the results were not statistically significant.

Table 4.32: Associations between air pollutant sources at residences and reported respiratory symptoms

Sources at Residences	Respiratory Symptoms			
	Cough OR (95% CI)	Phlegm OR (95% CI)	Wheezing OR (95% CI)	Chest Tightness OR (95% CI)
Indoor Painting within the Past 12 Months	0.7 (0.40 – 1.34)	0.8 (0.33 – 1.71)	1.4 (0.65 – 3.01)	1.8 (0.62 – 5.11)
Floor Renovation within the Past 12 Months	0.9 (0.37 – 2.28)	0.6 (0.13 – 2.51)§	3.2 (1.27 – 8.02)	0.7 (0.09 – 5.47)§
Pets	0.8 (0.40 – 1.47)	0.9 (0.37 – 2.06)	3.1 (1.45 – 6.44)	1.3 (0.40 – 4.15)§
Mosquitoes Coils	0.6 (0.19 – 1.67)§	0.6 (0.14 – 2.59)§	0.7 (0.15 – 2.89)§	1.3 (0.47 – 3.75)§
Carpet Usage	0.6 (0.37 – 1.07)	1.1 (0.55 – 2.20)	1.8 (0.89 – 3.69)	1.3 (0.47 – 3.75)
Chemical Solvent	1.1 (0.60 – 1.96)	1.1 (0.49 – 2.38)	1.5 (0.66 – 3.18)	2.1 (0.74 – 6.14)
Moth Balls	1.2 (0.66 – 2.35)	2.5 (1.20 – 5.36)	1.5 (0.65 – 3.52)	1.1 (0.30 – 3.99)§
Air Freshener	1.4 (0.81 – 2.30)	1.1 (0.54 – 2.21)	2.3 (1.09 – 4.74)	1.7 (0.60 – 4.76)
House Cleaning Frequency Per Week	1.0 (0.60 – 1.70)	1.1 (0.54 – 2.16)	0.9 (0.43 – 1.82)	2.1 (0.70 – 6.19)
Indoor Smoking	1.1 (0.61 – 1.79)	1.2 (0.64 – 2.43)	2.5 (1.18 – 5.09)	1.6 (0.58 – 4.42)
Indoor Cooling System	2.1 (1.19 – 3.81)	1.4 (0.64 – 3.16)	0.7 (0.27 – 1.92)	2.1 (0.68 – 6.17)
Cooking Stove	1.3 (0.72 – 2.43)	0.8 (0.32 – 1.98)	1.3 (0.58 – 3.09)	1.4 (0.45 – 4.65)§
Daily Cooking Activity	0.7 (0.41 – 1.15)	1.0 (0.49 – 1.96)	0.7 (0.34 – 1.46)	0.9 (0.33 – 2.64)
Usage of Cooking Hood and Hob	2.2 (1.18 – 4.02)	1.0 (0.41 – 2.57)	1.4 (0.59 – 3.46)	0.8 (0.17 – 3.42)§
Open Window or Door While Cooking	0.9 (0.26 -3.40)§	0.4 (0.11 – 1.49)§	n/a§	0.3 (0.05 – 1.22)
Distance from Main Roads	1.0 (0.49 – 2.14)	1.9 (0.56 – 6.43)§	0.7 (0.28 – 1.82)	n/a§
Distance from Highways	**2.7 (1.58 – 4.53)***	**3.3 (1.63 – 6.87)***	**3.3 (1.54 – 6.87)**	**3.7 (1.24 – 11.09)***
Distance from Factories	1.9 (0.49 – 3.88)	0.5 (0.06 – 3.61)§	1.1 (0.25 – 5.08)§	1.2 (0.15 – 9.65)

N=392; ***Significant at $p<0.05$**; § By χ^2 test with Yates' correction for expected value <5; n/a: Data could not be calculated

4.4.5.2 Associations between Air Pollutant Sources at Residences and Lung Function Status

Table 4.33 displays the associations between lung function status and air pollutant sources at residences. There was no significant association between FVC% and air pollutant sources at residences at $p<0.05$ except for the distance of residences from highways. FVC% was 1.4 times more likely to increase when the residences were near highways ($p=0.002$, 95% CI=0.20-1.71). Meanwhile, there was no significant association between FEV_1% and air pollutant sources at residences at $p<0.05$ except for the distance of residences from main roads and highways. FEV_1% was 1.3 and 1.5 times more likely to increase when the residences were located near to main roads ($p=0.046$, 95% CI=0.09-2.04) and highways ($p=0.021$, 95% CI=0.26-1.90), respectively. In contrast, no significant association was reported between FEV_1/FVC% and air pollutant sources at residences at $p<0.05$.

The current findings are generally in the same vein as previous studies linking residential TRAP exposure to increased risk for lung function impairment in persons living closer to heavy-traffic roads such as highways (Fadzir & Jalaludin, 2013; Gauderman et al., 2004). The deficits in lung function among the children living close to highways could be due to chronic airway inflammation (Schultz, Litonjua, & Melén, 2017). In a cohort study among children by Urman et al. (2014), living within 500 m of a highway was associated with a nearly 2% reduction in FVC ($p=0.009$, 95% CI −3.41% to −0.49%) compared with those living at least 1500 m from a highway. Nevertheless, there was no significant association between mean FEV_1 and living within 500 m of a highway. In another cohort study among children by Gauderman et al. (2004), they observed a significantly lower percentage of predicted lung function in 8-year FEV_1 growth ($p=0.013$, 97.0%, 95% CI=94.6-99.4) but no significant lower percentage of predicted lung function in 8-year FVC growth for those who lived <500 m of a highway.

Table 4.33: Associations between air pollutant sources at residences and lung function status

Sources at Residences	Lung Function Status		
	FVC% OR (95% CI)	FEV_1% OR (95% CI)	FEV_1/FVC% OR (95% CI)
Indoor Painting within the Past 12 Months	0.8 (0.40 – 1.58)	0.8 (0.42 – 1.63)	0.8 (0.31 – 2.26)
Floor Renovation within the Past 12 Months	1.1 (0.34 – 3.34)§	1.1 (0.35 – 3.43)§	n/a§
Pets	0.9 (0.44 – 1.82)	0.8 (0.40 – 1.63)	1.9 (0.55 – 6.76)§
Mosquitoes Coils	1.4 (0.47 – 4.40)§	1.1 (0.39 – 3.08)	2.4 (0.30 – 18.41)§
Carpet Usage	0.9 (0.50 – 1.70)	0.9 (0.48 – 1.61)	0.7 (0.28 – 1.68)
Chemical Solvent	1.5 (0.69 – 3.16)	1.8 (0.81 – 8.89)	3.3 (0.75 – 14.76)§
Moth Balls	0.7 (0.32 – 1.42)	0.9 (0.43 – 2.05)	0.3 (0.12 – 0.82)
Air Freshener	1.1 (0.59 – 2.07)	0.7 (0.38 – 1.29)	0.5 (0.19 – 1.17)
House Cleaning Frequency Per Week	1.0 (0.55 – 1.88)	1.2 (0.64 – 2.17)	1.1 (0.46 – 2.76)
Indoor Smoking	1.0 (0.54 – 1.95)	0.8 (0.42 – 1.46)	0.5 (0.19 – 1.13)
Indoor Cooling System	1.6 (0.70 – 3.63)	0.8 (0.36 – 1.58)	2.7 (0.61 – 11.91)§
Cooking Stove	2.4 (0.97 – 6.02)	1.7 (0.74 – 3.84)	0.9 (0.30 – 2.46)
Daily Cooking Activity	1.0 (0.55 – 1.86)	0.7 (0.39 – 1.34)	1.1 (0.44 – 2.63)
Usage of Cooking Hood and Hob	1.2 (0.50 – 2.67)	1.4 (0.60 – 3.50)	4.4 (0.58 – 33.79)§
Open Window or Door While Cooking	0.4 (0.15 – 1.32)§	0.4 (0.05 – 3.21)§	1.2 (0.15 – 10.05)§
Distance from Main Roads	0.4 (0.15 – 1.32)§	**1.3 (0.09 – 2.04)*§**	0.3 (0.04 – 2.19)§
Distance from Highways	**1.4 (0.20 – 1.71)***	**1.5 (0.26 – 1.90)***	0.8 (0.33 – 2.06)
Distance from Factories	0.8 (0.24 – 2.54)§	0.8 (0.25 – 2.60)§	1.4 (0.18 – 11.28)§

N=248; ***Significant at $p<0.05$**; § By χ^2 test with Yates' correction for expected value <5; n/a: Data could not be calculated

4.4.5.3 Associations between Air Pollutant Sources at Residences and Histone H3 Level

Table 4.34 displays the associations between histone H3 level and air pollutant sources at residences. Histone H3 level was divided into high or low according to its median concentration (710.3 ng/ml). Any concentration below the median was considered a low concentration. There was no significant association between histone H3 level and air pollutant sources at residences at $p<0.05$ except for the distance of residences from highways. Histone H3 level was 2.2 times more likely to increase when the residences were located near highways ($p=0.003$, 95% CI=1.31-3.84). No published data exist to support an association between the distance of residences from highways and Histone H3 level. However, previous evidence has identified histone H3 modification after exposure to TRAP (Ding et al., 2017; Zheng et al., 2017).

Table 4.34. Associations between air pollutant sources at residences and histone H3 level

Sources at Residences	Histone H3 OR (95% CI)
Indoor Painting within the Past 12 Months	0.5 (0.29 – 0.94)
Floor Renovation within the Past 12 Months	0.8 (0.32 – 2.01)
Pets	1.1 (0.61 – 1.97)
Mosquitoes Coils	0.6 (0.28 – 1.48)
Carpet Usage	0.8 (0.50 – 1.36)
Chemical Solvent	1.5 (0.83 – 2.66)
Moth Balls	1.3 (0.69 – 2.51)
Air Freshener	1.1 (0.64 – 1.78)
House Cleaning Frequency Per Week	1.2 (0.71 – 1.93)
Indoor Smoking	1.3 (0.76 – 2.16)
Indoor Cooling System	2.0 (1.07 – 3.76)
Cooking Stove	0.8 (0.43 – 1.45)
Daily Cooking Activity	0.9 (0.57 – 1.54)
Usage of Cooking Hood and Hob	0.8 (0.41 – 1.55)
Open Window or Door While Cooking	1.5 (0.42 – 5.54)
Distance from Main Roads	1.1 (0.52 – 2.19)
Distance from Highways	**2.2 (1.31 – 3.84)***
Distance from Factories	1.3 (0.47 – 3.63)

N=248; ***Significant at $p<0.05$**

4.4.5.4 Associations between Air Pollutant Sources at Residences and DNAm Status

Table 4.35 displays the associations between DNAm status and air pollutant sources at residences. There was no significant association between DNAm status of both genes and air pollutant sources at residences at $p<0.05$. No similar study has been conducted before on the association between the distance of residences from highways and DNAm of *TNFα* and *CYP1A1*. However, a previous cohort study among children has identified Ten-Eleven Translocation 1 (*TET1*) gene, a key enzyme in DNA demethylation, displayed an increased methylation for the measurement of 24 h after exposure to TRAP, which proposed a negative association between methylation and gene expression (Somineni et al., 2016). Furthermore, they revealed that increased global 5hmC in bronchial epithelial cells was significantly associated with asthma.

Table 4.35: Associations between air pollutant sources at residences and DNAm status

Sources at Residences	DNAm Status	
	TNFα OR (95% CI)	*CYP1A1* OR (95% CI)
Indoor Painting within the Past 12 Months	0.8 (0.44 – 1.41)	0.6 (0.32 – 1.05)
Floor Renovation within the Past 12 Months	0.8 (0.31 – 1.98)	0.5 (0.19 – 1.35)
Pets	0.7 (0.36 – 1.21)	1.0 (0.56 – 1.81)
Mosquitoes Coils	1.3 (0.58 – 3.05)	0.6 (0.23 – 1.32)
Carpet Usage	0.8 (0.48 – 1.20)	0.7 (0.42 – 1.14)
Chemical Solvent	1.1 (0.62 – 1.98)	1.2 (0.65 – 2.07)
Moth Balls	1.6 (0.85 – 3.13)	1.9 (0.99 – 3.74)
Air Freshener	0.6 (0.38 – 1.07)	1.3 (0.80 – 2.22)
House Cleaning Frequency Per Week	1.0 (0.61 – 1.65)	0.8 (0.47 – 1.28)
Indoor Smoking	1.5 (0.87 – 2.48)	1.2 (0.69 – 1.96)
Indoor Cooling System	0.8 (0.41 – 1.43)	1.1 (0.58 – 1.98)
Cooking Stove	0.8 (0.44 – 1.50)	0.6 (0.30 – 1.06)
Daily Cooking Activity	1.1 (0.69 – 1.88)	1.1 (0.69 – 1.89)
Usage of Cooking Hood and Hob	0.5 (0.27 – 1.09)	0.6 (0.69 – 1.89)
Open Window or Door While Cooking	0.8 (0.23 – 2.95)	1.9 (0.49 – 7.68)§
Distance from Main Roads	0.7 (0.32 – 1.36)	1.7 (0.79 – 3.52)
Distance from Highways	1.4 (0.83 – 2.39)	1.6 (0.94 – 2.70)
Distance from Factories	0.5 (0.18 – 1.55)	0.4 (0.12 – 1.24)§

N=248; § By χ^2 test with Yates' correction for expected value <5

4.4.6 Relationships between Reported Respiratory Symptoms and Epigenetic Modifications

Table 4.36 shows the associations between reported respiratory symptoms and epigenetic modifications (histone H3 level, DNAm status). There was no significant association between reported respiratory symptoms and histone H3 level at $p<0.05$ except for cough. Cough was 3 times more likely to occur among those with higher histone H3 level ($p=0.004$, 95% CI=1.39-6.58). Meanwhile, there were significant associations between the reported cough and DNAm of *TNFα* and *CYP1A1* at $p<0.05$. Cough was 2.1 times and 2.9 times more likely to occur among those with methylated *TNFα* ($p=0.043$, 95% CI=1.01-4.31) and methylated *CYP1A1* ($p=0.004$, 95% CI=1.36-6.05), respectively.

Table 4.36: Associations between reported respiratory symptoms and epigenetic modifications

Respiratory Symptoms	Histone H3 OR (95% CI)	DNAm Status	
		TNFα OR (95% CI)	*CYP1A1* OR (95% CI)
Cough	**3.0 (1.39 – 6.58)***	**2.1 (1.01 – 4.31)***	**2.9 (1.36 – 6.05)***
Phlegm	2.8 (0.96 – 8.07)	1.2 (0.46 – 3.16)	2.0 (0.77 – 5.46)
Wheezing	2.1 (0.69 – 6.30)	1.0 (0.37 – 2.99)	1.1 (0.38 – 3.09)
Chest Tightness	1.3 (0.30 – 6.14)§	0.5 (0.09 – 2.46)§	0.9 (0.20 – 4.22)§

N=248; ***Significant at $p<0.05$;** § By χ^2 test with Yates' correction for expected value <5

No similar study has been conducted before on associations between respiratory symptoms and epigenetic modifications. It is still poorly known whether epigenetic modifications are involved in the track of cough, phlegm, wheezing and chest tightness pathophysiology. Nevertheless, exposure to air pollution generally causes oxidative stress (Gawda, Majka, Nowak, & Marcinkiewicz, 2017), later regulating histone H3 and DNAm (Niu, Desmarais, Tong, Yao, & Costa, 2015). These epigenetic mechanisms modulate the gene expression by being responsive to changes in the environment of a cell, including oxidative stress that leads to respiratory health effects (Ji et al., 2016). Air pollutants in the form of a foreign matter could be the original elicitor that provokes cough; hence, it causes traumatic inflammation in the airways (Jo & Song, 2019). Asthma is one of the most prevalent inflammatory diseases, characterised by respiratory symptoms of varying severity. Many asthma-related genes undergo histone modifications (Peng, Zong, Zhou, & Chen, 2016). Moreover, existing research suggests that epigenetic marks change gene expression in the lungs, which could be linked to respiratory diseases (Jirtle & Skinner, 2007).

4.4.7 Relationships between Dietary Intake and Epigenetic Modifications

Table 4.37 displays the associations between dietary intake and epigenetic modifications (histone H3 level, DNAm status). There was no significant association between histone H3 level and dietary intake at $p<0.05$. Besides, there was no significant association between DNAm status of *TNFα* and dietary intake at $p<0.05$. There was also no significant association between DNAm status of *CYP1A1* and dietary intake at $p<0.05$.

Table 4.37: Associations between dietary intake and epigenetic modifications

Dietary Intake	Histone H3 OR (95% CI)	DNAm Status	
		TNFα OR (95% CI)	*CYP1A1* OR (95% CI)
Eat Chicken or Meat	1.6 (0.61 – 3.94)	1.2 (0.49 – 3.03)	0.8 (0.32 – 2.05)
Eat Fish	3.1 (0.31 – 29.73)§	1.2 (0.17 – 8.64)§	1.2 (0.17 – 8.94)§
Eat Seafood	2.1 (0.92 – 4.64)	2.1 (0.96 – 4.74)	1.4 (0.63 – 2.98)
Eat Fruits	0.6 (0.14 – 2.53)§	1.2 (0.29 – 4.92)§	0.7 (0.17 – 3.14)§
Eat Vegetables	1.3 (0.68 – 2.63)	1.7 (0.86 – 3.03)	1.4 (0.70 – 2.67)
Drink Milk	1.3 (0.64 – 2.51)	1.6 (0.80 – 3.10)	1.6 (0.83 – 3.23)
Eat Milk Products	0.9 (0.46 – 1.61)	1.6 (0.86 – 3.03)	1.7 (0.90 – 3.15)
Eat Fast Foods	0.5 (0.04 – 5.54)§	0.6 (0.05 – 6.64)§	2.5 (0.22 – 27.89)§
Drink Fruit Juices	1.2 (0.53 – 2.68)	0.9 (0.38 – 1.96)	2.1 (0.93 – 4.92)
Drink Carbonated Drinks	0.7 (0.53 – 2.68)	0.9 (0.50 – 1.55)	1.0 (0.57 – 1.76)
Eat Health Supplements	1.1 (0.65 – 1.76)	1.0 (0.60 – 1.62)	0.9 (0.56 – 1.53)

N=248; § By χ^2 test with Yates' correction for expected value <5

No similar study has been conducted before on associations between dietary intake and epigenetic modifications. Epigenetic modifications and dietary intake have a complicated relationship and are affected by sensitivity to the timing of exposure (Montrose et al., 2017). However, the exact relationship between diet and the epigenome remains unclear for most nutrients. Dietary intake may influence epigenetic modifications by directly affecting catalytic activities of enzymes responsible for regulating epigenetic modifications (Zhang & Kutateladze, 2018). Vitamin C is widely known to boost the immune system; Chong et al. (2019) revealed the roles of vitamin C as an epigenetic regulator by targeting aberrant histone and DNAm patterns related to cancer progression. Vitamin C is naturally available in many foods such as orange, papaya, broccoli, tomato, and potato.

From the results of this subsection, the fourth hypothesis in this study is true for the data; there were significant associations between the concentrations of air

pollutants, reported respiratory symptoms, lung function status, histone H3 level and DNAm status among the respondents in high and low traffic areas.

4.5 Multivariate Analyses

This section presents the results and discusses the main findings of the study pertaining to the fifth hypothesis. The fifth objective was to identify the factors significantly associated with the respiratory symptoms, lung function status, histone H3 level and DNAm in response to TRAP-induced systemic inflammation after controlling the confounders (sociodemographic factors, socioeconomic factors, dietary intake, the environment at residences). These statistically significant variables at the bivariate level were analysed further at various multivariate level using multiple logistic regression for categorical dependent variables (cough, phlegm, wheezing, *TNFα* DNAm and *CYP1A1* DNAm) and multiple linear regression for continuous dependent variables (FVC%, FEV_1% and histone H3).

4.5.1 Multiple Logistic Regressions

4.5.1.1 Respiratory Symptoms

In order to estimate the probability of children getting respiratory symptoms (cough, phlegm, wheezing), a binary logistic regression was performed. The probability of children getting cough was estimated using TRAP (PM_{10}, $PM_{2.5}$, PM_1, BC, NO_2, O_3, TVOC), the distance of residences from the highway and sociodemographic factors (age, gender, BMI). Assumption testing operated before the analysis did not indicate any violations. The omnibus model for the logistic regression analysis was statistically significant, χ^2 (df=17, N=248)=33.56, p=0.010, Cox and Snell R^2=0.127, Nagelkerke R^2=0.225. The model was 85.5% accurate in its predictions of children getting cough. Hosmer and Lemeshow test results showed that the model was a good fit for the data, χ^2 (df=8, N=248)=2.72, p=0.951. Coefficients for the model's predictors are displayed in Table 4.38. Two variables appear to significantly influence children's probability of getting cough, which are BC and O_3. The rests did not contribute significantly to the model. A stronger predictor of cough would be BC with an OR of 2.2, which indicates that children who had higher exposure to BC were over 2.2 times more likely to experience cough than those who had lower exposure to BC, controlling for all other factors in the model.

BC has been associated with cough among children (Patel et al., 2009). BC is a nanoparticle classified as a carcinogenic air pollutant (Arif & Parveen, 2020). The morphology of BC particles offers the potential for BC to penetrate deep into the alveoli and can potentially enter the brain directly through the olfactory nerve (Bourganis, Kammona, Alexopoulos, & Kiparissides, 2018). Meanwhile, O_3

contributes to cough by initiating action potentials in nerve fibres, which are situated to sense the inhaled air environment (Taylor-Clark & Undem, 2010).

Table 4.38: Factors that influenced respiratory symptoms among children after controlling the confounders

	β	SE	p	OR	95% CI	R^2
			Cough			
Constant	13.34					
BC[a]	0.21	0.08	**0.009***	2.2	1.09 - 2.45	0.225
O_3[a]	0.50	0.24	**0.039***	1.6	1.43 – 2.02	
			Phlegm			
Constant	3.58					0.211
$PM_{2.5}$[a]	0.45	0.20	**0.024***	1.6	0.40 – 1.93	
			Wheezing			
Constant	2.33					
Gender[b]	1.40	0.69	**0.043***	2.1	1.04 – 15.81	0.117
NO_2[a]	0.38	0.17	**0.026***	2.5	1.10 – 2.97	

N=248; β: Regression Coefficient; SE: Standard Error; Nagelkerke R^2= 0.225;
***Significant at p<0.05**; a: continuous variable; b: categorical variable

The probability of children catching phlegm was estimated using TRAP (PM_{10}, $PM_{2.5}$, PM_1, BC, O_3), the distance of residences from the highway and sociodemographic factors (age, gender, BMI). Assumption testing conducted before the analysis did not express any violations. However, the omnibus model for the logistic regression analysis was statistically significant, χ^2 (df=11, N=248)=22.19, p=0.023, Cox and Snell R^2=0.086, Nagelkerke R^2=0.211. The model was 92.7% accurate in its predictions of children getting phlegm. Hosmer and Lemeshow test results certified that the model was a good fit for the data, χ^2 (df=8, N=248)=8.02, p=0.431. Coefficients for the model's predictors are displayed in Table 4.38. Only one variable appears to significantly influence children's probability of getting phlegm, which is $PM_{2.5}$ with an OR of 1.6. The rests did not contribute significantly to the model. Children who had higher exposure to $PM_{2.5}$ were over 1.6 times more likely to get phlegm than those who had lower exposure to $PM_{2.5}$, controlling for all other factors in the model.

$PM_{2.5}$ has been associated with phlegm among children (Suhaimi et al., 2015). Owing to its fine consistency, $PM_{2.5}$ can be brought more deeply into the lungs and deposited in alveolar sacs (Deng et al., 2019). The entry of $PM_{2.5}$ into the respiratory system has triggered a robust inflammatory response via phlegm as an indicator of mucus production (Liu et al., 2020). Particles are primarily deposited in the respiratory tract via sedimentation, impaction and diffusion (Brown, 2015). The deposition of inhaled particles depends on the dimension of the airways; hence, there are differences in the dose deposited in various regions of the respiratory tract (Lippmann, 2011). This mechanism explains why higher concentrations of particles may accumulate in children's lower respiratory tract compared to adults.

The probability of children experiencing wheezing was estimated using TRAP (PM_{10}, $PM_{2.5}$, PM_1, BC, NO_2, O_3, TVOC), the distance of residences from the highway and sociodemographic factors (age, gender, BMI). Assumption testing operated before the analysis did not demonstrate any violations. The omnibus model for the logistic regression analysis was not statistically significant, χ^2 (df=9, N=248)=10.88, p=0.284, Cox and Snell R^2=0.043, Nagelkerke R^2=0.117. The model was 94.0% accurate in its predictions of children getting wheezing. Hosmer and Lemeshow test results validated that the model was a good fit for the data, χ^2 (df=8, N=248)=4.00, p=0.858. Coefficients for the model's predictors are shown in Table 4.38. Two variables appear to significantly influence children's probability of getting wheezing, which are gender and NO_2. The rests did not contribute significantly to the model. A stronger predictor of wheezing would be NO_2 with an OR of 2.5, which indicates that children who had higher exposure to NO_2 were over 2.5 times more likely to get wheezing than those who had lower exposure to NO_2, controlling for all other factors in the model. NO_2 has been associated with wheezing among children (Kamaruddin et al., 2016) and implicated in the aetiology of oxidative damage (Nathan & Cunningham-Bussel, 2013). By inducing ROS formation, NO_2 causes cellular injury that triggers a cytokine response, which results in inflammation (Petit et al., 2017) and wheezing as one of the respiratory symptoms.

4.5.1.2 DNAm

In order to estimate the probability of children getting methylated *TNFα* and *CYP1A1*, a binary logistic regression was performed for each of the genes. The probability of children getting methylated *TNFα* was estimated using TRAP (PM_{10}, $PM_{2.5}$, PM_1, NO_2, TVOC, CO, BC) and sociodemographic factors (age, gender, BMI). Assumption testing administered before the analysis did not indicate any violations. The omnibus model for the logistic regression analysis was statistically significant, χ^2 (df=16, N=248)=26.35, p=0.049, Cox and Snell R^2=0.101, Nagelkerke R^2=0.135. The model was 54.4% accurate in its predictions of children getting methylated *TNFα*. Hosmer and Lemeshow test results confirmed that the model was a good fit for the data, χ^2 (df=8, N=248)=9.17, p=0.328. Coefficients for the model's predictors are displayed in Table 4.39. Two variables appear to significantly influence children's probability of having methylated *TNFα*, which are BC and NO_2. The rests did not contribute significantly to the model. A stronger predictor of methylated *TNFα* would be NO_2 with OR of 3, which indicates that children who had higher exposure to NO_2 were almost 3 times more likely to get methylated *TNFα* than those who had lower exposure to NO_2, controlling for all other factors in the model.

The probability of children getting methylated *CYP1A1* was estimated using TRAP (PM_{10}, $PM_{2.5}$, PM_1, NO_2, O_3, TVOC, CO) and sociodemographic factors (age, gender, BMI). Assumption testing conducted before the analysis did not indicate any violations. The omnibus model for the logistic regression analysis was not statistically significant, χ^2 (df=8, N=248)=10.04, p=0.262, Cox and Snell R^2=0.043, Nagelkerke R^2=0.053. The model was 55.2% accurate in its

predictions of children getting *CYP1A1* DNAm. Hosmer and Lemeshow test results confirmed that the model was a good fit for the data, χ^2 (df=8, N=248)=6.47, p=0.595. Coefficients for the model's predictors are shown in Table 4.39. One variable appears to significantly influence the probability of children having *CYP1A1* DNAm, which is BC. The rests did not contribute significantly to the model. A stronger predictor of methylated *CYP1A1* would be BC with OR of 2, which indicates that children who had higher exposure to BC were almost 2 times more likely to get methylated *CYP1A1* than those who had lower exposure to BC, controlling for all other factors in the model.

Table 4.39: Factors that influenced DNAm among children after controlling the confounders

	β	SE	p	OR	95% CI	R^2
			TNFα			
Constant	1.61					
BC	0.07	0.03	**0.032***	1.9	0.92 – 2.02	0.135
NO_2	0.04	0.02	**0.036***	3.0	0.88 – 1.95	
			CYP1A1			
Constant	0.01					0.053
BC	0.03	0.01	**0.019***	2.0	1.02 – 2.03	

N=248, ***Significant at p<0.05**

NO_2 and BC have been associated with DNAm among children (Jung et al., 2017; Prunicki et al., 2018). NO_2 prompts a long-term inflammatory condition mediated by the immune system. DNAm levels in various spots on the genome point to significant mechanisms for NO_2 exposure, specifically in the immune system (Plusquin et al., 2017). Meanwhile, BC exposure modifies the genes included in the immunoregulatory cells; thus, rooting the channel to asthmatic reactions (Lovinsky-Desir et al., 2017). Since BC has a short lifespan of about one week, its dispersion in the atmosphere is heterogeneous (Kholod & Evans, 2016). BC concentrations are higher near plentiful emission sources, and BC in Klang Valley is mostly due to the burning of fossil fuels (Amil et al., 2016).

4.5.2 Multiple Linear Regressions

4.5.2.1 Lung Function

These statistically significant variables at the bivariate level were analysed further at the multivariate level using multiple linear regression. The enter method was employed, which embed two out of nine variables (PM_{10}, $PM_{2.5}$, PM_1, NO_2, O_3, TVOC, SO_2, CO, BC) in the final model. Table 4.40 shows two variables representing TRAP in schools, which were significantly associated with FVC% among children. BC and NO_2 were revealed as the most significant TRAP that influenced the FVC% among children, as portrayed in Equation 4.3.

$$FVC\% = 94.32 - 0.033(BC) - 0.008(NO_2)$$ **(Equation 4.3)**

For the model, the beta value was significant at the 0.05 level. Variance Inflation Factor (VIF) value was <5, which proved no problem with multicollinearity. There was a significant direct linear relationship between BC and NO_2 with FVC% ($p<0.001$). 40.5% of variance in FVC% can be explained by BC and NO_2, adjusted R^2 = 0.335, F (9, 238)=5.266, $p<0.001$. An integrated effect of this magnitude can be regarded as medium ($f^2=0.68$); hence, we could not prove the large contribution of BC and NO_2 in FVC%.

Table 4.40 shows three variables representing TRAP in schools, which was significantly associated with FEV_1% among children. The enter method was used, which inserted three out of nine variables (PM_{10}, $PM_{2.5}$, PM_1, NO_2, O_3, TVOC, SO_2, CO, BC) in the final model. Results from multivariate level analysis using multiple linear regression unveiled that BC, PM_1 and $PM_{2.5}$ were was the most significant TRAP that influenced the FEV_1% among children, as portrayed in Equation 4.4.

$$FEV_1\% = 95.71 - 0.028(BC) - 0.008(PM_1) - 0.002(PM_{2.5})$$ **(Equation 4.4)**

For the model, the beta value was significant at the 0.05 level. VIF value was <5, which implied that there was no problem with multicollinearity. There was a significant direct linear relationship between BC, $PM_{2.5}$ and PM_1 with FEV_1% ($p<0.001$). 41.2% of the variance in FEV_1% can be explained by BC, adjusted R^2=0.322, F (9, 238)=4.818, $p<0.001$. A combined effect of this magnitude can be considered large ($f^2=0.70$); hence, we could demonstrate the large contribution of BC, $PM_{2.5}$ and PM_1 in FEV_1%.

Table 4.40: Multiple linear regression for the association between TRAP and lung function abnormality among children

Independent Variables	B (SE)	Standardised Coefficients	*p*	95% CI	VIF	R^2	Adjusted R^2
			FVC%				
Constant	94.32 (1.24)	-	**<0.001***	90.56 – 95.45	-		
BC	-0.033 (0.04)	-0.26	**0.012***	-0.04 – -0.02	3.39	0.416	0.405
NO_2	-0.008 (0.02)	-0.19	**0.041***	-0.02 – -0.06	3.27		
			FEV_1%				
Constant	95.71 (1.39)	-	**<0.001***	92.33 – 97.78	-		
BC	-0.028 (0.06)	-0.26	**<0.001***	-0.12 – -0.02	4.23	0.424	0.412
PM_1	-0.018 (0.10)	-0.11	**0.024***	-0.10 – -0.01	3.66		
$PM_{2.5}$	-0.012 (0.21)	-0.06	**0.025***	-0.10 – 0.01	2.34		

***Significant at $p<0.05$**; Method: Enter

There is no precise theory on how air pollution upsets the lungs and airways. A mechanism has been proposed that inhaled pollutants trigger the formation of ROS. Highly oxidative gases such as NO_2 activate inflammatory responses (Schultz et al., 2017) and airflow obstruction (Gaffin et al., 2017). Besides, high exposure to NO_2 has been previously reported to be significantly associated with lower FVC% (Hou et al., 2020). On the other hand, BC has been associated with decreased FVC% and FEV_1% among children (Paunescu et al., 2019). BC has an acute effect on the inflammation of the airways, which may reduce lung function (Yoda et al., 2017). They can reach lung alveoli and are toxic through mechanisms of oxidative stress, cell signalling and activation, and the release of mediators commencing inflammatory processes in the respiratory tract (Meldrum et al., 2017). Previous studies had demonstrated that exposure to $PM_{2.5}$ was associated with poorer FEV_1% among children (Arifuddin et al., 2019; Yang et al., 2020). Nevertheless, a recent study by Yang et al. (2020) had proven that PM_1 is more harmful than $PM_{2.5}$, which is possibly contributed by its finer size than $PM_{2.5}$. Experimental data had reported that finer particles have a larger surface area to volume ratio than coarser particles; hence, they are possibly causing more harmful health risks (Mei et al., 2018).

4.5.2.2 Histone H3

Afterwards, these statistically significant variables at the bivariate level were analysed further at the multivariate level using multiple linear regression. The enter method was used, which inserted one out of nine variables (PM_{10}, $PM_{2.5}$, PM_1, NO_2, O_3, TVOC, SO_2, CO, BC) in the final model. Table 4.41 shows one variable representing TRAP in schools, which was significantly associated with histone H3 level among children. It was revealed that NO_2 was the most significant TRAP that influenced the histone H3 level among children, as portrayed in Equation 4.5.

$$Histone\ H3\ Level = 466.35 + 3.98(NO_2) \qquad \textbf{(Equation 4.5)}$$

For every unit (1 $\mu g/m^3$) increase in NO_2, histone H3 level will increase by 3.98. For the model, the beta value was significant at the 0.05 level. VIF value was < 5, which suggested that there was no problem with multicollinearity. There was a significant direct linear relationship between NO_2 with histone H3 level ($p<0.001$). NO_2 explained 33.7% of the variance in histone H3 level, adjusted $R^2=0.337$, F (1, 246)=40.07, $p<0.001$. A mixed effect of this magnitude can be considered medium ($f^2=0.51$); hence, we could not attest to the vast contribution of NO_2 in histone H3 level.

Table 4.41. Multiple linear regression for the association between NO_2 and histone H3 level among children

Independent Variables	B (SE)	Standardised Coefficients	*p*	95% CI	VIF	R^2	Adjusted R^2
Constant	466.35 (50.55)	-	**<0.001***	366.80 – 565.90	-	0.340	0.337
NO_2	3.98 (0.63)	0.374	**<0.001***	2.67 – 5.21	1.00		

***Significant at $p<0.05$**; Method: Enter

Minimal studies have investigated the effects of air pollutants on histone H3 modification; most of these studies were performed on occupational exposure (Zhang et al., 2016; Zheng et al., 2017). No previous study reported the association between NO_2 and histone H3 level among children, but Vrijens et al. (2020) reported such a relationship among newborns. NO_2 is classically regarded as a proxy for road traffic in outdoor city backgrounds, although it is not undoubtedly independent of other TRAP-related exposures (Rider & Carlsten, 2019).

On the whole, the results from this study may be generalised to suggest that the increasing concentrations of air pollutants in schools, specifically BC, $PM_{2.5}$, PM_1, NO_2, and O_3, had significantly influenced the prevalence of cough, phlegm, wheezing, DNAm, histone H3 level and decreased lung function among the children in this study. These air pollutants may combine in the air and work together to produce synergistic effects, which results in more significant impacts than those impacts caused by a single air pollutant alone. Klang Valley is considered the largest hub of road networks in Malaysia due to rapid development and urbanisation, and this condition could be the main contributor to high concentrations of TRAP in the vicinity. Besides, this study has confirmed the validity of histone H3 and DNAm as a mediator in linking exposure to TRAP and respiratory health impacts (respiratory symptoms, lung function), as proposed earlier by Rider & Carlsten (2019).

From the results of this subsection, the fifth hypothesis in this study is true for the data; TRAP exposure could be the main factor influencing respiratory health and epigenetic modifications among children in response to TRAP-induced systemic inflammation after controlling the confounders.

CHAPTER 5

SUMMARY, CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Summary

Childhood is a crucial life stage because exposure to environmental contaminants may impact on growth and development of a human and may thus serve as determinants for an increased risk of health issues. Therefore, two groups of children were purposely recruited for this study; they were categorised into high traffic (HT) or low traffic (LT) group. This cross-sectional comparative study investigated TRAP distribution and its potential in causing epigenetic modifications and respiratory health impacts among primary school children. Four schools in the HT area and four schools in the LT area were selected in this study based on their distances from nearby major roadways or highways.

The air quality monitoring in this study was performed using several instruments. DustTrak DRX was used for 6-h real-time monitoring of particulate matter (PM_{10}, $PM_{2.5}$ and PM_1), Aeroqual S500 was used for real-time monitoring of gases (NO_2, SO_2, O_3 and CO), whereas PpbRAE3000 was used for real-time monitoring of TVOC. Meanwhile, air sampling pumps that utilise the filter-based method were used for 24-h measurements of $PM_{2.5}$–bound BC in schools, and particulate matters in residences. The results from school monitoring were validated using the data from the local Continuous Air Quality Monitoring (CAQM) stations. Besides, Principal Component Analysis (PCA) was applied to identify the source of air pollution in the studied areas, highlighting that the air quality in the HT area had been affected by the combustion of fuel engines.

The health impacts of TRAP in this study were explored through reported respiratory symptoms from the distributed questionnaires and lung function status as a lung performance indicator. Meanwhile, histone H3 level and DNA methylation (DNAm) status collected from saliva samples were investigated for their possibilities as epigenetic mechanisms in connecting exposure to TRAP and respiratory health effects. The individual children's information, as obtained from the questionnaires, was statistically analysed to explore the effect of individual factors on reported respiratory symptoms, lung function status, histone H3 level and DNAm status. Bivariate and multivariate analyses were computed to provide information on the influence of TRAP exposure on the studied health impacts. The followings are the summary of the outcome of this study. On the whole, the hypotheses set in the early stage of this study had been tested statistically, and those proven to be true are as follows:

1. There are significant differences in sociodemographic and socioeconomic information, house condition and location, family background and dietary intake between the respondents in high and low traffic areas.
2. There are significant differences in the concentrations of air pollutants inside respondents' classrooms and residences between high and low traffic areas.
3. There are significant differences in reported respiratory symptoms, lung function status, level of histone H3 modification and DNAm status among the respondents between high and low traffic areas.
4. There are significant associations between TRAP exposure with respiratory symptoms, lung function status and epigenetic modifications assessed in the study.
5. TRAP exposure could be the main factor influencing respiratory health and epigenetic modifications among children in response to TRAP-induced systemic inflammation after controlling the confounders.

5.1.1 Measurements of Indoor TRAP and Physical Parameters

There were significant differences in the concentrations of indoor TRAP between the HT and LT schools. The indoor air pollutant concentrations were significantly higher at the HT schools when compared to the LT schools. The median concentrations of PM_{10}, $PM_{2.5}$, and PM_1 and BC recorded at the HT schools were 112.0 $\mu g/m^3$, 79.0 $\mu g/m^3$, 72.0 $\mu g/m^3$ and 35.2 $\mu g/m^3$ respectively. In comparison, the median concentrations of PM_{10}, $PM_{2.5}$, PM_1 and BC recorded at the LT schools were 63.0 $\mu g/m^3$, 45.0 $\mu g/m^3$, 35.0 $\mu g/m^3$ and 9.7 $\mu g/m^3$, respectively. The median concentrations of NO_2, SO_2, O_3, CO and TVOC recorded at the HT schools were 33.0 ppb, 20.0 ppb, 24.0 ppb, 23.0 ppb and 318.0 ppb, respectively. In comparison, the median concentrations of NO_2, SO_2, O_3, CO and TVOC recorded at the LT schools were 12.0 ppb, 10.0 ppb, 13.0 ppb, 10.0 ppb and 0.0 ppb, respectively. Some of the schools recorded readings of SO_2, TVOC and CO below LOD.

As for the physical parameters, HT schools had significantly higher temperature with a median of 29.9°C than LT schools with a median of 27.9°C. Meanwhile, LT schools had significantly higher RH with a median of 71.7% than HT schools with a median of 67.5%. The air velocity had no significant difference between both groups of schools with an equal median of 0.3 m/s.

5.1.2 Measurements of Outdoor TRAP and Meteorological Parameters

There were significant differences in the concentrations of outdoor TRAP between the HT and LT schools. The outdoor air pollutant concentrations were significantly higher at the HT schools when compared to the LT schools. The median concentrations of PM_{10}, $PM_{2.5}$, PM_1 and BC recorded at the HT schools were 124.0 $\mu g/m^3$, 97.0 $\mu g/m^3$, 86.0 $\mu g/m^3$, and 54.5 $\mu g/m^3$, respectively. In

comparison, the median concentrations of PM_{10}, $PM_{2.5}$, PM_1 and BC recorded at the LT schools were 63.0 μg/m^3, 40.0 μg/m^3, 33.0 μg/m^3, and 14.1 μg/m^3, respectively. The median concentrations of NO_2, SO_2, O_3, CO and TVOC recorded at the HT schools were 53.0 ppb, 10.0 ppb, 25.0 ppb, 350.0 ppb and 749.0 ppb, respectively. In comparison, the median concentrations of NO_2, SO_2, O_3, CO and TVOC recorded at the LT schools were 22.0 ppb, 0.0 ppb, 14.0 ppb, 10.0 ppb and 29.0 ppb, respectively. Some of the schools recorded readings of TVOC and CO below LOD.

As for the data retrieved from the nearby CAQM stations, Cheras station had significantly higher PM_{10} and O_3 with median concentrations of 28.6 μg/m^3 and 16.5 ppb than measurements at Batu Muda. In contrast, the Batu Muda station had significantly higher concentrations of SO_2 and CO with median concentrations of 3.0 ppb and 700.0 ppb than measurements at Cheras. $PM_{2.5}$ and NO_2 had no significant difference between the two stations. None of the outdoor measurements of TRAP at the CAQM stations exceeded the recommended values by MAAQS. In contrast, the median of PM_{10} and $PM_{2.5}$ at the HT schools exceeded the recommended values by MAAQS.

As for the meteorological parameters, HT schools had significantly higher RH with a median of 70.4% than LT schools with a median of 64.4%. HT schools had higher AT with a median of 28.3°C than LT schools with a median of 28.2°C, but the difference was not significant. Meanwhile, LT schools had higher WS with a median of 0.8 m/s than HT schools with a median of 0.6 m/s. AT had no significant difference between both groups of schools, with HT schools recorded a higher median AT of 28.3°C than LT schools with a median of 28.2°C.

5.1.3 Measurements of Indoor Particulate Matter in Residences

There were significant differences in the concentrations of particulate matter between the HT and LT residences. HT residences had significantly higher concentrations with the median at 73.9 μg/m^3 and 50.3 μg/m^3 compared to LT residences at 18.7 μg/m^3 and 12.6 μg/m^3.

5.1.4 Indoor/Outdoor (I/O) Ratios

The I/O ratios ranged between 0.00 and 1.19, which suggested that indoor concentrations of air pollutants were not necessarily influenced by outdoor air pollutants. Only PM_{10}, $PM_{2.5}$ and PM_1 in LT schools recorded I/O ratios > 1, which signifies that concentrations of other TRAP could be related to the proximity of the schools to major roads or with traffic intensity. Activities in the classrooms, such as occupants' movement around the classrooms, could generate indoor air pollutants.

5.1.5 Trends in Nearby CAQM Stations

There is no CAQM station near the selected LT schools; hence, only CAQM stations near the HT schools were included in this study. Both Batu Muda and Cheras stations show that PM_{10} and $PM_{2.5}$ concentrations tend to peak around 9 a.m. There is a very pronounced increase in NO_2 concentrations during the peak morning rush hour. O_3 shows very different behaviour than NO_2 as it showed an increase in O_3 concentrations in the afternoon between 2 to 3 p.m. Meanwhile, CO and SO_2 had similar trends daily. Just like NO_2, CO showed a very pronounced increase in concentrations during the peak morning rush hour. These trends imply very similar source origins, which are most likely vehicular emissions.

5.1.6 Principal Component Analysis (PCA)

PCA has been applied as a multivariate statistical tool to identify the major sources for air pollutants, which was further progressed with MLR. Only data from CAQM stations had API values; hence, the MLR models were developed based on measurements recorded from the CAQM stations only. The traffic intensity has influenced the air quality in the HT region since NO_2, CO, O_3 and TVOC were included in the factor 1 group of the PCA results. This finding is also supported by PCA results of CAQM stations nearby the selected HT schools. Only SO_2 is not contributing to any PCA results – either selected schools or CAQM stations nearby selected HT schools; hence, this finding confirms the low contribution of industrial emissions in the selected locations and high contribution of vehicle emissions.

5.1.7 Reported Respiratory Symptoms

Chi-Square Test disclosed a significant difference between the two groups of children, with a notably higher number of respondents in the HT group for reported cough (χ^2=15.28, p<0.001), phlegm (χ^2=5.16, p=0.023) and wheezing (χ^2=4.77, p=0.029) compared to the respondents in the LT group. However, there was no significant difference in chest tightness.

5.1.8 DNA Methylation (DNAm) Status

Chi-Square Test indicated an increased risk between the two groups of children, with a remarkably higher number of respondents in the HT group with methylated *TNFα* (χ^2=7.17, p=0.007) and methylated *CYP1A1* (χ^2=4.71, p=0.030) compared to the respondents in the LT group.

5.1.9 Lung Function Status

Mann-Whitney *U* Test unveiled that the abnormality of FVC% of the respondents in the HT group (*Mean Rank*=100.70) was significantly higher than those of the respondents in the LT group (*Mean Rank*=148.30), $p<0.001$. The abnormality of FEV_1% of the respondents in the HT group (*Mean Rank*=101.67) was significantly higher than those of the respondents in the LT group (*Mean Rank*=147.33), $p<0.001$. However, there was no significant difference in the abnormality of FEV_1/FVC%.

5.1.10 Histone H3 Level

Mann-Whitney *U* Test revealed that the level of histone H3 among the respondents in the HT group (*Mean Rank*=147.87) was significantly higher than those of the respondents in the LT group (*Mean Rank*=101.13), $p<0.001$.

5.1.11 Relationships between TRAP Exposure and Health Impacts

Chi-Square Test of Independence discovered significant associations between the reported cough and TRAP at $p<0.05$, except for SO_2 and CO. Besides, there were significant associations between reported phlegm and TRAP at $p<0.05$, except for NO_2, SO_2, CO and TVOC. There were also significant associations between reported wheezing and TRAP at $p<0.05$, except for SO_2 and CO. On the contrary, there was no significant association between reported chest tightness and TRAP at $p<0.05$. There was also no significant association between any reported respiratory symptoms and TRAP inside residences at $p<0.05$. The same bivariate analysis also shows significant associations between methylated *TNFα* and TRAP in schools at $p<0.05$, except for SO_2. Besides, there was no significant association between methylated *CYP1A1* and TRAP in schools at $p<0.05$, except for PM_{10}, $PM_{2.5}$, PM_1 and TVOC. On the contrary, there was no significant association between DNAm of *TNFα* and *CYP1A1* and TRAP inside residences at $p<0.05$.

Spearman Rho's Test discovered that FVC% were negatively and weakly correlated with PM_1 (r= -0.14), $PM_{2.5}$ (r= -0.15), PM_{10} (r= -0.16), NO_2 (r= -0.16), TVOC (r= -0.16), CO (r= -0.19), SO_2 (r= -0.19),O_3 (r= -0.22) and BC (r= -0.22). FEV_1% were negatively and weakly correlated with concentrations of TVOC (r= -0.19), PM_1 (r= -0.20), $PM_{2.5}$ (r= -0.20), PM_{10} (r= -0.22), NO_2 (r= -0.24), CO (r= -0.26), O_3 (r= -0.27), SO_2 (r= -0.27) and BC (r= -0.29). FEV_1/FVC% were negatively and weakly correlated with concentrations of CO (r= -0.14), SO_2 (r= -0.17), and NO_2 (r= -0.17). By contrast, Spearman Rho's Test revealed that histone H3 level were positively and weakly correlated with concentrations of NO_2 (r=0.37), CO (r=0.36), PM_1 (r=0.35), $PM_{2.5}$ (r=0.34), SO_2 (r=0.34), PM_{10} (r=0.33), O_3 (r=0.33), TVOC (r=0.25) and BC (r=0.19).

Multiple logistic regression revealed that BC (OR=2.2, 95% CI=1.09-2.45) and O_3 (OR=1.6, 95% CI=1.43-2.02) were the most significant factors influencing the probability of children getting cough. The same analysis found that $PM_{2.5}$ (OR=1.6, 95% CI=0.40-1.93) was the most significant factor influencing children's probability of getting phlegm. NO_2 (OR=2.5, 95% CI=1.10-2.97) and gender (OR=2.1, 95% CI=1.04-15.81) were the most significant factors influencing the children's probability of getting wheezing. The same multivariate analysis also revealed that BC (OR=1.9, 95% CI=0.92-2.02) and NO_2 (OR=3.0, 95% CI=0.88-1.95) were the most significant factors which influence the probability of children in getting methylated *TNFα*. The same analysis found that BC (OR=2.0, 95% CI=1.02-2.03) was the most significant factor influencing children's probability of getting methylated *CYP1A1*.

Multiple linear regression revealed that BC and NO_2 (R^2=0.416, p<0.001, f^2=0.71) were the most significant factors influencing the probability of children getting abnormality of FVC%. Besides, the same analysis found that BC, PM_1 and $PM_{2.5}$ (R^2=0.424, p<0.001, f^2=0.74) were the most significant factors which influence the probability of children getting abnormality of FEV_1%. The same multivariate analysis also revealed that NO_2 (R^2=0.340, p<0.001, f^2=0.52) was the most significant factor which influence the probability of children getting high histone H3 level.

5.2 Conclusion

This study strongly suggests that school children in the HT area of Klang Valley had higher exposure to air pollutants (PM_{10}, $PM_{2.5}$, PM_1, BC, NO_2, SO_2, CO, O_3, and TVOC); hence, they had an increased risk of developing epigenetic modifications (histone H3 and DNAm), which induced respiratory health effects (respiratory symptoms and lung function), as proposed in Figure 1.2. The children were exposed to TRAP from their transport mode to schools, round-trip travel period from residences to schools, and time spent at schools or homes. As such, children are considered one of the populations vulnerable to TRAP as their exposure starts at an early age. All nine air pollutants monitored in this study were significantly higher among the schools and residences in the HT area than those in the LT area. Moreover, the prevalence of respiratory symptoms (cough, phlegm, and wheezing) and DNAm (*TNFα* and *CYP1A1*) and level of histone H3 were significantly higher among the children in the HT group than those in the LT group. In contrast, the percentage predicted of lung function (FVC% and FEV_1%) was significantly lower among the children in the HT group than those in the LT group.

NO_2 and BC in schools were the most significant factors to play a role in the epigenetic modifications as mediators of respiratory health impacts. Meanwhile, BC, NO_2, $PM_{2.5}$, and O_3 were the most significant factors to cause respiratory symptoms and decreased lung function. Nevertheless, the definite causal agents are challenging to be elucidated because there were several limitations in the nature of this study, which are elaborated further in the limitations section.

Despite those limitations, histone H3 and DNAm of *TNFα* and *CYP1A1* are validated as mediators in linking TRAP exposure and respiratory health effects. The epigenetic modifications discussed herein support future epidemiological studies on the pathways indicating a high likelihood of detrimental health impacts related to residing near a high-traffic region.

Furthermore, the teamwork of epidemiologists and laboratory-based researchers could develop effective interventions on the reversible potential of epigenetic modifications. Meanwhile, the saliva method is proven to be a reliable, safe, and non-invasive procedure for children with their reproducibility and sensitivity, as portrayed in this study. On the whole, epigenetic mechanisms are very complex, and their functions in the pathophysiology of respiratory diseases are not well understood. Therefore, novel approaches and ongoing enhancements are necessary for this evolving field.

5.3 Limitations

There are several limitations in this study, which predominantly resulted from the design of the study. For the nature of a cross-sectional study, the exposure and the outcome were concurrently assessed. Even though the data in the study shows that certain relationships were established between the exposure and the outcome, there was insufficient evidence to prove them. The accurate measure of association can only be determined if the entire population participate, and precise data on exposure and outcome were gathered. However, this issue was controlled by choosing a sample of school children aged 7-11 years old in Selangor and Kuala Lumpur, which was representative of the population. This cross-sectional study was also advantageous as a baseline for designing cohort studies involving school children in Malaysia. Besides, the population comprises Malay ethnicity, which means the study results cannot be generalised for other ethnicities in Malaysia because the characteristics of the population were definite. However, since many school children in Selangor and Kuala Lumpur primary schools are Malays, the results may represent the states in general.

Furthermore, there could be a possibility for recall bias from the parents or guardians when they did not recall some of their children's actual experiences as they were filling in the questionnaires. However, this bias was minimised by assessing children's past exposures from the biomarkers collected. As the possibility for information and researcher bias, the bias was controlled by adapting similar questions to both groups of respondents using questionnaires adapted from ATS and ISAAC, which are constructed to maximise accuracy and completeness. Other than that, as the respondents and residences were recruited voluntarily, the study had a drawback when the respondents refused to continue the procedures involving human samples or when the parents or guardians refused to have indoor air pollutants monitoring in their residences. These conditions may initiate selection bias in the study, particularly termed self-selection bias, which is linked to non-agreement to engage in the study. Besides that, not all children who attended the schools in the HT area were living near

the HT area, as well as not all children who attended the schools in the LT area were living further away from the HT area. There are also many children with both working parents, and some even travel far from home daily. Therefore, more children now spend time after school at transit centres for afterschool supervision and are only back home together with their parents in the late evening.

There are no standards established yet for naturally ventilated school buildings in Malaysia regarding indoor air assessment. The current ICOP by DOSH Malaysia is only applicable to the occupants of an air-conditioned indoor setting. Therefore, the concentrations of air pollutants exposure in this study were only determined from the measurements taken. However, measurements from local monitoring stations were included in this study to validate the findings in schools. With environmental health issues gaining more attention nowadays, there will likely be standards for naturally ventilated schools in Malaysia in future to protect the health of school children and teachers.

Although MS-PCR is a well-developed method, it is limited to qualitative results; thus, DNAm could not be determined quantitatively. For instance, the level and percentage of DNAm. After PCR amplification, MS-PCR results can be validated through pyrosequencing to confirm the full conversion of every non-CpG cytosine. Unlike MS-PCR, pyrosequencing results are quantitative, and the technique can detect even small differences in methylation (down to 5%). The only limitations are the cost and requirements of specialised equipment. In Malaysia, the pyrosequencing instrument is only available in the Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia.

5.4 Recommendations

There is no single solution to the management of TRAP exposure among children. The air pollution issues differ for each school, depending on their size and location. Though the general guidelines can be followed, each issue has to be analysed and dealt with accordingly. With communication and participation between schools, children, parents, communities, and regulatory bodies, a holistic approach is key to overall change and effective exposure reduction.

Table 5.1 tabulates the mitigation strategies for reducing TRAP exposure at schools in Malaysia. Recommendations are based on the findings of this current study. Figure 5.1 shows that the pick-up zone was also where the school children waited to be fetched by their parents or guardians after school. Meanwhile, Figure 5.2 shows some of the school children were seen nearby the idling vehicles while waiting to be fetched by their parents or guardians. This scenario, in turn, creates an increased level of air emissions in the school environment. Moreover, there were street vendors selling junk foods, snacks, and drinks outside the school gate.

Table 5.1: Mitigation strategies for reducing TRAP exposure at schools in Malaysia

Issues		Recommendations
1. Cars queuing/idling during drop-off hours in the morning and pick-up hours in the afternoon are correlated with concentrations of TRAP in the school premises	Children	• Stay away from a car or a queue of cars when their engines are running. • Wear a suitable face mask to filter air pollutants.
	School Management	• Regulate the number of entry and exit points in school. Choose the area where the road is wider and not heavily congested. • Implement an anti-idling approach to control vehicle emissions. • Mark exclusion areas within which parents or guardians are not allowed to park, even if only briefly. • Assign school staffs on a rotation basis to escort children from vehicle to school compound. • Provide separate entrances and exits for children walking or cycling to school. • Assign patrol officers to monitor the traffic during rush hour and ensure drivers' adherence to the anti-idling policy. • Display posters about the air quality around the school, especially in the areas where children and parents frequently visit (e.g., guard post, canteen).
	Community	• Switch off the vehicle engine while waiting to drop-off or pick-up children, even if it is only briefly. • Park cars away from the school entrance, and then walk the rest of the way to drop-off or pick-up children.
2. TRAP concentrations in a road-facing classroom are comparable to those on the main road throughout the school hours	Children	• Avoid opening doors or windows in a road-facing classroom during peak traffic times such as morning rush hours. • Open the windows later in the day if the classroom starts to feel hot and stuffy, probably due to CO_2 build-up in the classroom.
	School Management	• Assign drop-off and pick-up points away from the classroom buildings. • Plant vegetation barriers between the school compound and nearby road but ensure that the plants do not emit air pollutants. The plants should also be thick and have a full leaf from the ground to the top of the canopy along the entire length. • Prevent children from playing near any fence bordering a busy road. • Enforce housekeeping programs in the classrooms, such as cleaning and maintaining the cleanliness of the classrooms before and after school sessions. • Change the curtains and clean the fan blades regularly depending on the dustiness level (e.g., once a month). • Introduce awards for the cleanest classroom weekly or monthly and the grand prize for the cleanest classroom of the year. • Draw fresh air into the classroom if the children start to show symptoms of high CO_2 concentrations in the classroom (e.g., tiredness, dizziness, inability to focus).

Table 5.1: Continued

Issues		Recommendations
	Community	• Support the school in planting vegetation barriers around the school and other suitable control measures. • Approach local authority for additional space which might invade into neighbouring areas. Negotiate to utilise the space only during drop-off and pick-up hours. • Re-route the roads leading to the school with 'no entry' or 'one-way' signages during drop-off and pick-up hours. •
3. Children breathe more polluted air than adults because of the smaller size of their bodies, which causes higher ventilation per minute in their lungs	Children	• Try to keep away from the edge of the road, curb, and traffic intersections. Walk on the far side of the pavement. • Inform teachers if not feeling well during school hours.
	School Management	• Collaborate with the Health Promotion Unit in District Health Office and community to hold a campaign related to the awareness of air pollutants, health risks, and actions to take. • Conduct spring cleaning throughout the school compounds that involve children and their family members (e.g., four times a year). • Ensure the layout of furniture in the classroom does not obstruct ventilation.
	Community	• For new school developments, consider locations further from major roads but still within the community. • Parents or guardians should refer to medical doctors for any abnormal health symptoms shown by their children. • Parents or guardians should clean their houses regularly, and it is advisable to be carried out without the presence of children at home. • Install air-conditioners and air purifiers at home to reduce the dispersion of air pollutants from outdoors to purify the indoor air that could have been polluted with air pollutants. • For new developments on private land nearby the school compound, the developer should leave space for a 'buffer zone' between the school compound and construction sites.
	Researcher	• If the results of health impacts are abnormal, the parents or guardians will be informed face-to-face regarding the issue. • As for lung function abnormality, the medical doctor will suggest other tests to determine whether impaired breathing is caused by a breathing disorder. • As for histone H3 modification and DNAm, these epigenetic changes interact with each other and involve molecular changes that can signify the presence or future disease development. The medical doctor will suggest other specific tests to diagnose abnormal epigenetics.

Figure 5.1: The pick-up zone was also the location where the children waited for their parents or guardians after school

Figure 5.2: There were a few idling vehicles near the location where the children waited for their parents or guardians after school

Figure 5.3 shows the layout of Sekolah Kebangsaan Damansara Utama (SKDU), Selangor. This school is located next to a highway with an average daily traffic volume of ≥100,000 vehicles. Nonetheless, this school has already applied some mitigation strategies, as recommended in this study. Wall and vegetation barriers are bordering the school compound from the high-density highway. Moreover, the school has placed metal signages for road restrictions during drop-off and pick-up hours.

Legends
A: Drop-off/pick-up points
B: School canteen
C: Administration building and classrooms
D: Classrooms
E: Assembly hall
F: Computer lab
G: Store rooms
H: School field
I: Sign boards for road restrictions during drop-off and pick-up hours
J: Vegetation and wall barriers
K: Damansara-Puchong Expressway (LDP)
L: Community park

Figure 5.3: The layout of a school near a highway that has already applied some of the mitigation strategies

On the other hand, Figure 5.4 shows the self-illustrated recommended layout for a new school and other land uses, as adapted from the publication by US EPA (United States EPA, 2015). The classrooms are located no less than 152.4 m away from a highway. The school canteen located at point B is designated as the 'waiting zone' for parents, guardians, and children. Therefore, the children are less likely to be exposed to TRAP while waiting to be fetched after school. Moreover, this act would benefit the parents or guardians to increase their physical activity by walking. The pick-up and drop-off zone were separated between those who travelled by motor vehicles (motorcycles, cars, vans, and buses) at point E and those who travelled by bicycles or walking at point F; hence, this would reduce the TRAP exposure among walking parents, guardians and children or children who cycle to school. There are anti-idling waiting zones at point G, located at a wider road near the pick-up and drop-off zone at point E. At least two patrol officers are assigned to monitor the traffic at the pick-up and drop-off zone and ensure that all drivers adhere to the strict 'No Idling' policy; these officers could be the school guards or officers from The People's Volunteer Corps (RELA).

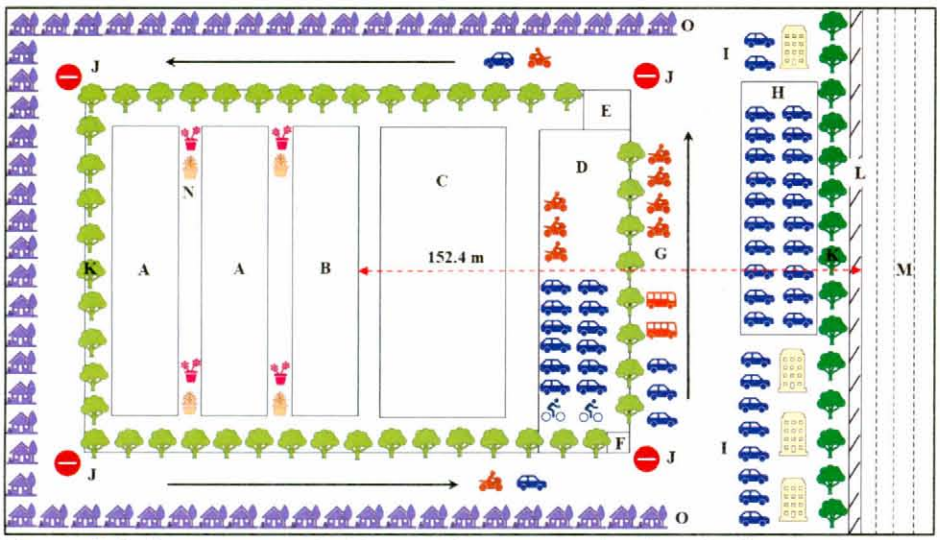


Figure 5.4: A recommended layout for a new school and other land uses
A: Classrooms; B: Administration building, canteen, and classrooms; C: School field; D: Parking for school staffs (motorcycles and cars) and children (bicycles); E: Pick-up and drop-off zone (motorcycles, cars, vans, and buses); F: Pick-up and drop-off zone (walking), entrance and exit for children (walking and cycling); G: Anti-idling waiting zone (motorcycles, cars, vans, and buses); H: Temporary parking space during drop-off and pick-up hours; I: Retail spaces or office buildings; J: Signages for road restrictions during drop-off and pick-up hours; K: Vegetation barriers; L: Wall barriers; M: Highway; N: Plants around the school compound; O: Residential area.

In this recommended layout, there is a temporary parking space for parents or guardians during drop-off and pick-up hours, located in an area near the school compound. However, this would need negotiation with the local authority on the land-use of that space temporarily only during school drop-off and pick-up hours. Besides, air-conditioned buildings such as retail spaces or office buildings are suggested to be built next to the highway to utilise the land available fully. Air-conditioned buildings are deemed more suitable next to the highway than the school buildings because most schools in Malaysia are naturally-ventilated; hence, TRAP could easily penetrate the school buildings compared to the air-conditioned buildings' filters against air pollutants. Furthermore, planting trees around the school compound improves the overall aesthetics of the school premise and improves air quality. Malaysia has hot and humid weather all-year-round; thus, planting trees would help provide a cooler environment away from the sunshine.

Maximising the distance between schools and heavily-travelled roadways will greatly reduce the level of exposure children may have to TRAP. Although it is better if schools are located further from heavily travelled roads, it is not easy to relocate existing schools, especially in Klang Valley where the populations are increasing but the available lands are reducing. Therefore, road traffic density

should be lessened around existing schools. This concept of reducing road traffic density also applies for a school in other types of terrain structure, depending on design characteristics for roadside applications such as green infrastructure. Figure 5.5 shows one of the signages placed near SKDU to notify other road users of the road usage restrictions during certain hours of the day. The school ground should have metal signages to remind parents or guardians about the 'No Idling' policy at schools. Figure 5.6 shows such metal signage, which is placed at a rest area in Selangor but is also applicable to the school area.

Figure 5.5: Signage for road restriction during drop-off and pick-up hours

Figure 5.6: An example of a metal 'No Idling' signage for the school ground

Figure 5.7 shows examples of wall and vegetation barriers bordering Damansara-Puchong Expressway (LDP) and a neighbourhood in Damansara Utama, Selangor. They use solid barriers and high-level vegetations, which are trees with a canopy lifted from the ground level. Moreover, these trees are taller than the solid barriers; hence, these are excellent combinations. These trees can improve pedestrian-side air quality, although any effects are varied by wind conditions, temperature, RH, and physical characteristics of the barrier (Baldauf, 2017). Tall and low-porosity vegetation with no gaps or breaks in the barrier can limit pollution removal by restricting infiltration and forcing air pollutants to flow above and around the barrier (Abhijith et al., 2017). Conversely, high-porosity vegetation barriers can reduce wind speed as it penetrates gaps, potentially resulting in pollutant accumulation downwind (Deshmukh et al., 2019).

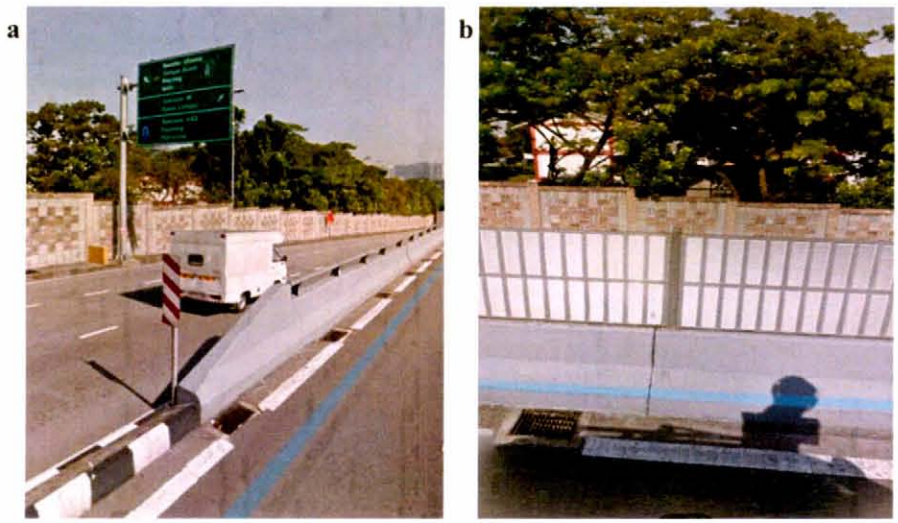


Figure 5.7: Wall and vegetation barriers near (a) a residential area and (b) a school compound

Based on their modelling, Neft et al. (2016) recommends the barrier thickness with an optimal thickness of 10 m or more to remove approximately 50% of nanoparticles, and the barrier length should extend beyond the area of concern. Modelling performed by Ghasemian et al. (2017) found that near-road air quality dense canopies reduced concentrations of roadway emissions downwind by 10%. In contrast, the solid barrier with the same height improved the air quality by 58%. Moreover, a study by Yang et al. (2015) on the trees planted in the urban environment in several cities in the world has proven that a broadleaf species can improve $PM_{2.5}$ reduction. Kuala Lumpur was included in their study. They discovered that London plane *(P. acerifolia)*, silver maple *(A. saccharinum)*, and honey locust *(G. triacanthos)* were among the top ten most frequently planted trees in the studied cities and had a $PM_{2.5}$ removal efficiency that was above average. One of the plants that is deemed suitable to be planted in Malaysia as a vegetation barrier is silly oak (*Grevillea robusta*). As shown by the air pollution tolerance index, this evergreen tree is intermediately tolerant towards vehicular emission (Singh et al., 2020). Moreover, this perennial species

is easy to propagate, fast-growing and require little maintenance. It can grow up to 40 m height and spread up to 20 m width (Centre for Agriculture and Bioscience International, 2021). Based on previous literature suggestions, Figure 5.8 shows the self-illustrated recommended design for roadside vegetation and a solid barrier.

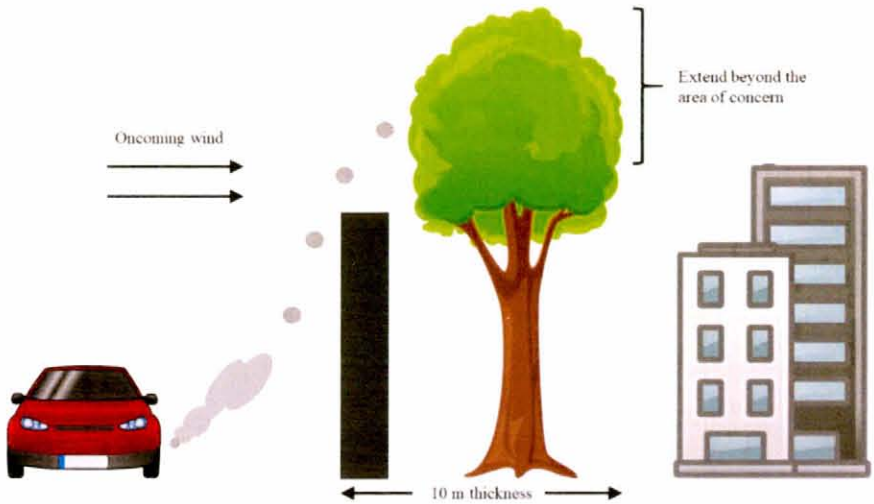


Figure 5.8: A recommended design for roadside vegetation and solid barrier

REFERENCES

Ab Jamil, N., Jalaludin, J., Kamaruddin, A. S., & Ibrahim, M. H. (2015). Exposure to Air Pollutants (PM10, NO2, SO2 and VOCs) on the Lung Functions among School Children Living Nearby the Petrochemical Industry Area in Kemaman, Terengganu. *Advances in Environmental Biology*, *9*(26), 55–63.

Abdul Hamid, H., Ho, G., & Ismail, S. (2019). *Demarcating Households: An Integrated Income and Consumption Analysis*. Retrieved from http://www.krinstitute.org/assets/contentMS/img/template/editor/Presentation Slides_Demarcating Households_Discussion Paper.pdf

Abdul Manan, M. M., Várhelyi, A., Çelik, A. K., & Hashim, H. H. (2018). Road Characteristics and Environment Factors Associated with Motorcycle Fatal Crashes in Malaysia. *IATSS Research*, *42*(4), 207–220. https://doi.org/10.1016/j.iatssr.2017.11.001

Abdul, Q. A., Yu, B. P., Chung, H. Y., Jung, H. A., & Choi, J. S. (2017). Epigenetic Modifications of Gene Expression by Lifestyle and Environment. *Archives of Pharmacal Research*, *40*(11), 1219–1237. https://doi.org/10.1007/s12272-017-0973-3

Abdul Rahman, Shamsiah, Hamzah, M. S., Elias, S., Ashifa, N., Salim, A., Hashim, A., … Wood, A. K. (2015). A Long Term Study on Characterization and Source Apportionment of Particulate Pollution in Klang Valley, Kuala Lumpur. *Aerosol and Air Quality Research*, *15*, 2291–2304. https://doi.org/10.4209/aaqr.2015.03.0188

Abdul Rahman, Shamsiah, Hamzah, M. S., Wood, A. K., Elias, M. S., Salim, N. A. A., & Sanuri, E. (2011). Sources Apportionment of Fine and Coarse Aerosol in Klang Valley, Kuala Lumpur using Positive Matrix Factorization. *Atmospheric Pollution Research*, *2*(2), 197–206. https://doi.org/10.5094/APR.2011.025

Abdul Rahman, SR, Ismail, S. N. S., Sahani, M., Firuz, Rm., & Latif, M. (2017). A Case Crossover Analysis of Primary Air Pollutants Association on Acute Respiratory Infection (ARI) among Children in Urban Region of Klang Valley, Malaysia. *Annals of Tropical Medicine and Public Health*, *10*(1), 44. https://doi.org/10.4103/ATMPH.ATMPH_75_17

Abdul Wahab, M. I., Razak, W. M. A. A., Sahani, M., & Khan, M. F. (2020). Characteristics and Health Effect of Heavy Metals on Non-Exhaust Road Dusts in Kuala Lumpur. *Science of the Total Environment*, *703*, 135535. https://doi.org/10.1016/j.scitotenv.2019.135535

Abdullah, R. (2020). Malaysia: Country Report on Children's Environmental Health. *Reviews on Environmental Health*. De Gruyter. https://doi.org/10.1515/reveh-2019-0077

Abdullah, S., Mansor, A. A., Napi, N. N. L. M., Mansor, W. N. W., Ahmed, A. N., Ismail, M., & Ramly, Z. T. A. (2020). Air Quality Status during 2020 Malaysia Movement Control Order (MCO) Due To 2019 Novel Coronavirus (2019-nCoV) Pandemic. *Science of the Total Environment*, *729*, 139022. https://doi.org/10.1016/j.scitotenv.2020.139022

Abhijith, K. V., Kumar, P., Gallagher, J., McNabola, A., Baldauf, R., Pilla, F., ... Pulvirenti, B. (2017). Air Pollution Abatement Performances of Green Infrastructure in Open Road and Built-Up Street Canyon Environments – A Review. *Atmospheric Environment*, *162*, 71–86. https://doi.org/10.1016/j.atmosenv.2017.05.014

Abrams, S. T., Zhang, N., Manson, J., Liu, T., Dart, C., Baluwa, F., ... Toh, C. H. (2013). Circulating Histones are Mediators of Trauma-Associated Lung Injury. *American Journal of Respiratory and Critical Care Medicine*, *187*, 160–169. https://doi.org/10.1164/rccm.201206-1037OC

Ahamad, F., Latif, M. T., Tang, R., Juneng, L., Dominick, D., & Juahir, H. (2014). Variation of Surface Ozone Exceedance around Klang Valley, Malaysia. *Atmospheric Research*, *139*, 116–127. https://doi.org/10.1016/j.atmosres.2014.01.003

Al-Qerem, W., & Ling, J. (2018). Pulmonary Function Tests in Egyptian Schoolchildren in Rural and Urban Areas. *Eastern Mediterranean Health Journal*, *24*(4), 325–332. https://doi.org/10.26719/2018.24.4.325

Alfano, R., Herceg, Z., Nawrot, T. S., Chadeau-Hyam, M., Ghantous, A., & Plusquin, M. (2018). The Impact of Air Pollution on Our Epigenome: How Far Is the Evidence? (A Systematic Review). *Current Environmental Health Reports*, *5*(4), 544–578. https://doi.org/https://doi.org/10.1007/s40572-018-0218-8

Alhamdi, Y., Abrams, S. T., Cheng, Z., Jing, S., Su, D., Liu, Z., ... Toh, C.-H. (2015). Circulating Histones Are Major Mediators of Cardiac Injury in Patients With Sepsis. *Critical Care Medicine*, *43*(10), 2094–2103. https://doi.org/10.1097/CCM.0000000000001162

Alvarado-Cruz, I., Alegría-Torres, J. A., Montes-Castro, N., Jiménez-Garza, O., & Quintanilla-Vega, B. (2018). Environmental Epigenetic Changes, As Risk Factors for The Development of Diseases in Children: A Systematic Review. *Annals of Global Health*, *84*(2), 212–224. https://doi.org/https://doi.org/10.29024/aogh.909

Alvarado-Cruz, I., Sánchez-Guerra, M., Hernández-Cadena, L., De Vizcaya-Ruiz, A., Mugica, V., Pelallo-Martínez, N. A., ... Quintanilla-Vega, B. (2017). Increased Methylation of Repetitive Elements and DNA Repair Genes Is Associated with Higher DNA Oxidation in Children in An Urbanized, Industrial Environment. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *813*, 27–36. https://doi.org/https://doi.org/10.1016/j.mrgentox.2016.11.007

American Lung Association. (2020). Living Near Highways and Air Pollution. Retrieved April 24, 2020, from https://www.lung.org/clean-air/outdoors/who-is-at-risk/highways

American Thoracic Society. (2019). Pulmonary Function Testing in Children.

Amil, N., Latif, M. T., Khan, M. F., & Mohamad, M. (2016). Seasonal Variability of PM2.5 Composition and Sources in the Klang Valley Urban-Industrial Environment. *Atmospheric Chemistry and Physics*, *16*(8), 5357–5381. https://doi.org/10.5194/acp-16-5357-2016

Amin, M. S. R., Tamima, U., & Amador Jimenez, L. (2017). Understanding Air Pollution from Induced Traffic During and After The Construction of A New Highway: Case Study of Highway 25 in Montreal. *Journal of Advanced Transportation*. https://doi.org/10.1155/2017/5161308

Ang, K. H. (2018). Applied Chemometric Approach in Identification Sources of Air Quality Pattern in Selangor, Malaysia. *Sains Malaysiana*, *47*(3), 471–479. https://doi.org/10.17576/jsm-2018-4703-06

Arif, M., & Parveen, S. (2020). Carcinogenic Effects of Indoor Black Carbon and Particulate Matters (PM2.5 and PM10) in Rural Households of India. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-020-10668-5

Arifuddin, A. A., Jalaludin, J., & Hisamuddin, N. H. (2019). Air Pollutants Exposure with Respiratory Symptoms and Lung Function among Primary School Children Nearby Heavy Traffic Area in Kajang. *Asian Journal of Atmospheric Environment*, *13*(1), 21–29. https://doi.org/https://doi.org/10.5572/ajae.2019.13.1.021

Arphorn, Ishimaru, Hara, & Mahasandana. (2018). Considering the Effects of Ambient Particulate Matter on The Lung Function of Motorcycle Taxi Drivers in Bangkok, Thailand. *Journal of the Air & Waste Management Association*, *68*(2), 139–145. https://doi.org/10.1080/10962247.2017.1359217

Asrul, S., & Juliana, J. (2017). Indoor Air Quality and Its Association with Respiratory Health among Preschool Children in Urban and Suburban Area. *Malaysian Journal of Public Health Medicine*, *1*, 78–88. Retrieved from https://www.mjphm.org.my/mjphm/journals/2017 - Special Volume (1)/INDOOR AIR QUALITY AND ITS ASSOCIATION WITH RESPIRATORY HEALTH AMONG PRESCHOOL CHILDREN IN URBAN AND SUBURBAN AREA.pdf

Attorney General Chamber of Malaysia. (2013). *Births and Deaths Registration Act 1957*.

Awang, M. F., Jalaludin, J., Abu Bakar, S., Latif, M. T., Mohamad Fandi, N. F., & Abdul Hamid, H. H. (2020). Assessment of Micronucleus Frequency and Respiratory Health Symptoms among Traffic Policemen Exposed to BTEX and PM2.5 in Klang Valley, Malaysia. *Jurnal Teknologi*, *82*(4), 73–82. https://doi.org/10.11113/jt.v82.14493

Awang, M. F., Jalaludin, J., Latif, M. T., & Mohamad Fandi, N. F. (2019). Exposure to PM2.5 in Urban Area and Respiratory Health Symptoms among Urban Workers in Klang Valley. *IOP Conference Series: Earth and Environmental Science*, *228*, 1–5. https://doi.org/10.1088/1755-1315/228/1/012015

Azeez, O. S., Pradhan, B., & Shafri, H. Z. M. (2018). Vehicular CO Emission Prediction using Support Vector Regression Model and GIS. *Sustainability*, *10*(10). https://doi.org/10.3390/su10103434

Azhari, A., Latif, M. T., & Mohamed, A. F. (2018). Road Traffic as An Air Pollutant Contributor within An Industrial Park Environment. *Atmospheric Pollution Research*, *9*, 680–687. https://doi.org/10.1016/j.apr.2018.01.007

Azid, A., Juahir, H., Ezani, E., Toriman, M. E., Endut, A., Rahman, M. N. A., ... Umar, R. (2015). Identification Source of Variation on Regional Impact of Air Quality Pattern Using Chemometric. *Aerosol and Air Quality Research*, *15*(4), 1545–1558. https://doi.org/10.4209/aaqr.2014.04.0073

Azizi, B. H. O., & Henry, R. L. (1994). Ethnic Differences in Normal Spirometric Lung Function of Malaysian Children. *Respiratory Medicine*, *88*(5), 349–356. https://doi.org/10.1016/0954-6111(94)90040-X

Baccarelli, A., & Bollati, V. (2009). Epigenetics and Environmental Chemicals. *Current Opinion in Pediatrics*, *21*(2), 243–251. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3035853/

Baldauf, R. (2017). Roadside Vegetation Design Characteristics That Can Improve Local, Near-Road Air Quality. *Transportation Research Part D: Transport and Environment*, *52*, 354–361. https://doi.org/10.1016/j.trd.2017.03.013

Banerjee, T., & Christian, R. (2017). On-Field and Laboratory Measurement of Nanoparticle Emission in The Wake of Gasoline Vehicle. *Atmospheric Pollution Research*, *8*(6), 1179–1192. https://doi.org/10.1016/j.apr.2017.05.007

Barnes, N. M., Ng, T., Ma, K. K., & Lai, K. M. (2018). In-Cabin Air Quality During Driving and Engine Idling in Air-Conditioned Private Vehicles in Hong Kong. *International Journal of Environmental Research and Public Health*, *15*(4), 611. https://doi.org/10.3390/ijerph15040611

Barwise, Y., & Kumar, P. (2020). Designing Vegetation Barriers for Urban Air Pollution Abatement: A Practical Review for Appropriate Plant Species Selection. *Npj Climate and Atmospheric Science 2020 3:1*, *3*(1), 1–19. https://doi.org/10.1038/s41612-020-0115-3

Bernama. (2019). Pengumuman Dasar Automotif Nasional Baharu Suku Kedua. Retrieved November 13, 2019, from https://www.sinarharian.com.my/article/22441/BISNES/Pengumuman-Dasar-Automotif-Nasional-baharu-suku-kedua

Bhargava, A., Bunkar, N., Aglawe, A., Pandey, K. C., Tiwari, R., Chaudhury, K., … Mishra, P. K. (2018). Epigenetic Biomarkers for Risk Assessment of Particulate Matter Associated Lung Cancer. *Current Drug Targets*, *19*(10), 1127–1147. https://doi.org/10.2174/1389450118666170911114342

Bhatti, U., Laghari, Z. A., & Syed, B. M. (2019). Effect of Body Mass Index on Respiratory Parameters: A Cross-Sectional Analytical Study. *Pakistan Journal of Medical Sciences*, *35*(6), 1724–1729. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6861468/pdf/PJMS-35-1724.pdf

Bintu, L., Yong, J., Antebi, Y. E., McCue, K., Kazuki, Y., Uno, N., … Elowitz, M. B. (2016). Dynamics of Epigenetic Regulation at The Single-Cell Level. *Science*, *351*(6274), 720–724. https://doi.org/10.1126/science.aab2956

Both, A. F., Westerdahl, D., Fruin, S., Haryanto, B., & Marshall, J. D. (2013). Exposure to Carbon Monoxide, Fine Particle Mass, and Ultrafine Particle Number in Jakarta, Indonesia: Effect of Commute Mode. *Science of the Total Environment*, *443*, 965–972. https://doi.org/10.1016/j.scitotenv.2012.10.082

Bourganis, V., Kammona, O., Alexopoulos, A., & Kiparissides, C. (2018). Recent Advances in Carrier Mediated Nose-to-Brain Delivery of Pharmaceutics. *European Journal of Pharmaceutics and Biopharmaceutics*, *128*, 337–362. https://doi.org/10.1016/j.ejpb.2018.05.009

Brauer, M., Reynolds, C., & Hystad, P. (2013). Traffic-Related Air Pollution and Health in Canada. *CMAJ: Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne*, *185*(18), 1557–1558. https://doi.org/10.1503/cmaj.121568

Brown, J. S. (2015). Deposition of Particles. In *Comparative Biology of the Normal Lung: Second Edition* (pp. 513–536). Elsevier Inc. https://doi.org/10.1016/B978-0-12-404577-4.00027-8

Brunst, K. J., Leung, Y. K., Ryan, P. H., Khurana Hershey, G. K., Levin, L., Ji, H., … Ho, S. M. (2013). Forkhead Box Protein 3 (FOXP3) Hypermethylation is Associated with Diesel Exhaust Exposure and Risk for Childhood Asthma. *Journal of Allergy and Clinical Immunology*, *131*(2), 592-594.e3. https://doi.org/10.1016/j.jaci.2012.10.042

Carrion-Matta, A., Kang, C. M., Gaffin, J. M., Hauptman, M., Phipatanakul, W., Koutrakis, P., & Gold, D. R. (2019). Classroom Indoor PM2.5 Sources and Exposures in Inner-City Schools. *Environment International*, *131*, 104968. https://doi.org/10.1016/j.envint.2019.104968

Centre for Agriculture and Bioscience International. (2021). Grevillea robusta (Silky Oak). Retrieved July 29, 2021, from https://www.cabi.org/isc/datasheet/25866

Checkley, W., Robertson, N. M., Kayongo, A., Siddharthan, T., Pollard, S. L., Gomez Villalobos, J., ... Kirenga, B. (2019). The Role of Epigenetics in Respiratory Health in Urban Populations in Low and Middle-Income Countries. *Global Health, Epidemiology and Genomics*, *4*(e8), 1–7. https://doi.org/10.1017/gheg.2019.7

Chin, Y. S. J., De Pretto, L., Thuppil, V., & Ashfold, M. J. (2019). Public Awareness and Support for Environmental Protection—A Focus on Air Pollution in Peninsular Malaysia. *PLOS ONE*, *14*(3), e0212206. https://doi.org/10.1371/journal.pone.0212206

Chong, H. S., Park, Y., Kwon, S., & Hong, Y. (2018). Analysis of Real Driving Gaseous Emissions from Light-Duty Diesel Vehicles. *Transportation Research Part D: Transport and Environment*, *65*(October), 485–499. https://doi.org/10.1016/j.trd.2018.09.015

Chong, T. L., Ahearn, E. L., & Cimmino, L. (2019). Reprogramming the Epigenome With Vitamin C. *Frontiers in Cell and Developmental Biology*, *7*, 128. https://doi.org/10.3389/fcell.2019.00128

Chowdhury, B., Cho, I.-H., & Irudayaraj, J. (2017). Technical Advances in Global DNA Methylation Analysis in Human Cancers. *Journal of Biological Engineering*, *11*(1), 10. https://doi.org/https://doi.org/10.1186/s13036-017-0052-9

Chu, M. Y., Law, T. H., Hamid, H., Law, S. H., & Lee, J. C. (2019). The Motorcycle to Car Ownership Ratio: Inflation and Its Indirect Effects. *IOP Conference Series: Materials Science and Engineering*, *512*, 012015. https://doi.org/10.1088/1757-899X/512/1/012015

Cordero, P., Campion, J., Milagro, F. I., Goyenechea, E., Steemburgo, T., Javierre, B. M., & Martinez, J. A. (2011). Leptin and TNF-Alpha Promoter Methylation Levels Measured by MSP Could Predict The Response to A Low-Calorie Diet. *Journal of Physiology and Biochemistry*, *67*(3), 463–470. https://doi.org/10.1007/s13105-011-0084-4

Das, N., Verstraete, K., Topalovic, M., Aerts, J.-M., & Janssens, W. (2019). Estimating Airway Resistance from Forced Expiration in Spirometry. *Applied Sciences*, *9*(14), 2842. https://doi.org/10.3390/app9142842

Deng, Q., Deng, L., Miao, Y., Guo, X., & Li, Y. (2019). Particle Deposition in The Human Lung: Health Implications of Particulate Matter from Different Sources. *Environmental Research*, *169*, 237–245. https://doi.org/10.1016/j.envres.2018.11.014

Department of Statistics Malaysia. (2020). Household Income & Basic Amenities Survey Report 2019. Retrieved October 23, 2020, from https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=120&bul_id=TU00TmRhQ1N5TUxHVWN0T2VjbXJYZz09&menu_id=amVoWU54UTl0a21NWmdhMjFMMWcyZz09

Deshmukh, P., Isakov, V., Venkatram, A., Yang, B., Zhang, K. M., Logan, R., & Baldauf, R. (2019). The Effects of Roadside Vegetation Characteristics on Local, Near-Road Air Quality. *Air Quality, Atmosphere and Health*, *12*, 259–270. https://doi.org/10.1007/s11869-018-0651-8

Ding, R., Jin, Y., Liu, X., Ye, H., Zhu, Z., Zhang, Y., … Xu, Y. (2017). Dose- and Time- Effect Responses of DNA Methylation and Histone H3K9 Acetylation Changes Induced by Traffic-Related Air Pollution. *Scientific Reports*, *7*, 43737. https://doi.org/10.1038/srep43737

Ding, R., Jin, Y., Liu, X., Zhu, Z., Zhang, Y., Wang, T., & Xu, Y. (2016). H3K9 Acetylation Change Patterns in Rats After Exposure to Traffic-Related Air Pollution. *Environmental Toxicology and Pharmacology*, *42*, 170–175. https://doi.org/10.1016/j.etap.2016.01.016

DOE Malaysia. (2020). New Malaysia Ambient Air Quality Standard. Retrieved May 9, 2020, from https://www.doe.gov.my/portalv1/en/category/info-umum/info-kualiti-udara

Dominick, D., Juahir, H., Latif, M. T., Zain, S. M., & Aris, A. Z. (2012). Spatial Assessment of Air Quality Patterns in Malaysia using Multivariate Analysis. *Atmospheric Environment*, *60*, 172–181. https://doi.org/10.1016/j.atmosenv.2012.06.021

DOS Malaysia. (2018a). *Children Statistics Malaysia 2018. Department of Statistics Malaysia*. Putrajaya: Department of Statistics Malaysia.

DOS Malaysia. (2018b). *Compendium of Environment Statistics. Department of Statistics Malaysia*. Putrajaya. https://doi.org/10.5860/choice.29-3644

DOS Malaysia. (2019). *Current Population Estimates Malaysia 2019. Department of Statistics Malaysia*. Putrajaya: Department of Statistics Malaysia. Retrieved from www.dosm.gov.my

DOS Malaysia. (2021). *Current Population Estimates Malaysia 2021*.

DOSH Malaysia. (2020). *Official Website Department of Occupational Safety and Health - Indoor Air Quality*. Retrieved from http://www.dosh.gov.my/index.php/chemical-management-v/indoor-air-quality

Erath, A., Fourie, P., Van Eggermond, M., & Ordoñez Medina, S. (2018). *Potential of Motorcycles As Urban Means of Transport*. Future Cities Laboratory (FCL). https://doi.org/10.3929/ethz-b-000306547

Ezani, E., Abdul Hisham, N. A., Syed Ismail, S. N., & Abdul Wahab, M. I. (2018). Air Pollution and Traffic Measurements within University Areas in Serdang, Malaysia. *Asia Pacific Environmental and Occupational Health Journal*, *4*(3), 58–65.

Fadzir, N. A. S., & Jalaludin, J. (2013). Indoor Particulate Matter 2.5 (PM2.5) and Lung Function among Children Living Near Busy Road in Cheras, Kuala Lumpur. *Health and the Environment Journal*, *4*(2), 1–19. Retrieved from https://www.researchgate.net/publication/304109295_Indoor_Particulate_Matter_25_PM25_and_lung_function_among_children_living_near_busy_road_in_Cheras_Kuala_Lumpur

Ferrari, L., Carugno, M., & Bollati, V. (2019). Particulate Matter Exposure Shapes DNA Methylation Through the Lifespan. *Clinical Epigenetics*, *11*(129). https://doi.org/https://doi.org/10.1186/s13148-019-0726-x

Gaffin, J. M., Hauptman, M., Lai, P., Petty, C. R., Wolfson, J. M., Kang, C.-M., … Phipatanakul, W. (2017). Nitrogen Dioxide Exposure in School Classrooms Is Associated with Airflow Obstruction in Students with Asthma. *Journal of Allergy and Clinical Immunology*, *139*(2), AB174. https://doi.org/10.1016/j.jaci.2016.12.570

Galvez, M. C., Tipan, D. P., Valera, A. A., Vallar, E., & Nacua, A. (2020). Ozone, Nitrogen Dioxide, and PM2.5 Measurement at Three Urban Parks in Manila, Philippines using Portable Sensors. *Advances in Science, Technology and Engineering Systems Journal*, *5*(6), 1027–1032. https://doi.org/10.25046/aj0506124

Gauderman, W. J., Avol, E., Gilliland, F., Vora, H., Thomas, D., Berhane, K., … Peters, J. (2004). The Effect of Air Pollution on Lung Development from 10 to 18 Years of Age. *New England Journal of Medicine*, *351*(11), 1057–1067. https://doi.org/10.1056/nejmoa040610

Gawda, A., Majka, G., Nowak, B., & Marcinkiewicz, J. (2017). Air Pollution, Oxidative Stress, and Exacerbation of Autoimmune Diseases. *Central European Journal of Immunology*, *42*(3), 305–312. https://doi.org/10.5114/ceji.2017.70975

Ghasemian, M., Amini, S., & Princevac, M. (2017). The Influence of Roadside Solid and Vegetation Barriers on Near-Road Air Quality. *Atmospheric Environment*, *170*, 108–117. https://doi.org/10.1016/j.atmosenv.2017.09.028

Gowher, H., & Jeltsch, A. (2018). Mammalian DNA Methyltransferases: New Discoveries and Open Questions. *Biochemical Society Transactions*, *46*(5), 1191–1202. https://doi.org/10.1042/BST20170574

Graham, B. L., Steenbruggen, I., Miller, M. R., Barjaktarevic, I. Z., Cooper, B. G., Hall, G. L., ... Thompson, B. R. (2019). Standardization of Spirometry 2019 Update: An Official American Thoracic Society and European Respiratory Society Technical Statement. *American Journal of Respiratory and Critical Care Medicine*, *200*(8), e70–e88. https://doi.org/10.1164/rccm.201908-1590ST

Gsell, C., Richly, H., Coin, F., & Naegeli, H. (2020). A Chromatin Scaffold for DNA Damage Recognition: How Histone Methyltransferases Prime Nucleosomes for Repair of Ultraviolet Light-Induced Lesions. *Nucleic Acids Research*, *48*(4), 1652–1668. https://doi.org/10.1093/nar/gkz1229

Gupta, A., Bush, A., & Nagakumar, P. (2020). Asthma in Children During The COVID-19 Pandemic: Lessons from Lockdown and Future Directions for Management. *The Lancet Respiratory Medicine*, *8*(11), 1070–1071. https://doi.org/10.1016/S2213-2600(20)30278-2

Hamid, H. H. A., Latif, M. T., Uning, R., Nadzir, M. S. M., Khan, M. F., Ta, G. C., & Kannan, N. (2020). Observations of BTEX in The Ambient Air of Kuala Lumpur by Passive Sampling. *Environmental Monitoring and Assessment*, *192*(6), 342. https://doi.org/10.1007/s10661-020-08311-4

Haque, M. S., & Singh, R. B. (2017). Air Pollution and Human Health in Kolkata, India: A Case Study. *Climate*, *5*(4), 77. https://doi.org/10.3390/cli5040077

Haryanto, B. (2018). Climate Change and Urban Air Pollution Health Impacts in Indonesia. In *Springer Climate* (pp. 215–239). Springer. https://doi.org/10.1007/978-3-319-61346-8_14

Haryanto, B. (2020). Indonesia: Country Report on Children's Environmental Health. *Reviews on Environmental Health*, *35*(1), 41–48. https://doi.org/10.1515/reveh-2019-0088

Haryanto, B., & Pratiwi, D. A. (2020). Effect of Particulate Matter 2.5 Exposure to Urinary Malondialdehyde Levels of Public Transport Drivers in Jakarta. *Reviews on Environmental Health*, *35*(3), 295–300. https://doi.org/10.1515/reveh-2020-0017

Hazenkamp-Von Arx, M. E., Schindler, C., Ragettli, M. S., Künzli, N., Braun-Fahrländer, C., & Liu, L. J. S. (2011). Impacts of Highway Traffic Exhaust in Alpine Valleys on The Respiratory Health in Adults: A Cross-Sectional Study. *Environmental Health: A Global Access Science Source*, *10*(1), 1–9. https://doi.org/10.1186/1476-069X-10-13

He, L., Gao, L., Shi, Z., Li, Y., Zhu, L., Li, S., ... Feng, F. (2015). Involvement of Cytochrome P450 1A1 and Glutathione S-transferase P1 Polymorphisms and Promoter Hypermethylation in The Progression of Anti-Tuberculosis Drug-Induced Liver Injury: A Case-Control Study. *PLoS ONE*, *10*(3), 1–11. https://doi.org/10.1371/journal.pone.0119481

Hegseth, M. N., Oftedal, B. M., Höper, A. C., Aminoff, A. L., Thomassen, M. R., Svendsen, M. V., & Fell, A. K. M. (2019). Self-Reported Traffic-Related Air Pollution and Respiratory Symptoms among Adults in An Area with Modest Levels of Traffic. *PLOS ONE*, *14*(12), e0226221. https://doi.org/10.1371/journal.pone.0226221

Hind, C. R. K. (2013). Neurogenic respiratory failure. In *Handbook of Clinical Neurology* (Vol. 110, pp. 295–302). Elsevier B.V. https://doi.org/10.1016/B978-0-444-52901-5.00024-1

Hirota, K., & Kashima, S. (2020). How are Automobile Fuel Quality Standards Guaranteed? Evidence from Indonesia, Malaysia and Vietnam. *Transportation Research Interdisciplinary Perspectives*, *4*, 100089. https://doi.org/10.1016/j.trip.2019.100089

Hosaini, P. N., Khan, M. F., Mustaffa, N. I. H., Amil, N., Mohamad, N., Jaafar, S. A., … Latif, M. T. (2017). Concentration and Source Apportionment of Volatile Organic Compounds (VOCs) in The Ambient Air of Kuala Lumpur, Malaysia. *Natural Hazards*, *85*(1), 437–452. https://doi.org/10.1007/s11069-016-2575-7

Hou, D., Ge, Y., Chen, C., Tan, Q., Chen, R., Yang, Y., … Song, Y. (2020). Associations of Long-Term Exposure to Ambient Fine Particulate Matter and Nitrogen Dioxide with Lung Function: A Cross-Sectional Study in China. *Environment International*, *144*, 105977. https://doi.org/10.1016/j.envint.2020.105977

Hussin, F. S., & Jalaludin, J. (2016). Association of PM10 and PM2.5 Exposure with Respiratory Health of the Children Living Near Palm Oil Mill, Dengkil. *Malaysian Journal of Public Health Medicine*, *16*, 20–26.

Ichikawa, Y., Connelly, C. F., Appleboim, A., Miller, T. C. R., Jacobi, H., Abshiru, N. A., … Kaufman, P. D. (2017). A Synthetic Biology Approach To Probing Nucleosome Symmetry. *ELife*, *6*. https://doi.org/10.7554/eLife.28836

Jalaludin, J., Hashim, Z., Taib, N. M., Lubis, S., & Hashim, J. H. (2002). Comparison on the Influence of Residential Environment to Asthma Attacks among Children in Kuala Lumpur and Terengganu, Malaysia. *Malaysian Journal of Community Health*, *8*, 51–62.

Jalaludin, J., & Jasme, N. J. M. (2015). Exposure to Indoor Partculate Matter 2.5 (PM2.5) and Volatile Organic Compounds (VOC) among Preschool Children at an Industrial Area in Petaling Jaya, Selangor. *International Journal of Applied and Natural Sciences*, *4*(6), 41–50. Retrieved from www.iaset.us

Jalaludin, J., Syed Noh, S. N., Suhaimi, N. F., & Md Akim, A. (2014). Tumor Necrosis Factor-Alpha as Biomarkers of Exposure to Indoor Pollutants among Primary School Children in Klang Valley. *American Journal of Applied Sciences*, *11*(9), 1616–1630. https://doi.org/10.3844/ajassp.2014.1616.1630

Janssen, B. G., Gyselaers, W., Byun, H. M., Roels, H. A., Cuypers, A., Baccarelli, A. A., & Nawrot, T. S. (2017). Placental Mitochondrial DNA and CYP1A1 Gene Methylation As Molecular Signatures for Tobacco Smoke Exposure in Pregnant Women and The Relevance for Birth Weight. *Journal of Translational Medicine*, *15*(1), 1–10. https://doi.org/10.1186/s12967-016-1113-4

Jasim, H., Olausson, P., Hedenberg-Magnusson, B., Ernberg, M., & Ghafouri, B. (2016). The Proteomic Profile of Whole and Glandular Saliva in Healthy Pain-Free Subjects. *Scientific Reports*, *6*. https://doi.org/10.1038/srep39073

Jeong, H., & Park, D. (2017). Characteristics of Elementary School Children's Daily Exposure to Black Carbon (BC) in Korea. *Atmospheric Environment*, *154*, 179–188. https://doi.org/10.1016/j.atmosenv.2017.01.045

Ji, H., Biagini Myers, J. M., Brandt, E. B., Brokamp, C., Ryan, P. H., & Khurana Hershey, G. K. (2016). Air Pollution, Epigenetics, and Asthma. *Allergy, Asthma and Clinical Immunology*, *12*(1), 1–14. https://doi.org/https://doi.org/10.1186/s13223-016-0159-4

Jiang, R., Jones, M. J., Sava, F., Kobor, M. S., & Carlsten, C. (2014). Short-Term Diesel Exhaust Inhalation in A Controlled Human Crossover Study is Associated with Changes in DNA Methylation of Circulating Mononuclear Cells in Asthmatics. *Particle and Fibre Toxicology*, *11*, 71. https://doi.org/10.1186/s12989-014-0071-3

Jirtle, R. L., & Skinner, M. K. (2007). Environmental Epigenomics and Disease Susceptibility. *Nature Reviews Genetics*, *8*, 253–262. https://doi.org/10.1038/nrg2045

Jo, E.-J., & Song, W.-J. (2019). Environmental Triggers for Chronic Cough. *Asia Pacific Allergy*, *9*(2). https://doi.org/10.5415/apallergy.2019.9.e16

Jun, K. E., Hamzah, N. A., & Anua, S. M. (2017). Indoor Air Quality and Symptoms of Sick Building Syndrome in Two Selected Building (New Versus Old). *Journal of Occupational Safety and Health*, *14*(2), 7–14.

Jung, K. H., Lovinsky-Desir, S., Yan, B., Torrone, D., Lawrence, J., Jezioro, J. R., … Miller, R. L. (2017). Effect of Personal Exposure to Black Carbon on Changes in Allergic Asthma Gene Methylation Measured 5 Days Later in Urban Children: Importance of Allergic Sensitization. *Clinical Epigenetics*, *9*, 61. https://doi.org/https://doi.org/10.1186/s13148-017-0361-3

Kadambari, S., Abo, Y.-N., Phuong, L. K., Osowicki, J., & Bryant, P. A. (2020). Decrease in Infection-Related Hospital Admissions During COVID-19: Why Are Parents Avoiding the Doctor? *Pediatric Infectious Disease Journal*, *39*(11), e385–e386. https://doi.org/10.1097/INF.0000000000002870

Kamaruddin, A. S., Jalaludin, J., & Choo, C. P. (2015). Indoor Air Quality and Its Association with Respiratory Health among Malay Preschool Children in Shah Alam and Hulu Langat. *Advances in Environmental Biology*, *9*(9), 17–26. Retrieved from http://www.aensiweb.net/AENSIWEB/aeb/aeb/2015/Special IPN BANDUNG/17-26.pdf

Kamaruddin, A. S., Jalaludin, J., & Hamedon, T. R. (2016). Exposure to Industrial Air Pollutants and Respiratory Health School and Home Exposure among Primary School Children in Kemaman, Terengganu. *International Journal of Applied Chemistry*, *12*(1), 45–50. Retrieved from https://www.ripublication.com/ijacspl/ijacv12n1spl_09.pdf

Kamaruddin, A. S., Jalaludin, J., Hamedon, T. R., & Hisamuddin, N. H. (2019). FeNO as a Biomarker for Airway Inflammation Due to Exposure to Air Pollutants among School Children Nearby Industrial Areas in Terengganu. *Pertanika Journal of Science & Technology*, *27*(2), 589–600. Retrieved from http://www.pertanika.upm.edu.my/Pertanika PAPERS/JST Vol. 27 (2) Apr. 2019/03 JST(S)-0477-2019.pdf

Kanchanasuta, S., Sooktawee, S., Patpai, A., & Vatanasomboon, P. (2020). Temporal Variations and Potential Source Areas of Fine Particulate Matter in Bangkok, Thailand. *Air, Soil and Water Research*, *13*, 117862212097820. https://doi.org/10.1177/1178622120978203

Keen, C. L., Holt, R. R., Oteiza, P. I., Fraga, C. G., & Schmitz, H. H. (2005). Cocoa Antioxidants and Cardiovascular Health. *The American Journal of Clinical Nutrition*, *81*(1), 298S-303S. https://doi.org/10.1093/ajcn/81.1.298S

Kelkar, H., Sharma, A. K., & Chaturvedi, S. (2019). Association of Air Pollution and Lung Function of Young Adult Females in New Delhi. *Journal of Health and Pollution*, *9*(22), 190611. https://doi.org/10.5696/2156-9614-9.22.190611

Kenyon, C. C., Hill, D. A., Henrickson, S. E., Bryant-Stephens, T. C., & Zorc, J. J. (2020). Initial Effects of The COVID-19 Pandemic on Pediatric Asthma Emergency Department Utilization. *Journal of Allergy and Clinical Immunology: In Practice*, *8*(8), 2774-2776.e1. https://doi.org/10.1016/j.jaip.2020.05.045

Khan, M. F., Hamid, A. H., Bari, M. A., Tajudin, A. B. A., Latif, M. T., Nadzir, M. S. M., ... Kumar, P. (2019). Airborne Particles in The City Center of Kuala Lumpur: Origin, Potential Driving Factors, and Deposition Flux in Human Respiratory Airways. *Science of the Total Environment*, *650*, 1195–1206. https://doi.org/10.1016/j.scitotenv.2018.09.072

Kheirbek, I., Haney, J., Douglas, S., Ito, K., & Matte, T. (2016). The Contribution of Motor Vehicle Emissions to Ambient Fine Particulate Matter Public Health Impacts in New York City: A Health Burden Assessment. *Environmental Health: A Global Access Science Source*, *15*(1), 89. https://doi.org/10.1186/s12940-016-0172-6

Kholod, N., & Evans, M. (2016). Reducing Black Carbon Emissions from Diesel Vehicles in Russia: An Assessment and Policy Recommendations. *Environmental Science & Policy*, *56*, 1–8. https://doi.org/https://doi.org/10.1016/j.envsci.2015.10.017

Kietzmann, T., Petry, A., Shvetsova, A., Gerhold, J. M., & Görlach, A. (2017). The Epigenetic Landscape Related to Reactive Oxygen Species Formation in The Cardiovascular System. *British Journal of Pharmacology*, *174*(12), 1533–1554. https://doi.org/10.1111/bph.13792

Kim, D., Chen, Z., Zhou, L.-F., & Huang, S.-X. (2018). Air Pollutants and Early Origins of Respiratory Diseases. *Chronic Diseases and Translational Medicine*, *4*(2), 75–94. https://doi.org/10.1016/j.cdtm.2018.03.003

Kim, H. J., Choi, M. G., Park, M. K., & Seo, Y. R. (2017). Predictive and Prognostic Biomarkers of Respiratory Diseases due to Particulate Matter Exposure. *Journal of Cancer Prevention*, *22*(1), 6–15. https://doi.org/10.15430/JCP.2017.22.1.6

Kim, J. J., Huen, K., Adams, S., Smorodinsky, S., Hoats, A., Malig, B., ... Ostro, B. (2008). Residential Traffic and Children's Respiratory Health. *Environmental Health Perspectives*, *116*(9), 1274–1279. https://doi.org/10.1289/ehp.10735

Krishnaswamy, J., Koon, S. S., & Annamalai, N. (2019). The Shadow Education System: Students' Responses to Private Tutorial Learning. *Malaysian Journal of Learning and Instruction*, *16*(2), 201–225.

Kumar, S., Joos, G., Boon, L., Tournoy, K., Provoost, S., & Maes, T. (2017). Role of Tumor Necrosis Factor-α and Its Receptors in Diesel Exhaust Particle-Induced Pulmonary Inflammation. *Scientific Reports*, *7*, 11508. https://doi.org/10.1038/s41598-017-11991-7

Kusumaningtyas, S. D. A., Aldrian, E., Wati, T., Atmoko, D., & Sunaryo, S. (2018). The Recent State of Ambient Air Quality in Jakarta. *Aerosol and Air Quality Research*, *18*(9), 2343–2354. https://doi.org/10.4209/aaqr.2017.10.0391

Langie, S. A. S., Moisse, M., Szarc vel Szic, K., Van Der Plas, E., Koppen, G., De Prins, S., ... De Boever, P. (2018). GLI2 Promoter Hypermethylation in Saliva of Children with a Respiratory Allergy. *Clinical Epigenetics*, *10*(1), 1–13. https://doi.org/10.1186/s13148-018-0484-1

Latif, M. T., Abd Hamid, H. H., Ahamad, F., Khan, M. F., Mohd Nadzir, M. S., Othman, M., ... Tahir, N. M. (2019). BTEX Compositions and Its Potential Health Impacts in Malaysia. *Chemosphere*, *237*, 124451. https://doi.org/10.1016/j.chemosphere.2019.124451

Lee, W. J., & Zhu, B. T. (2006). Inhibition of DNA Methylation by Caffeic Acid and Chlorogenic Acid, Two Common Catechol-Containing Coffee Polyphenols. *Carcinogenesis*, *27*(2), 269–277. https://doi.org/10.1093/carcin/bgi206

Lee, Y.-Y., Lin, S.-L., Yuan, C.-S., Lin, M.-Y., & Chen, K.-S. (2018). Reduction of Atmospheric Fine Particle Level by Restricting The Idling Vehicles around A Sensitive Area. *Journal of the Air & Waste Management Association*, *68*(7), 656–670. https://doi.org/10.1080/10962247.2018.1438320

Lei, X., Chen, R., Wang, C., Shi, J., Zhao, Z., Li, W., ... Kan, H. (2020). Necessity of Personal Sampling for Exposure Assessment on Specific Constituents of PM2.5: Results of A Panel Study in Shanghai, China. *Environment International*, *141*, 105786. https://doi.org/10.1016/j.envint.2020.105786

Lemeshow, S., Hosmer Jr, D. W., Klar, J., & Lwanga, S. K. (1990). *Adequacy of Sample Size in Health Studies*. Chichester: World Health Organization. Retrieved from http://apps.who.int/iris/bitstream/handle/10665/41607/0471925179_eng.pdf?sequence=1&isAllowed=y

Leung, T. H., Hoffmann, A., & Baltimore, D. (2004). One Nucleotide in A κB Site Can Determine Cofactor Specificity for NF-κB Dimers. *Cell*, *118*(4), 453–464. https://doi.org/10.1016/j.cell.2004.08.007

Li, Y., Mu, Z., Wang, H., Liu, J., & Jiang, F. (2018). The Role of Particulate Matters on Methylation of IFN-γ and IL-4 Promoter Genes in Pediatric Allergic Rhinitis. *Oncotarget*, *9*(25), 17406–17419. https://doi.org/10.18632/oncotarget.24227

Lin, C., Gillespie, J., Schuder, M. D., Duberstein, W., Beverland, I. J., & Heal, M. R. (2015). Evaluation and Calibration of Aeroqual Series 500 Portable Gas Sensors for Accurate Measurement of Ambient Ozone and Nitrogen Dioxide. *Atmospheric Environment*, *100*, 111–116. https://doi.org/10.1016/j.atmosenv.2014.11.002

Lin, V. W., Baccarelli, A. A., & Burris, H. H. (2016). Epigenetics - A Potential Mediator between Air Pollution and Preterm Birth. *Environmental Epigenetics*, *2*(1), dvv008. https://doi.org/10.1093/eep/dvv008

Lin, Y. C., Chi, W. J., & Lin, Y. Q. (2020). The Improvement of Spatial-Temporal Resolution of PM2.5 Estimation Based on Micro-Air Quality Sensors By Using Data Fusion Technique. *Environment International*, *134*, 105305. https://doi.org/10.1016/j.envint.2019.105305

Lippmann, M. (2011). Regional Deposition of Particles in the Human Respiratory Tract. In *Comprehensive Physiology* (pp. 213–232). Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/cphy.cp090114

Liu, Q., Ma, T., Olson, M. R., Liu, Y., Zhang, T., Wu, Y., & Schauer, J. J. (2016). Temporal Variations of Black Carbon During Haze and Non-Haze Days in Beijing. *Scientific Reports*, *6*(1), 1–10. https://doi.org/10.1038/srep33331

Liu, S. K., Cai, S., Chen, Y., Xiao, B., Chen, P., & Xiang, X. D. (2016). The Effect of Pollutional Haze on Pulmonary Function. *Journal of Thoracic Disease*, *8*(1), E41–E56. https://doi.org/10.3978/j.issn.2072-1439.2016.01.18

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB Signaling in Inflammation. *Signal Transduction and Targeted Therapy*, *2*(1), 1–9. https://doi.org/10.1038/sigtrans.2017.23

Liu, Y., Zhang, B., Zhang, T., Wang, H., Peng, L., & Zhou, L. (2020). Effect of NF-κB Signal Pathway on Mucus Secretion Induced by Atmospheric PM2.5 in Asthmatic Rats. *Ecotoxicology and Environmental Safety*, *190*, 110094. https://doi.org/10.1016/j.ecoenv.2019.110094

Long, M., Park, S. G., Strickland, I., Hayden, M. S., & Ghosh, S. (2009). Nuclear Factor-κB Modulates Regulatory T Cell Development by Directly Regulating Expression of Foxp3 Transcription Factor. *Immunity*, *31*(6), 921–931. https://doi.org/10.1016/j.immuni.2009.09.022

Lovinsky-Desir, S., Jung, K. H., Jezioro, J. R., Torrone, D. Z., de Planell-Saguer, M., Yan, B., … Miller, R. L. (2017). Physical Activity, Black Carbon Exposure, and DNA Methylation in the FOXP3 Promoter. *Clinical Epigenetics*, *9*(1), 65. https://doi.org/https://doi.org/10.1186/s13148-017-0364-0

Lu, C., Jain, S. U., Hoelper, D., Bechet, D., Rosalynn, C., Ran, L., … Bruce, R. (2016). Histone H3K36 Mutations Promote Sarcomagenesis through Altered Histone Methylation Landscape. *Science*, *352*(6287), 844–849. https://doi.org/10.1126/science.aac7272.Histone

Lutfi, M. F. (2017). The Physiological Basis and Clinical Significance of Lung Volume Measurements. *Multidisciplinary Respiratory Medicine*, *12*(1), 3. https://doi.org/10.1186/s40248-017-0084-5

Lv, Y., Wang, H., Wei, S., Zhang, L., & Zhao, Q. (2017). The Correlation between Indoor and Outdoor Particulate Matter of Different Building Types in Daqing, China. *Procedia Engineering*, *205*, 360–367. https://doi.org/10.1016/j.proeng.2017.10.002

Ma, D., Jiang, C., Hu, X., Liu, H., Li, Q., Li, T., … Li, O. (2014). Methylation Patterns of the IFN-γ Gene in Cervical Cancer Tissues. *Scientific Reports*, *4*, 1–6. https://doi.org/10.1038/srep06331

Mafort, T. T., Rufino, R., Costa, C. H., & Lopes, A. J. (2016). Obesity: Systemic and Pulmonary Complications, Biochemical Abnormalities, and Impairment of Lung Function. *Multidisciplinary Respiratory Medicine*, *11*. https://doi.org/10.1186/s40248-016-0066-z

Matz, C. J., Stieb, D. M., Egyed, M., Brion, O., & Johnson, M. (2018). Evaluation of Daily Time Spent in Transportation and Traffic-Influenced Microenvironments by Urban Canadians. *Air Quality, Atmosphere and Health*, *11*(2), 209–220. https://doi.org/10.1007/s11869-017-0532-6

McCarthy, K. (2018). Pulmonary Function Testing. Retrieved November 13, 2019, from https://emedicine.medscape.com/article/303239-overview

McConnell, R., Berhane, K., Yao, L., Jerrett, M., Lurmann, F., Gilliland, F., … Peters, J. (2006). Traffic, Susceptibility, and Childhood Asthma. *Environmental Health Perspectives*, *114*(5), 766–772. https://doi.org/10.1289/ehp.8594

McNamara, M. L., Noonan, C. W., & Ward, T. J. (2011). Correction Factor for Continuous Monitoring of Wood Smoke Fine Particulate Matter. *Aerosol and Air Quality Research*, *11*(3), 316–323. https://doi.org/10.4209/aaqr.2010.08.0072

Mei, M., Song, H., Chen, L., Hu, B., Bai, R., Xu, D., … Chen, C. (2018). Early-life Exposure to Three Size-Fractionated Ultrafine and Fine Atmospheric Particulates in Beijing Exacerbates Asthma Development in Mature Mice. *Particle and Fibre Toxicology*, *15*(1), 13. https://doi.org/10.1186/s12989-018-0249-1

Meldrum, K., Guo, C., Marczylo, E. L., Gant, T. W., Smith, R., & Leonard, M. O. (2017). Mechanistic Insight into The Impact of Nanomaterials on Asthma and Allergic Airway Disease. *Particle and Fibre Toxicology*, *14*(1), 1–35. https://doi.org/10.1186/s12989-017-0228-y

Ministry of Education Malaysia. (2021). Senarai Sekolah Kementerian Pendidikan Malaysia. Retrieved February 3, 2021, from https://www.moe.gov.my/en/muat-turun/laporan-dan-statistik/senarai-sekolah

Ministry of Health Malaysia. (2021). Priority List of Environmental Health Issues in Malaysia. Retrieved February 5, 2021, from http://nehapmalaysia.moh.gov.my/priority-list-of-environmental-health-issues-in-malaysia/?fbclid=IwAR0l5bFMeHR5hStF94p_c2gwJVoqn0O7yV8QjA_qNPjpfFhHVcqTkg803v0

Moen, E. L., Mariani, C. J., Zullow, H., Jeff-Eke, M., Litwin, E., Nikitas, J. N., & Godley, L. A. (2015). New Themes in The Biological Functions of 5-Methylcytosine and 5-Hydroxymethylcytosine. *Immunological Reviews*, *263*(1), 36–49. https://doi.org/10.1111/imr.12242

MOH Malaysia. (2020). *Health Facts 2020 (Reference Data for 2019). Ministry of Health Malaysia.* Putrajaya. Retrieved from https://www.moh.gov.my/moh/resources/Penerbitan/Penerbitan Utama/HEALTH FACTS/Health Facts 2020.pdf

Mohamad Fandi, N. F., Jalaludin, J., Latif, M. T., Abd Hamid, H. H., & Awang, M. F. (2020). BTEX Exposure Assessment and Inhalation Health Risks to Traffic Policemen in The Klang Valley Region, Malaysia. *Aerosol and Air Quality Research*, *20*(9), 1922–1937. https://doi.org/10.4209/aaqr.2019.11.0574

Mohamad Fandi, N. F., Wan Mansor, W. A., & Jalaludin, J. (2018). Work Exposure to Traffic Air Pollutants (PM10, Benzene, Toluene, and Xylene) and Respiratory Health Implications among Urban Traffic Policemen in Klang Valley, Malaysia. *Malaysian Journal of Medicine and Health Sciences*, *14*(SP2), 2636–9346. Retrieved from http://www.medic.upm.edu.my/upload/dokumen/2018120408583509_MJMHS_SP_Nov_2018.pdf

Mohamad Jamil, P. A. S., Mohammad Yusof, N. A. D., Karuppiah, K., Rasdi, I., Jalaludin, J., Mohd Tamrin, S. B., ... Hashim, N. M. (2020). Malaysian Traffic Police in Highly Populated Areas: Is It Safe Working Outdoors on A Daily Basis? *Aerosol and Air Quality Research*, *20*(9), 2003–2011. https://doi.org/10.4209/aaqr.2020.02.0080

Mohamad, ND, Ash'aari, Z., & Othman, M. (2015). Preliminary Assessment of Air Pollutant Sources Identification at Selected Monitoring Stations in Klang Valley, Malaysia. *Procedia Environmental Sciences*, *30*, 121–126. https://doi.org/10.1016/j.proenv.2015.10.021

Mohamad, Noorlin, Latif, M. T., & Khan, M. F. (2016). Source Apportionment and Health Risk Assessment of PM10 in A Naturally Ventilated School in A Tropical Environment. *Ecotoxicology and Environmental Safety*, *124*, 351–362. https://doi.org/10.1016/j.ecoenv.2015.11.002

Mohammadyan, M., Alizadeh-Larimi, A., Etemadinejad, S., Latif, M. T., Heibati, B., Yetilmezsoy, K., & Abdul-Wahab, A. (2017). Particulate Air Pollution at Schools: Indoor-Outdoor Relationship and Determinants of Indoor Concentrations. *Aerosol and Air Quality Research*, *17*, 857–864. https://doi.org/10.4209/aaqr.2016.03.0128

Mohd Isa, K. N., Hashim, Z., Jalaludin, J., Lung Than, L. T., & Hashim, J. H. (2020). The Effects of Indoor Pollutants Exposure on Allergy and Lung Inflammation: An Activation State of Neutrophils and Eosinophils in Sputum. *International Journal of Environmental Research and Public Health*, *17*(15), 5413. https://doi.org/10.3390/ijerph17155413

Mohd Nadzir, M. S., Ooi, M. C. G., Alhasa, K. M., Abu Bakar, M. A., Ahmad Mohtar, A. A., Mohd Nor, M. F. F., ... Mohd Nor, M. Z. (2020). The Impact of Movement Control Order (MCO) during Pandemic COVID-19 on Local Air Quality in an Urban Area of Klang Valley, Malaysia. *Aerosol and Air Quality Research*, *20*, 1237–1248. https://doi.org/10.4209/aaqr.2020.04.0163

Mohd Nor Rawi, N. A., Jalaludin, J., & Chua, P. C. (2015). Indoor Air Quality and Respiratory Health among Malay Preschool Children in Selangor. *BioMed Research International*, 248178. https://doi.org/https://doi.org/10.1155/2015/248178

Mohd Shafie, S. H., & Mahmud, M. (2017). Public Perception on Traffic Pollution in Federal Territory of Kuala Lumpur, Malaysia. *Planning Malaysia*, *15*(4), 105–114. https://doi.org/10.21837/pmjournal.v15.i4.321

Montrose, L., Ward, T. J., Semmens, E. O., Cho, Y. H., Brown, B., & Noonan, C. W. (2017). Dietary Intake is Associated with Respiratory Health Outcomes and DNA Methylation in Children with Asthma. *Allergy, Asthma and Clinical Immunology*, *13*(1), 1–12. https://doi.org/10.1186/s13223-017-0187-8

Morawska, L., Thai, P. K., Liu, X., Asumadu-Sakyi, A., Ayoko, G., Bartonova, A., ... Williams, R. (2018). Applications of Low-Cost Sensing Technologies for Air Quality Monitoring and Exposure Assessment: How Far Have They Gone? *Environment International*, *116*, 286–299. https://doi.org/10.1016/j.envint.2018.04.018

Morgenstern, V., Zutavern, A., Cyrys, J., Brockow, I., Koletzko, S., Krämer, U., ... Schäfer, T. (2008). Atopic Diseases, Allergic Sensitization, and Exposure to Traffic-Related Air pollution in Children. *American Journal of Respiratory and Critical Care Medicine*, *177*(12), 1331–1337. https://doi.org/10.1164/rccm.200701-036OC

Muhamad Daud, S. A., Jalaludin, J., & Sopian, N. A. (2018). Air Pollutants Exposure and Frequency of Micronuclei (MN) among Primary School Children Nearby Industrial Area. *Malaysian Journal of Medicine and Health Sciences*, *14*(SP2), 2636–9346. Retrieved from http://www.medic.upm.edu.my/upload/dokumen/2018120408575208_MJMHS_SP_Nov_2018.pdf

Nakayama, T., Matsumi, Y., Kawahito, K., & Watabe, Y. (2018). Development and Evaluation of a Palm-Sized Optical PM2.5 Sensor. *Aerosol Science and Technology*, *52*(1), 2–12. https://doi.org/10.1080/02786826.2017.1375078

Nathan, C., & Cunningham-Bussel, A. (2013). Beyond Oxidative Stress: An Immunologist's Guide to Reactive Oxygen Species. *Nature Reviews Immunology*, *13*(5), 349–361. https://doi.org/10.1038/nri3423

National Center for Biotechnology Information. (2019). Ozone. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Ozone

Nédélec, Y., Sanz, J., Baharian, G., Szpiech, Z. A., Pacis, A., Dumaine, A., ... Barreiro, L. B. (2016). Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell*, *167*(3), 657-669.e21. https://doi.org/10.1016/j.cell.2016.09.025

Neft, I., Scungio, M., Culver, N., & Singh, S. (2016). Simulations of Aerosol Filtration by Vegetation: Validation of Existing Models with Available Lab Data and Application to Near-Roadway Scenario. *Aerosol Science and Technology*, *50*(9), 937–946. https://doi.org/10.1080/02786826.2016.1206653

Neves, A., & Brand, C. (2019). Assessing The Potential for Carbon Emissions Savings from Replacing Short Car Trips with Walking and Cycling Using A Mixed GPS-Travel Diary Approach. *Transportation Research Part A: Policy and Practice*, *123*, 130–146. https://doi.org/10.1016/j.tra.2018.08.022

NIOSH. (1994). *NMAM 0500: Particulates Not Otherwise Regulated, Total*. Retrieved from https://www.cdc.gov/niosh/docs/2003-154/pdfs/0500.pdf

NIOSH. (1998). *NMAM 0600: Particulates Not Otherwise Regulated, Respirable*.

Niu, Y., Desmarais, T. L., Tong, Z., Yao, Y., & Costa, M. (2015). Oxidative Stress Alters Global Histone Modification and DNA Methylation. *Free Radical Biology and Medicine*, *82*, 22–28. https://doi.org/10.1016/j.freeradbiomed.2015.01.028

Nucleus Medical Media. (2016). Respiratory System of Child. Retrieved November 8, 2019, from https://ebsco-smartimagebase-com.ezadmin.upm.edu.my/respiratory-system-of-child/view-item?ItemID=13299

Ohguchi, H., Hideshima, T., & Anderson, K. C. (2018). The Biological Significance of Histone Modifiers in Multiple Myeloma: Clinical Applications. *Blood Cancer Journal*, *8*(9), 1–13. https://doi.org/10.1038/s41408-018-0119-y

Othman, M., & Latif, M. T. (2020). Pollution Characteristics, Sources, and Health Risk Assessments of Urban Road Dust in Kuala Lumpur City. *Environmental Science and Pollution Research*, *27*(10), 11227–11245. https://doi.org/10.1007/s11356-020-07633-7

Othman, M., Latif, M. T., & Matsumi, Y. (2019). The Exposure of Children to PM2.5 and Dust in Indoor and Outdoor School Classrooms in Kuala Lumpur City Centre. *Ecotoxicology and Environmental Safety*, *170*, 739–749. https://doi.org/10.1016/j.ecoenv.2018.12.042

Patel, M. M., Hoepner, L., Garfinkel, R., Chillrud, S., Reyes, A., Quinn, J. W., ... Miller, R. L. (2009). Ambient Metals, Elemental Carbon, and Wheeze and Cough in New York City Children through 24 Months of Age. *American Journal of Respiratory and Critical Care Medicine*, *180*(11), 1107–1113. https://doi.org/10.1164/rccm.200901-0122OC

Paunescu, A. C., Casas, M., Ferrero, A., Pañella, P., Bougas, N., Beydon, N., ... Momas, I. (2019). Associations of Black Carbon with Lung Function and Airway Inflammation in Schoolchildren. *Environment International*, *131*, 104984. https://doi.org/10.1016/j.envint.2019.104984

Pellegrino, R., Viegi, G., Brusasco, V., Crapo, R. O., Burgos, F., Casaburi, R., ... Wanger, J. (2005). Interpretative Strategies for Lung Function Tests. *European Respiratory Journal*, *26*, 948–968. https://doi.org/10.1183/09031936.05.00035205

Peng, H., Zong, D., Zhou, Z., & Chen, P. (2016). Pulmonary Diseases and Epigenetics. In *Medical Epigenetics* (pp. 221–242). Elsevier Inc. https://doi.org/10.1016/B978-0-12-803239-8.00013-2

Petit, P. C., Fine, D. H., Vásquez, G. B., Gamero, L., Slaughter, M. S., & Dasse, K. A. (2017). The Pathophysiology of Nitrogen Dioxide During Inhaled Nitric Oxide Therapy. *ASAIO Journal*, *63*(1), 7–13. https://doi.org/10.1097/MAT.0000000000000425

Plusquin, M., Guida, F., Polidoro, S., Vermeulen, R., Raaschou-Nielsen, O., Campanella, G., ... Chadeau-Hyam, M. (2017). DNA Methylation and Exposure to Ambient Air Pollution in Two Prospective Cohorts. *Environment International*, *108*(August), 127–136. https://doi.org/10.1016/j.envint.2017.08.006

Prunicki, M., Stell, L., Dinakarpandian, D., de Planell-Saguer, M., Lucas, R. W., Hammond, S. K., ... Nadeau, K. C. (2018). Exposure to NO2, CO and PM2.5 is Linked to Regional DNA Methylation Differences in Asthma. *Clinical Epigenetics*, *10*, 2. https://doi.org/10.1186/s13148-017-0433-4

Public Works Department Malaysia. (2019). *Statistik Jalan Malaysia Edisi 2019*. (M. E. Abd Kasim, M. I. Ismail, N. S. Abdullah, H. F. Hamzah, F. Abdullah, V. Muniandy, ... Z. Hashim, Eds.) (2019th ed.). Kuala Lumpur: Cawangan Senggara Fasiliti Jalan, Ibu Pejabat JKR Malaysia.

Quincey, P. (2007). A Relationship between Black Smoke Index and Black Carbon Concentration. *Atmospheric Environment*, *41*(36), 7964–7968. https://doi.org/10.1016/j.atmosenv.2007.09.033

Rani, N. L. A., Azid, A., Khalit, S. I., Juahir, H., & Samsudin, M. S. (2018). Air Pollution Index Trend Analysis in Malaysia, 2010-15. *Polish Journal of Environmental Studies*, *27*(2), 801–808. https://doi.org/10.15244/pjoes/75964

Reddy, D., Khade, B., Pandya, R., & Gupta, S. (2017). A Novel Method for Isolation of Histones from Serum and Its Implications in Therapeutics and Prognosis of Solid Tumours. *Clinical Epigenetics*, *9*(1), 1–11. https://doi.org/https://doi.org/10.1186/s13148-017-0330-x

Ren, N., Atyah, M., Chen, W. Y., & Zhou, C. H. (2017). The Various Aspects of Genetic and Epigenetic Toxicology: Testing Methods and Clinical Applications. *Journal of Translational Medicine*, *15*(1), 110. https://doi.org/10.1186/s12967-017-1218-4

Rice, M. B., Rifas-Shiman, S. L., Litonjua, A. A., Oken, E., Gillman, M. W., Kloog, I., ... Gold, D. R. (2016). Lifetime Exposure to Ambient Pollution and Lung Function in Children. *American Journal of Respiratory and Critical Care Medicine*, *193*(8), 881–888. https://doi.org/10.1164/rccm.201506-1058OC

Rider, C. F., & Carlsten, C. (2019). Air Pollution and DNA Methylation: Effects of Exposure in Humans. *Clinical Epigenetics*, *11*(1). https://doi.org/https://doi.org/10.1186/s13148-019-0713-2

Roldán-Henao, N., Hoyos, C. D., Herrera-Mejía, L., & Isaza, A. (2020). An Investigation of The Precipitation Net Effect on The Particulate Matter Concentration in A Narrow Valley: Role of Lower-Troposphere Stability. *Journal of Applied Meteorology and Climatology*, *59*(3), 401–426. https://doi.org/10.1175/JAMC-D-18-0313.1

Saad, N. J., Patel, J., Minelli, C., & Burney, P. G. J. (2017). Explaining Ethnic Disparities in Lung Function among Young Adults: A Pilot Investigation. *PLOS ONE*, *12*(6), e0178962. https://doi.org/10.1371/journal.pone.0178962

Sadiq, S., Rizvi, N. A., Soleja, F. K., & Abbasi, M. (2019). Factors Affecting Spirometry Reference Range in Growing Children. *Pakistan Journal of Medical Sciences*, *35*(6), 1587–1591. https://doi.org/10.12669/pjms.35.6.1212

Sampson, S. (2017). Spirometry: What to Expect and How to Interpret Your Results. Retrieved November 13, 2019, from https://www.healthline.com/health/spirometry

Sarigiannis, D. A. (2019). The Exposome: A New Tool for Improved Health Risk Assessment. *Management of Emerging Public Health Issues and Risks: Multidisciplinary Approaches to the Changing Environment*, xxiii–xlv. https://doi.org/10.1016/B978-0-12-813290-6.02002-3

Schalch, T., & Steiner, F. A. (2017). Structure of Centromere Chromatin: From Nucleosome to Chromosomal Architecture. *Chromosoma*, *126*(4), 443–455. https://doi.org/10.1007/s00412-016-0620-7

Schultz, E. S., Litonjua, A. A., & Melén, E. (2017). Effects of Long-Term Exposure to Traffic-Related Air Pollution on Lung Function in Children. *Current Allergy and Asthma Reports*, *17*(6), 996–1006. https://doi.org/10.1007/s11882-017-0709-y

Schulze, F., Gao, X., Virzonis, D., Damiati, S., Schneider, M. R., & Kodzius, R. (2017). Air Quality Effects on Human Health and Approaches for Its Assessment through Microfluidic Chips. *Genes*, *8*(10). https://doi.org/https://doi.org/10.3390/genes8100244

Sharavanan, V. J., Sivaramakrishnan, M., Sivarajasekar, N., Senthilrani, N., Kothandan, R., Dhakal, N., ... Naushad, M. (2020). Pollutants Inducing Epigenetic Changes and Diseases. *Environmental Chemistry Letters*, *18*(2), 325–343. https://doi.org/10.1007/s10311-019-00944-3

Shukla, A., Bunkar, N., Kumar, R., Bhargava, A., Tiwari, R., Chaudhury, K., ... Mishra, P. K. (2019). Air Pollution Associated Epigenetic Modifications: Transgenerational Inheritance and Underlying Molecular Mechanisms. *Science of The Total Environment*, *656*, 760–777. https://doi.org/https://doi.org/10.1016/J.SCITOTENV.2018.11.381

Singh, H., Yadav, M., Kumar, N., Kumar, A., & Kumar, M. (2020). Assessing Adaptation and Mitigation Potential of Roadside Trees Under The Influence of Vehicular Emissions: A Case Study of Grevillea robusta and Mangifera indica Planted in An Urban City of India. *PLOS ONE*, *15*(1), e0227380. https://doi.org/10.1371/JOURNAL.PONE.0227380

Sofer, T., Baccarelli, A., Cantone, L., Coull, B., Maity, A., & Schwartz, J. (2013). Methylation Pattern in the Asthma Pathway. *Epigenomics*, *5*(2), 147–154. https://doi.org/10.2217/epi.13.16.Exposure

Sofwan, N. M., & Latif, M. T. (2021). Characteristics of The Real-Driving Emissions from Gasoline Passenger Vehicles in the Kuala Lumpur Urban Environment. *Atmospheric Pollution Research*, *12*(1), 306–315. https://doi.org/10.1016/j.apr.2020.09.004

Somineni, H. K., Zhang, X., Biagini Myers, J. M., Kovacic, M. B., Ulm, A., Jurcak, N., ... Ji, H. (2016). Ten-Eleven Translocation 1 (TET1) Methylation Is Associated with Childhood Asthma and Traffic-Related Air Pollution. *The Journal of Allergy and Clinical Immunology*, *137*(3), 797-805.e5. https://doi.org/10.1016/j.jaci.2015.10.021

Sopian, N. A., Jalaludin, J., Tengku Mayusi, T. Z. A., & Latif, M. T. (2020). Increased Chromosomal Damage among Children in Proximity to Industrial Zone. *Aerosol and Air Quality Research*, *20*, 944–955. https://doi.org/10.4209/aaqr.2019.12.0653

Spinelle, L., Gerboles, M., Kok, G., Persijn, S., & Sauerwald, T. (2017). Review of Portable and Low-Cost Sensors for the Ambient Air Monitoring of Benzene and Other Volatile Organic Compounds. *Sensors*, *17*(7), 1520. https://doi.org/10.3390/s17071520

Srithawirat, T., Latif, M. T., & Sulaiman, F. R. (2016). Indoor PM10 and Its Heavy Metal Composition at A Roadside Residential Environment, Phitsanulok, Thailand. *Atmósfera*, *29*(4), 311–322. https://doi.org/10.20937/ATM.2016.29.04.03

Stover, P. J., James, W. P. T., Krook, A., & Garza, C. (2018). Emerging Concepts on The Role of Epigenetics in The Relationships between Nutrition and Health. *Journal of Internal Medicine*, *284*, 37–49. https://doi.org/10.1111/joim.12768

Suhaimi, N., Jalaludin, J., & Abu Bakar, S. (2015). Association between School and Residential Air Pollutants with Respiratory Symptoms among School Children at an Industrial Area. *Advances in Environmental Biology*, *9*(26), 77–86. Retrieved from https://www.researchgate.net/publication/291833606_Association_between_School_and_Residential_Air_Pollutants_with_Respiratory_Symptoms_among_School_Children_at_an_Industrial_Area

Sulong, N. A., Latif, M. T., Khan, M. F., Amil, N., Ashfold, M. J., Abdul Wahab, M. I., ... Sahani, M. (2017). Source Apportionment and Health Risk Assessment among Specific Age Groups During Haze and Non-Haze Episodes in Kuala Lumpur, Malaysia. *Science of the Total Environment*, *601–602*, 556–570. https://doi.org/10.1016/j.scitotenv.2017.05.153

Sulong, N. A., Latif, M. T., Sahani, M., Khan, M. F., Fadzil, M. F., Tahir, N. M., ... Tohno, S. (2019). Distribution, Sources and Potential Health Risks of Polycyclic Aromatic Hydrocarbons (PAHs) in PM2.5 Collected During Different Monsoon Seasons and Haze Episode in Kuala Lumpur. *Chemosphere*, *219*, 1–14. https://doi.org/10.1016/j.chemosphere.2018.11.195

Sultan, Z., Ngadiman, N., Dela, F., Kadir, A., Roslan, N., & Moeinaddini, M. (2016). *Factor Analysis of Motorcycle Crashes in Malaysia*. *Planning Malaysia* (Vol. 14). https://doi.org/10.21837/PM.V14I4.154

Sun, B., Shi, Y., Yang, X., Zhao, T., Duan, J., & Sun, Z. (2018). DNA Methylation: A Critical Epigenetic Mechanism Underlying the Detrimental Effects of Airborne Particulate Matter. *Ecotoxicology and Environmental Safety*, *161*(June), 173–183. https://doi.org/https://doi.org/10.1016/j.ecoenv.2018.05.083

Tajudin, M. A. B. A., Khan, M. F., Mahiyuddin, W. R. W., Hod, R., Latif, M. T., Hamid, A. H., ... Sahani, M. (2019). Risk of Concentrations of Major Air Pollutants on The Prevalence of Cardiovascular and Respiratory Diseases in Urbanized Area of Kuala Lumpur, Malaysia. *Ecotoxicology and Environmental Safety*, *171*, 290–300. https://doi.org/10.1016/j.ecoenv.2018.12.057

Tan, S. Y., Praveena, S. M., Abidin, E. Z., & Cheema, M. S. (2018). Heavy Metal Quantification of Classroom Dust in School Environment and Its Impacts on Children Health from Rawang (Malaysia). *Environmental Science and Pollution Research*, *25*(34), 34623–34635. https://doi.org/10.1007/s11356-018-3396-x

Tang, C. S., Lung, S. C. C., Chang, T. Y., Tu, H. H., & Chang, L. Te. (2019). Investigation of Microenvironmental Exposures to Particle-Bound Polycyclic Aromatic Hydrocarbons for Elementary School Children. *International Journal of Environmental Research and Public Health*, *16*. https://doi.org/10.3390/ijerph16224390

Taylor-Clark, T. E., & Undem, B. J. (2010). Ozone Activates Airway Nerves via The Selective Stimulation of TRPA1 Ion Channels. *The Journal of Physiology*, *588*(3), 423–433. https://doi.org/10.1113/jphysiol.2009.183301

Teleszewski, T. J., & Gładyszewska-Fiedoruk, K. (2020). Characteristics of Humidity in Classrooms with Stack Ventilation and Development of Calculation Models of Humidity Based on The Experiment. *Journal of Building Engineering*, *31*, 101381. https://doi.org/10.1016/j.jobe.2020.101381

Tirado-Magallanes, R., Rebbani, K., Lim, R., Pradhan, S., & Benoukraf, T. (2017). Whole Genome DNA Methylation: Beyond Genes Silencing. *Oncotarget*, *8*(3), 5629–5637. https://doi.org/10.18632/oncotarget.13562

Tran, P. T. M., Ngoh, J. R., & Balasubramanian, R. (2020). Assessment of the Integrated Personal Exposure to Particulate Emissions in Urban Micro-environments: A Pilot Study. *Aerosol and Air Quality Research*, *20*(2), 341–357. https://doi.org/10.4209/aaqr.2019.04.0201

Tuck, N. W., Zaki, S. A., Hagishima, A., Rijal, H. B., Zakaria, M. A., & Yakub, F. (2019). Effectiveness of Free Running Passive Cooling Strategies for Indoor Thermal Environments: Example From A Two-Storey Corner Terrace House in Malaysia. *Building and Environment*, *160*, 106214. https://doi.org/10.1016/j.buildenv.2019.106214

UniProt. (2020). H3C1 Histone H3.1 - Homo sapiens (Human). Retrieved November 13, 2020, from https://www.uniprot.org/uniprot/P68431

United Nations. (2016). *The World's Cities in 2016 Data Booklet* ((ST/ESA/ S). Department of Economic and Social Affairs, Population Division, United Nations. Retrieved from http://www.un.org/en/development/desa/population/publications/pdf/urbanization/the_worlds_cities_in_2016_data_booklet.pdf

United Nations Development Programme. (2021). Sustainable Development Goals. Retrieved February 5, 2021, from https://www.my.undp.org/content/malaysia/en/home/sustainable-development-goals.html

United States EPA. (2015). *Best Practices for Reducing Near-Road Pollution Exposure at Schools. US EPA*. Retrieved from https://www.epa.gov/schools/basic-information-about-best-practices-reducing-near-road-pollution-exposure-schools

United States EPA. (2016a). Basic Information about Carbon Monoxide (CO) Outdoor Air Pollution | Carbon Monoxide (CO) Pollution in Outdoor Air. Retrieved November 5, 2019, from https://www.epa.gov/co-pollution/basic-information-about-carbon-monoxide-co-outdoor-air-pollution

United States EPA. (2016b). Basic Information about NO2 | Nitrogen Dioxide (NO2) Pollution. Retrieved from https://www.epa.gov/no2-pollution/basic-information-about-no2

United States EPA. (2017). Technical Overview of Volatile Organic Compounds | Indoor Air Quality (IAQ). Retrieved November 5, 2019, from https://www.epa.gov/indoor-air-quality-iaq/technical-overview-volatile-organic-compounds

United States EPA. (2019). Sulfur Dioxide Basics | Sulfur Dioxide (SO2) Pollution. Retrieved November 5, 2019, from https://www.epa.gov/so2-pollution/sulfur-dioxide-basics#what is so2

United States EPA. (2021). Particulate Matter (PM) Basics. Retrieved February 1, 2021, from https://www.epa.gov/pm-pollution/particulate-matter-pm-basics

Urman, R., Eckel, S., Deng, H., Berhane, K., Avol, E., Lurmann, F., ... Gilliland, F. (2018). Risk Effects of Near-Roadway Pollutants and Asthma Status on Bronchitic Symptoms in Children. *Environmental Epidemiology*, *2*(2), e012. https://doi.org/10.1097/ee9.0000000000000012

Urman, R., Mcconnell, R., Islam, T., Avol, E. L., Lurmann, F. W., Vora, H., ... Gauderman, W. J. (2014). Associations of Children's Lung Function with Ambient Air Pollution: Joint Effects of Regional and Near-Roadway Pollutants. *Thorax*, *69*, 540–547. https://doi.org/10.1136/thoraxjnl-2012-203159

van der Zee, S. C., Strak, M., Dijkema, M. B. A., Brunekreef, B., & Janssen, N. A. H. (2017). The Impact of Particle Filtration on Indoor Air Quality in A Classroom Near A Highway. *Indoor Air*, *27*(2), 291–302. https://doi.org/10.1111/ina.12308

Van Vliet, E. D. S., Kinney, P. L., Owusu-Agyei, S., Schluger, N. W., Ae-Ngibise, K. A., Whyatt, R. M., ... Asante, K. P. (2019). Current Respiratory Symptoms and Risk Factors in Pregnant Women Cooking with Biomass Fuels in Rural Ghana. *Environment International*, *124*, 533–540. https://doi.org/10.1016/j.envint.2019.01.046

Vrijens, K., Trippas, A. J., Lefebvre, W., Vanpoucke, C., Penders, J., Janssen, B. G., & Nawrot, T. S. (2020). Association of Prenatal Exposure to Ambient Air Pollution With Circulating Histone Levels in Maternal Cord Blood. *JAMA Network Open*, *3*(5), e205156. https://doi.org/10.1001/jamanetworkopen.2020.5156

Wang, M., Liu, F., & Zheng, M. (2020). Air Quality Improvement from COVID-19 Lockdown: Evidence from China. *Air Quality, Atmosphere and Health*, 1–14. https://doi.org/10.1007/s11869-020-00963-y

Wang, Z., Calderón, L., Patton, A. P., Sorensen Allacci, M., Senick, J., Wener, R., ... Mainelis, G. (2016). Comparison of Real-Time Instruments and Gravimetric Method When Measuring Particulate Matter in A Residential Building. *Journal of the Air & Waste Management Association*, *66*(11), 1109–1120. https://doi.org/10.1080/10962247.2016.1201022

Wargocki, P., Porras-Salazar, J. A., Contreras-Espinoza, S., & Bahnfleth, W. (2020). The Relationships between Classroom Air Quality and Children's Performance in School. *Building and Environment*, *173*, 106749. https://doi.org/10.1016/j.buildenv.2020.106749

Wendt Hess, J., Bachler, G., Momin, F., & Sexton, K. (2019). Assessing Agreement in Exposure Classification between Proximity-Based Metrics and Air Monitoring Data in Epidemiology Studies of Unconventional Resource Development. *International Journal of Environmental Research and Public Health*, *16*(17), 3055. https://doi.org/10.3390/ijerph16173055

WHO. (2013). Definition of Key Terms. Retrieved from https://www.who.int/hiv/pub/guidelines/arv2013/intro/keyterms/en/

Winck, F. V., Ribeiro, A. C. P., Domingues, R. R., Ling, L. Y., Riaño-Pachón, D. M., Rivera, C., ... Leme, A. F. P. (2015). Insights into Immune Responses in Oral Cancer Through Proteomic Analysis of Saliva and Salivary Extracellular Vesicles. *Scientific Reports*, *5*. https://doi.org/10.1038/srep16305

Winkler, S., Anderson, J., Garza, L., Ruona, W., Vogt, R., & Wallington, T. (2018). Vehicle Criteria Pollutant (PM, NOx, CO, HCs) Emissions: How Low Should We Go? *Npj Climate and Atmospheric Science*, *1*, 26. https://doi.org/10.1038/s41612-018-0037-5

Wright, R. J., & Brunst, K. J. (2013). Programming of Respiratory Health in Childhood: Influence of Outdoor Air Pollution. *Current Opinion in Pediatrics*, *25*(2), 232–239. https://doi.org/https://doi.org/10.1097/MOP.0b013e32835e78cc

Yang, J., Chang, Y., & Yan, P. (2015). Ranking The Suitability of Common Urban Tree Species for Controlling PM2.5 Pollution. *Atmospheric Pollution Research*, *6*(2), 267–277. https://doi.org/10.5094/APR.2015.031

Yang, M., Guo, Y. M., Bloom, M. S., Dharmagee, S. C., Morawska, L., Heinrich, J., ... Dong, G. H. (2020). Is PM1 Similar to PM2.5? A New Insight into The Association of PM1 and PM2.5 with Children's Lung Function. *Environment International*, *145*, 106092. https://doi.org/10.1016/j.envint.2020.106092

Yang Razali, N. Y., Latif, M. T., Dominick, D., Mohamad, N., Sulaiman, F. R., & Srithawirat, T. (2015). Concentration of Particulate Matter, CO and CO2 in Selected Schools in Malaysia. *Building and Environment*, *87*, 108–116. https://doi.org/10.1016/j.buildenv.2015.01.015

Yarza, S., Hassan, L., Shtein, A., Lesser, D., Novack, L., Katra, I., ... Novack, V. (2020). Novel Approaches to Air Pollution Exposure and Clinical Outcomes Assessment in Environmental Health Studies. *Atmosphere*, *11*(2), 122. https://doi.org/10.3390/atmos11020122

Yen, Y., Yang, C., Mena, K., Cheng, Y., & Chen, P. (2019). Cooking/Window Opening and Associated Increases of Indoor PM2.5 and NO2 Concentrations of Children's Houses in Kaohsiung, Taiwan. *Applied Sciences*, *9*(20), 4306. https://doi.org/10.3390/app9204306

Yi, S.-J., Shon, C., Min, K.-D., Kim, H.-C., Leem, J.-H., Kwon, H.-J., ... Kim, S.-Y. (2017). Association between Exposure to Traffic-Related Air Pollution and Prevalence of Allergic Diseases in Children, Seoul, Korea. *BioMed Research International*. https://doi.org/10.1155/2017/4216107

Yoda, Y., Takagi, H., Wakamatsu, J., Ito, T., Nakatsubo, R., Horie, Y., ... Shima, M. (2017). Acute Effects of Air Pollutants on Pulmonary Function among Students: A Panel Study in An Isolated Island. *Environmental Health and Preventive Medicine*, *22*(1), 33. https://doi.org/10.1186/s12199-017-0646-3

Zainudin, M. A., Jalaludin, J., & Sopian, N. A. (2019). Indoor Air Quality (IAQ) in Preschools and Its Association with Respiratory Inflammation among Pre-schoolers. *Malaysian Journal of Medicine and Health Sciences*, *15*(SP4), 2636–9346.

Zakaria, M., Ezani, E., Hassan, N., Ramli, N., & Wahab, M. (2019). Traffic-Related Air Pollution (TRAP), Air Quality Perception and Respiratory Health Symptoms of Active Commuters in a University Outdoor Environment. *IOP Conf. Series: Earth and Environmental Science*, *228*, 012017. https://doi.org/10.1088/1755-1315/228/1/012017

Zaki, S. A., Azid, N. S., Shahidan, M. F., Hassan, M. Z., Md Daud, M. Y., Abu Bakar, N. A., ... Yakub, F. (2020). Analysis of Urban Morphological Effect on The Microclimate of The Urban Residential Area of Kampung Baru in Kuala Lumpur Using A Geospatial Approach. *Sustainability*, *12*(18). https://doi.org/10.3390/SU12187301

Zamri, M., Ismail, M., & Kim, Y. (2012). Mapping the Spatial Distribution of Criteria Air Pollutants in Peninsular Malaysia Using Geographical Information System (GIS). In *Air Pollution - Monitoring, Modelling and Health*. InTech. https://doi.org/10.5772/32902

Zhang, Y., & Kutateladze, T. G. (2018). Diet and The Epigenome. *Nature Communications*, *9*, 3375. https://doi.org/10.1038/s41467-018-05778-1

Zhang, Z., Chen, L., Xing, X., Li, D., Gao, C., He, Z., ... Chen, W. (2016). Specific Histone Modifications Were Associated with the PAH-Induced DNA Damage Response in Coke Oven Workers. *Toxicology Research*, *5*(4), 1193–1201. https://doi.org/10.1039/c6tx00112b

Zheng, Y., Sanchez-Guerra, M., Zhang, Z., Joyce, B. T., Zhong, J., Kresovich, J. K., ... Hou, L. (2017). Traffic-Derived Particulate Matter Exposure and Histone H3 Modification: A Repeated Measures Study. *Environmental Research*, *153*(December 2016), 112–119. https://doi.org/10.1016/j.envres.2016.11.015

Zimmermann, K. A. (2019). Respiratory System: Facts, Function and Diseases. Retrieved November 12, 2019, from https://www.livescience.com/22616-respiratory-system.html

Zordoky, B., & El-Kadi, A. (2009). Role of NF-kappaB in the Regulation of Cytochrome P450 Enzymes. *Current Drug Metabolism*, *10*(2), 164–178. https://doi.org/10.2174/138920009787522151

Zou, B., Zheng, Z., Wan, N., Qiu, Y., & Wilson, J. G. (2016). An Optimized Spatial Proximity Model for Fine Particulate Matter Air Pollution Exposure Assessment in Areas of Sparse Monitoring. *International Journal of Geographical Information Science*, *30*(4), 727–747. https://doi.org/10.1080/13658816.2015.1095921

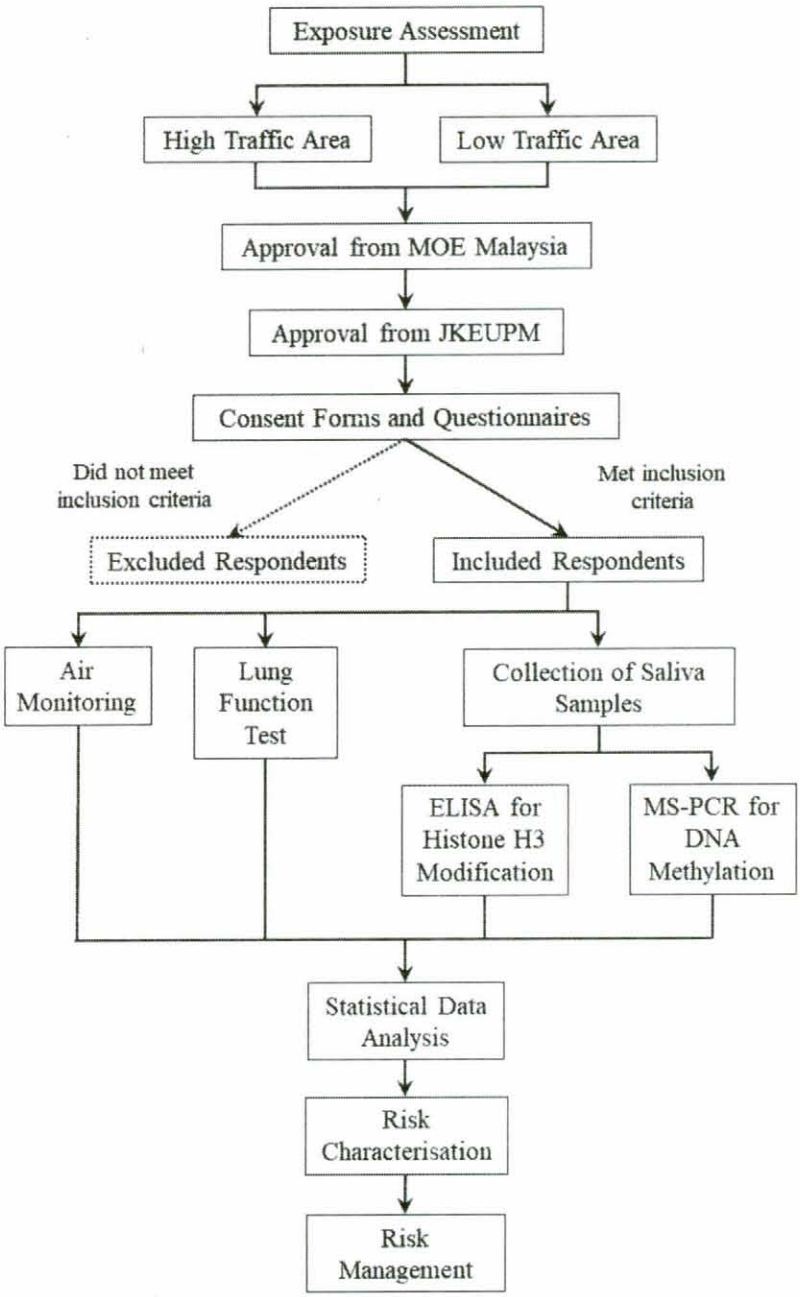
APPENDICES

APPENDIX 1
GANTT CHART

Year	2018												2019												2020												2021							
Project (Activities)	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Instruments and Methods Preparation, and Proposal Writing																																											
2. Presentation and Ethical Approval																																											
3. Execution of Exposure to Air Pollutants and Outcome Assessment																																											
4. Collection of Biomarkers Samples and Outcome Assessment																																											
5. Data and Sample Analysis																																											
6. Producing Thesis and Publication																																											

Year	2018												2019												2020												2021							
Project (Milestone)	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Complete Instruments and Methods Preparation, and Proposal Writing						♦																																					
2. Complete Presentation and Ethical Approval											♦																																	
3. Complete Execution of Exposure to Air Pollutants and Outcome Assessment																									♦																			
4. Complete Collection of Biomarkers Samples and Outcome Assessment																									♦																			
5. Complete Data Analysis																																			♦								
6. Complete Thesis and Publication																																											♦

APPENDIX 2
FLOW CHART OF RESEARCH



Appendix 2: Flow chart of research

APPENDIX 3
MOE APPROVAL LETTER

KEMENTERIAN PENDIDIKAN MALAYSIA
BAHAGIAN PERANCANGAN DAN PENYELIDIKAN DASAR PENDIDIKAN
ARAS 1-4, BLOK E8
KOMPLEKS KERAJAAN PARCEL E
PUSAT PENTADBIRAN KERAJAAN PERSEKUTUAN
62604 PUTRAJAYA

TEL : 0388846591
FAKS : 0388846579

Ruj. Kami : KPM.600-3/2/3-eras(1366)
Tarikh : 9 Julai 2018

NUR FASEEHA BINTI SUHAIMI
NO. KP : 910801146118

JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN, FAKULTI PERUBATAN DAN SAINS KESIHATAN,
UNIVERSITI PUTRA MALAYSIA 43400 UPM SERDANG
SELANGOR

Tuan,

KELULUSAN BERSYARAT UNTUK MENJALANKAN KAJIAN :
GLOBAL HISTONE H3 MODIFICATIONS AS EPIGENETIC BIOMARKER TO TRAFFIC-RELATED AIR POLLUTION (TRAP) AMONG PRIMARY SCHOOL CHILDREN IN KLANG VALLEY

Perkara di atas adalah dirujuk.

2. Sukacita dimaklumkan bahawa permohonan tuan untuk menjalankan kajian seperti di bawah telah diluluskan dengan syarat :

" KELULUSAN INI BERGANTUNG KEPADA PERTIMBANGAN PENTADBIR SEKOLAH. AKTIVITI PENGUTIPAN DATA TIDAK BOLEH MENGGANGGU PENGAJARAN DAN PEMBELAJARAN MURID. KEMENTERIAN PENDIDIKAN MALAYSIA TIDAK AKAN BERTANGGUNGJAWAB TERHADAP KESELAMATAN PERALATAN YANG AKAN DIGUNAKAN UNTUK PENGUTIPAN DATA DI SEKOLAH "

3. Kelulusan adalah berdasarkan kepada kertas cadangan penyelidikan dan instrumen kajian yang dikemukakan oleh tuan kepada bahagian ini. Walau bagaimanapun kelulusan ini bergantung kepada kebenaran Jabatan Pendidikan Negeri dan Pengetua / Guru Besar yang berkenaan.

4. Surat kelulusan ini sah digunakan bermula dari **15 Oktober 2018** hingga **5 April 2019**

5. Tuan dikehendaki menyerahkan senaskhah laporan akhir kajian dalam bentuk *hardcopy* bersama salinan *softcopy* berformat pdf dalam CD kepada Bahagian ini.Tuan juga diingatkan supaya mendapat kebenaran terlebih dahulu daripada Bahagian ini sekiranya sebahagian atau sepenuhnya dapatan kajian tersebut hendak diterbitkan di mana-mana forum, seminar atau diumumkan kepada media massa.

Sekian untuk makluman dan tindakan tuan selanjutnya. Terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menurut perintah,

Ketua Sektor
Sektor Penyelidikan dan Penilaian
b.p. Pengarah
Bahagian Perancangan dan Penyelidikan Dasar Pendidikan
Kementerian Pendidikan Malaysia

salinan kepada:-

JABATAN PENDIDIKAN SELANGOR
JABATAN PENDIDIKAN WILAYAH PERSEKUTUAN KUALA LUMPUR

* SURAT INI DIJANA OLEH KOMPUTER DAN TIADA TANDATANGAN DIPERLUKAN *

APPENDIX 4
ETHICAL APPROVAL LETTERS

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

Ref. : UPM/TNCPI/RMC/1.4.18.2 (JKEUPM)
Date : 19 December 2018

Assoc Prof. Dr. Juliana Jalaludin
Department of Environmental and Occupational Health
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
Serdang, Selangor

Dear Madam/Sir,

RESEARCH PROJECT: GLOBAL HISTONE H3 MODIFICATIONS AS EPIGNETIC BIOMARKER TO TRAFFIC-RELATED AIR POLLUTION (TRAP) AMONG PRIMARY SCHOOL CHILDREN IN KLANG VALLEY

RESEARCHER : NUR FASEEHA BINTI SUHAIMI
SUPERVISOR : ASSOC PROF. DR. JULIANA JALALUDIN

The Ethics Committee for Research involving Human Subjects of University Putra Malaysia (JKEUPM) has studied the proposal for the above project and found that there were no objectionable ethical issues involved in the proposed study.

Please find the list of documents received and reviewed with reference to the study and committee members who reviewed the documents (as attached).

Notwithstanding above, we will not be responsible for any misconduct on the part of researcher in the course of carrying out the research.

Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

Thank you.

"WITH KNOWLEDGE WE SERVE"

Sincerely yours,

PROF. DR. ZAMBERI SEKAWI
Chair
Ethics Committee for Research involving Human Subjects
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-8947 1002, Pejabat Pentadbiran TNCPI 603-8947 1608, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-8947 1601, Pejabat Pengarah, Putra Science Park (PSP) 603-8947 1291
http://www.tncpi.upm.edu.my

ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS
(JKEUPM)
UNIVERSITI PUTRA MALAYSIA

Research title	:	**Global Histone H3 Modifications as Epignetic Biomarker To Traffic-Related Air Pollution (TRAP) Among Primary School Children In Klang Valley**
Study Site	:	**Klang Valley**
JKEUPM Ref No.	:	**JKEUPM-2018-278**
Researcher	:	**Nur Faseeha binti Suhaimi**
Supervisor	:	**Assoc Prof. Dr. Juliana Jalaludin**

Documents received and reviewed with reference to the above study:

1. Ethics Application Form, Version 1 dated 15/8/2018
2. Respondent Information Sheet & Guardian's/Parent's Consent (English), Version 3 dated 10/12/2018
3. Respondent Information Sheet & Guardian's/Parent's Consent (Malay), Version 3 dated 10/12/2018
4. Respondent Information Sheet & Consent (Malay), Version 2 dated 17/10/2018
5. Proposal (English), Version 1 dated 15 /8/2018
6. Questionnaires/ Interviews (Malay), Version 1 dated 15/8/2018
7. Curriculum Vitae of:
    a. Assoc Prof. Dr. Juliana Jalaludin
    b. Dr. Suhaili Abu Bakar @ Jamaludin
    c. Dr. Nor Eliani Ezani
    d. Prof. Dr. Mohd Talib Latif
    e. Dr. Titi Rahmawati Hamedon
    f. Mr. Mohd Nasrul Che Hussin

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.

Decision by JKEUPM:

[X] Approved

[ ] **Permission MUST BE OBTAINED from the respective hospitals/ institutions before conducting the research**

[ ] Disapproved

APPENDIX 5
EXPLANATION LETTER AND CONSENT FORM

BORANG 2.5: PENERANGAN DAN PERSETUJUAN IBU BAPA/PENJAGA

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN

Pengubahsuaian Epigenetik dalam Kalangan Kanak-kanak Sekolah yang Terdedah kepada Pencemaran Udara Berkaitan Trafik di Lembah Klang

2. PENGENALAN

Pembangunan ekonomi yang pesat seperti pembangunan industri, kepadatan penduduk dan peningkatan bilangan kenderaan menyebabkan kawasan Lembah Klang menghadapi masalah kualiti udara yang tidak sihat berbanding kawasan lain di Malaysia. Penyebab utama pencemaran udara di Lembah Klang adalah dari pelepasan asap kenderaan, yang membentuk 70% daripada sumber pencemaran udara. Tujuan kajian ini adalah untuk menilai hubungan antara besarnya pendedahan kepada pencemaran udara berkaitan trafik dengan sistem pernafasan dalam kalangan kanak-kanak sekolah yang tinggal berdekatan dengan lebuh raya utama di Lembah Klang. Manakala kanak-kanak di kawasan luar Lembah Klang akan dijadikan kumpulan perbandingan. Pencemaran udara dapat menjejaskan semua golongan yang terdedah, tetapi hanya kanak-kanak sahaja yang akan diberi fokus dalam kajian ini kerana pencemaran udara dapat menghalang pertumbuhan paru-paru kanak-kanak. Kajian terdahulu telah menunjukkan bahawa kanak-kanak yang menghadiri sekolah berhampiran trafik sibuk mungkin terdedah kepada risiko kerosakan DNA berbanding kanak-kanak yang menghadiri sekolah di kawasan trafik kurang sibuk. Perhatian terhadap isu alam sekitar ini dapat membantu dalam mengurangkan masalah pernafasan dalam kalangan kanak-kanak sekolah. Ujian untuk mengetahui tahap kesihatan sistem pernafasan kanak-kanak akan dijalankan untuk menilai kesan pendedahan kanak-kanak ini terhadap pendedahan kepada pencemaran udara berkaitan trafik.

3. APAKAH YANG PERLU ANDA/ANAK/JAGAAN ANDA LAKUKAN?

Ibu bapa/penjaga kanak-kanak dikehendaki membaca dan memahami Borang 2.5 (Penerangan dan Persetujuan Ibu Bapa/Penjaga) ini sebelum mengisi borang soal selidik. Jika ibu bapa/penjaga bersetuju untuk membenarkan anak/jagaan mengambil bahagian dalam kajian ini, ibu bapa/penjaga perlu menandatangani perkara #9 di halaman 5 borang ini. Seterusnya, borang soal selidik perlu diisi dengan lengkap oleh ibu bapa/penjaga serta dikembalikan kepada guru sekolah. Pengisian soal selidik akan mengambil masa kira-kira 10 minit.

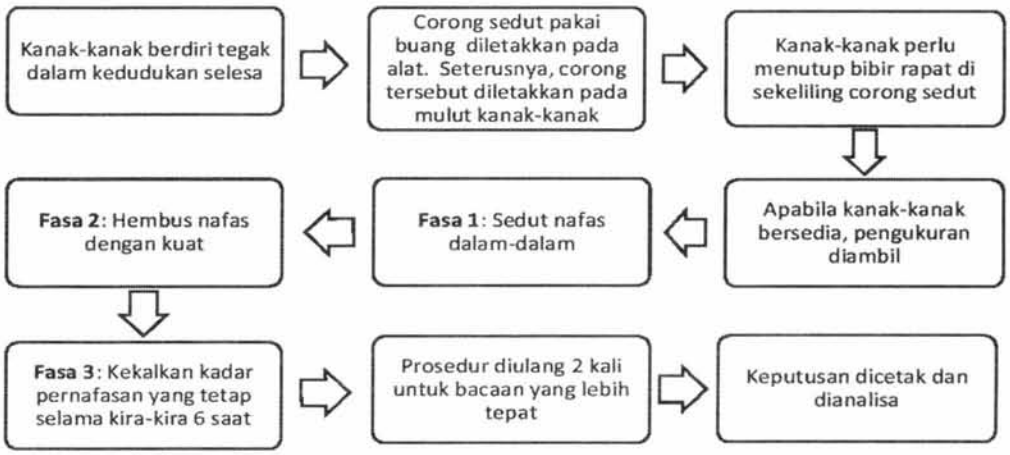
Sila maklum bahawa kanak-kanak diberi pilihan untuk terus menyertai kajian atau menarik diri pada bila-bila masa sahaja tanpa memberi sebarang sebab. Bagi menjaga kerahsiaan maklumat yang diberikan, ibu bapa/penjaga dikehendaki untuk mengembalikan soal selidik di dalam sampul yang diberikan, serta menurunkan tanda tangan pada penutup sampul yang telah digam pada belakang sampul surat tersebut. Penyelidik akan mengambil soal selidik yang dikembalikan kepada guru sekolah. Selepas proses penyaringan dijalankan dalam kalangan kanak-kanak yang mendapat kebenaran ibu bapa/penjaga, ujian fungsi paru-paru dan pengumpulan sampel air liur akan dijalankan di sekolah mengikut masa yang dipersetujui pihak sekolah. Prosedur ujian fungsi paru-paru dan pengambilan sampel kanak-kanak akan dijalankan oleh pembantu pegawai perubatan yang diiktiraf kelayakannya. Pembantu pegawai perubatan akan diselia oleh Pegawai Perubatan yang diiktiraf dan bertanggungjawab dalam membuat sebarang keputusan berkaitan perubatan dan menyemak teknik, peralatan serta prosedur yang akan digunakan.

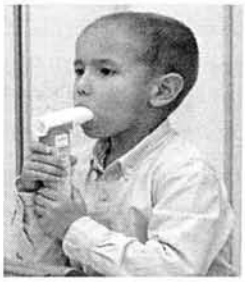
Kajian ini melibatkan 3 prosedur :

1) Pengukuran berat badan dan tinggi
2) Ujian fungsi paru-paru
3) Pengambilan sampel air liur

A) <u>Ujian Fungsi Paru - paru</u>

Ujian fungsi paru-paru akan diukur dengan menggunakan alat yang direka khas untuk merekodkan corak pernafasan. Kanak-kanak akan diminta untuk tidak melakukan aktiviti kecergasan dan tidak makan sekurang-kurangnya 30 minit sebelum ujian tersebut. Berikut merupakan prosedur ujian fungsi paru-paru mengikut *American Thoracic Society*:





Gambar 1: Corong sedut diletakkan pada mulut kanak-kanak

B) <u>Pengambilan Sampel Air Liur</u>

Sampel air liur akan dikumpul di dalam bekas sampel. Sebelum pengumpulan air liur, kanak-kanak perlu berkumur dengan air paip. Kemudian, kanak-kanak akan diminta untuk mengeluarkan air liur ke dalam bekas sampel sehingga kira-kira 10 mL air liur terkumpul.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

- Kanak-kanak yang tidak berumur antara 7 hingga 11 tahun semasa tahun persekolahan 2019
- Kanak-kanak yang bukan warganegara Malaysia
- Kanak-kanak yang ibu bapa/penjaganya enggan mengisi borang soal selidik sepenuhnya
- Kanak-kanak yang mempunyai sejarah alahan yang disahkan oleh doktor, atau penyakit pernafasan kronik seperti asma

- Kanak-kanak yang pernah menjalani pembedahan di bahagian abdomen (badan), mempunyai jantung yang tidak stabil atau pernah mengalami jangkitan pernafasan yang teruk seperti pneumonia, tuberkulosis dan sebagainya dalam tempoh 6 bulan sebelum tarikh sampel akan diambil

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANAK/JAGAAN SAYA SEBAGAI PESERTA?

Kajian ini akan membantu menentukan kualiti udara di sekolah rendah dan hubungannya dengan status kesihatan pernafasan kanak-kanak. Sekiranya kepekatan bahan pencemar udara tinggi dan dianggap memberi kesan kesihatan kepada kanak-kanak, tindakan susulan akan diambil berdasarkan hasil kajian ini. Selain itu, kajian ini juga akan membantu ibu bapa/penjaga dalam mengetahui tahap kesihatan sistem pernafasan anak/jagaan masing-masing. Kajian ini dapat memberikan langkah pencegahan awal kepada anak/jagaan yang terdedah kepada pencemaran udara berkaitan trafik. Ibu bapa/penjaga akan dimaklumkan tentang apa-apa keputusan yang tidak normal supaya tindakan yang sesuai dan langkah berjaga-jaga dapat diambil terhadap kesihatan anak/jagaan anda. Semua ujian yang terlibat dalam kajian ini akan dijalankan tanpa dikenakan bayaran kepada ibu bapa/penjaga.

b) KEPADA PENYELIDIK?

Secara keseluruhannya, hasil kajian ini akan menyumbang kepada komuniti dan negara. Langkah awal dalam mengurangkan risiko kesihatan dalam kalangan kanak-kanak yang bersekolah berdekatan dengan lebuh raya utama terutamanya di Lembah Klang dapat diperolehi. Agensi-agensi kerajaan boleh menggunakan data yang disediakan daripada penyelidikan ini untuk menyediakan pelan tindakan seperti program kesedaran berkaitan pencemaran udara trafik dan program kesihatan di sekolah bagi meningkatkan status kesihatan kanak-kanak yang terdedah kepada pencemaran udara trafik. Selain itu, hasil kajian ini dapat menyumbang kepada penjagaan perubatan, rawatan dan pencegahan masalah untuk golongan lain yang berisiko tinggi di masa hadapan seperti wanita mengandung dan warga emas.

6. ADAKAH KAJIAN INI BERISIKO?

Tahap risiko yang mungkin dikaitkan dengan kajian ini adalah rendah. Ujian fungsi paru-paru tidak membahayakan. Prosedur tersebut tidak mendatangkan sebarang kecederaan secara langsung terhadap kanak-kanak yang terlibat. Walau bagaimanapun, kerana ujian ini mungkin memerlukan kanak-kanak menarik dan mengeluarkan nafas dengan cepat, maka risiko yang mungkin terjadi termasuklah pening, sesak nafas atau batuk. Di samping itu, prosedur pengambilan air liur juga selamat. Hal ini demikian kerana prosedur pengambilan sampel air liur tidak melibatkan belahan atau memasukkan alatan ke dalam badan kanak-kanak. Setiap kanak-kanak akan diberikan alat persampelan masing-masing tanpa perkongsian dengan kanak-kanak lain. Penyertaan kanak-kanak akan dihentikan jika kanak-kanak enggan meneruskan kajian. Tiada pampasan yang akan diberikan kepada kanak-kanak kerana kajian ini bukan kajian klinikal dan tiada rawatan yang diberikan kepada kanak-kanak.

7. ADAKAH MAKLUMAT DAN IDENTITI ANAK/JAGAAN SAYA KEKAL RAHSIA?

Ya. Kami akan mengambil langkah-langkah keselamatan untuk melindungi kerahsiaan maklumat dan identiti anak/jagaan anda. Identiti dan maklumat yang diberikan dalam soal selidik, wawancara dan ujian akan tetap sulit termasuklah sekiranya keputusan kajian diterbitkan. Sebarang maklumat dan sampel yang dikumpul akan dilabelkan tanpa nama mengikut ID tertentu supaya tidak dapat dikenal pasti secara peribadi, dan hanya dapat diakses oleh penyelidik sahaja. Seterusnya borang penerangan dan persetujuan responden yang telah diisi dan ditandatangani akan disimpan di dalam almari besi berkunci di makmal

Kesihatan Persekitaran di Fakulti Perubatan dan Sains Kesihatan. Maklumat yang diperoleh akan digunakan untuk tujuan kajian ini sahaja. Maklumat peribadi yang diberikan akan dimusnahkan pada akhir kajian (dianggarkan pada akhir tahun 2020), manakala sampel biologi dan data yang dijana semasa kajian akan diarkibkan untuk tujuan penyelidikan lanjut. Maklumat peribadi setiap individu yang terlibat di dalam kajian ini tidak akan didedahkan di dalam mana-mana bahagian penyelidikan dan penerbitan kelak. Rekod kertas akan dihapuskan dengan mesin *shredder* dan dikitar semula, manakala rekod elektronik akan dihapuskan secara kekal.

8. SIAPA YANG PERLU SAYA HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEPANJANG PENYELIDIKAN INI?

Penyelidik hanya berfungsi sebagai penyiasat, dan bukan pengamal perubatan. Sekiranya anda mempunyai sebarang soalan mengenai kajian ini, anda boleh menghubungi:

Ketua Projek
Prof. Madya Dr. Juliana Jalaludin
Jabatan Kesihatan Persekitaran dan Pekerjaan, Fakulti Perubatan dan Sains Kesihatan,
Universiti Putra Malaysia (UPM) Serdang
Tel: 03-89472397/03-89472401
Emel: juliana@upm.edu.my

Penyelidik Kajian
Nur Faseeha Binti Suhaimi
Jabatan Kesihatan Persekitaran dan Pekerjaan,
Fakulti Perubatan dan Sains Kesihatan,
Universiti Putra Malaysia (UPM) Serdang.
Tel: 013-6264050
E-mel : gs50755@student.upm.edu.my

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini

_____

9. PERSETUJUAN

Saya ..... No. Kad Pengenalan .....

beralamat.....

.....

dengan ini secara sukarela bersetuju membenarkan ***anak/jagaan** saya ..... menyertai penyelidikan yang tersebut di atas ***(soal selidik/temuduga/ujian fungsi paru-paru/pengambilan sampel air liur).**

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti yang tercatat dalam Helaian Penerangan). Saya memahami bahawa ***anak/jagaan** saya berhak menarik diri daripada penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan. Saya memahami juga bahawa sebarang maklumat yang berkaitan identiti ***anak /jagaan** saya akan dirahsiakan.

Saya ***berminat/tidak berminat** untuk mengetahui keputusan kajian yang melibatkan ***anak/jagaan** saya. (Hanya keputusan ujian fungsi paru-paru sahaja yang akan dimaklumkan. Terdapat kemungkinan hasil kajian yang diberi terbatas dari segi tafsirannya)
Saya ***setuju/tidak bersetuju** untuk imej/gambar/rakaman video/ rakaman suara berkaitan dengan ***anak/jagaan** saya digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

Tandatangan: .....
(Ibu/Bapa/Penjaga)

Tarikh: .....

Tandatangan: .....
(Saksi)

Nama: .....

No. K/P: .....

***potong yang tidak berkenaan**

Saya mengesahkan bahawa saya telah menerangkan kepada ibubapa/penjaga responden mengenai sifat dan tujuan penyelidikan tersebut di atas.

Tarikh: .....

Tandatangan: .....
(Penyelidik)

APPENDIX 6
ADAPTED QUESTIONNAIRE

UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN
FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA

BORANG SOAL SELIDIK

TAJUK:

Pengubahsuaian Epigenetik dalam Kalangan Kanak-kanak Sekolah yang Terdedah kepada Pencemaran Udara Berkaitan Trafik di Lembah Klang

TARIKH SOAL SELIDIK DILENGKAPKAN:

		/			/				
Hari			Bulan			Tahun			

TARIKH PERSAMPELAN (diisi oleh penyelidik):

		/			/				
Hari			Bulan			Tahun			

Tandakan √ pada yang berkenaan

SOAL SELIDIK DILENGKAPKAN OLEH:

1. Ibu kanak-kanak
2. Bapa kanak-kanak
3. Penjaga kanak-kanak. Nyatakan. _____

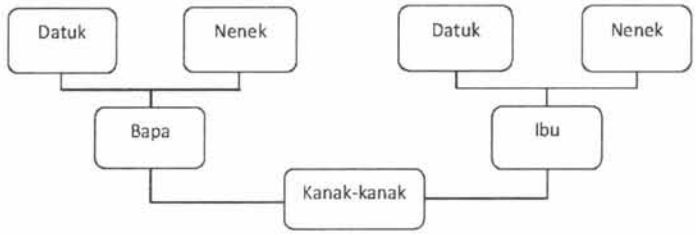
Terima kasih atas kesudian anda untuk menyertai penyelidikan saintifik ini. Kerjasama anda untuk memberikan jawapan yang jujur dan tepat amat diperlukan bagi menjayakan kajian ini. Selepas mengisi soal selidik ini, anda dikehendaki mengembalikan soal selidik dalam sampul yang diberikan serta menurunkan tandatangan di atas pembuka sampul yang telah digam pada belakang sampul tersebut.

Semua maklumat yang diperoleh dalam kajian ini akan dirahsiakan dan hanyalah untuk tujuan penyelidikan kesihatan semata-mata.

BAHAGIAN A: BUTIRAN DIRI (KANAK-KANAK DAN WARIS)

A1. BUTIRAN DIRI KANAK-KANAK

1. Jantina : a. Lelaki ☐ b. Perempuan ☐
2. Tarikh Lahir : _____ (Hari/Bulan/Tahun)
3. Sudah berapa lama tinggal di alamat sekarang : ☐☐ tahun ☐☐ bulan
4. Tahun berdaftar di sekolah ini : ☐☐☐☐
5. Keturunan **(sila isikan di dalam petak di bawah)**
   Melayu: **M** Cina : **C** India: **I** Lain lain: **O**



A2. BUTIRAN DIRI WARIS (sila isikan yang berkenaan untuk yang masih hidup sahaja)

1. Pendidikan Bapa/Penjaga Lelaki
   a. Tidak Bersekolah ☐
   b. Sekolah Rendah ☐
   c. Tingkatan 3 ☐
   d. Tingkatan 5 ☐
   e. Sijil ☐
   f. STPM/Diploma ☐
   g. Ijazah ☐
   h. Sarjana ☐
   i. Doktor Falsafah ☐
   j. Lain-lain. Nyatakan _____ ☐

2. Pendidikan Ibu/Penjaga Perempuan
   a. Tidak Bersekolah ☐
   b. Sekolah Rendah ☐
   c. Tingkatan 3 ☐
   d. Tingkatan 5 ☐
   e. Sijil ☐
   f. STPM/Diploma ☐
   g. Ijazah ☐
   h. Sarjana ☐
   i. Doktor Falsafah ☐
   j. Lain-lain. Nyatakan _____ ☐

3. Pekerjaan Bapa/Penjaga Lelaki
   a. Majikan ☐
   b. Kakitangan Kerajaan ☐
   c. Kakitangan Swasta ☐
   d. Bekerja Sendiri ☐
   e. Tidak Bekerja ☐
   f. Pesara ☐

4. Pekerjaan Ibu/Penjaga Perempuan
   a. Majikan ☐
   b. Kakitangan Kerajaan ☐
   c. Kakitangan Swasta ☐
   d. Bekerja Sendiri ☐
   e. Tidak Bekerja ☐
   f. Pesara ☐

5. Anggaran pendapatan kasar sebulan
   a. Bapa/Penjaga Lelaki RM _____ b. Ibu/Penjaga Perempuan RM _____

BAHAGIAN B: STATUS KESIHATAN

	YA	TIDAK
1. Adakah kanak-kanak ini pernah menjalani rawatan di bawah dalam tempoh berkenaan:		
a) Kemoterapi (dalam tempoh 6 bulan lepas)	☐	☐
b) Radioterapi (dalam tempoh 6 bulan lepas)	☐	☐

	YA	TIDAK
c) X-ray (dalam tempoh 3 bulan lepas)		
d) Lain-lain. Nyatakan: _____		
2. Adakah kanak-kanak ini menghidap penyakit kronik? **Jika YA**, sila nyatakan: _____		
3. Adakah ahli keluarga terdekat (ibu, bapa, adik-beradik, atuk, nenek) menghidap penyakit kronik? **Jika YA**, sila nyatakan:		

Penyakit: _____ Siapa: _____

BAHAGIAN C: SEJARAH KESIHATAN RESPIRATORI

C1. BATUK

		YA	TIDAK
1.	Adakah anak anda kerap mengalami batuk berserta selesema?		
2.	Adakah anak anda kerap mengalami batuk sahaja tanpa selesema?		
Jika YA untuk Soalan 1 atau/dan 2			
3.	Adakah anak anda batuk pada kebanyakan hari (≥ 4 hari dalam seminggu) selama 3 bulan berturut-turut dalam setahun?		
4.	Sudah berapa tahunkah anak anda mengalami batuk seperti ini?		tahun

C2. KAHAK

		YA	TIDAK
1.	Adakah anak anda kerap mengalami sesak nafas atau mengeluarkan kahak berserta selesema? **Jika YA sila jawab soalan seterusnya.**		
2.	Adakah anak anda mengalami sesak nafas dan mengeluarkan kahak pada kebanyakan hari (≥ 4 hari dalam seminggu) selama 3 bulan berturut-turut dalam setahun?		
3.	Sudah berapa tahunkah anak anda mengalami kahak seperti ini?		tahun
4.	Adakah anak anda pernah mengalami serangan batuk, sesak nafas atau berkahak selama seminggu atau lebih dalam masa setahun yang lalu?		
5.	**Jika YA (Soalan 4),** sudah berapa lamakah masalah ini berlaku?		

C3. DADA BERBUNYI

		YA	TIDAK
1.	Adakah anak anda selalu mengalami masalah pernafasan berbunyi di bahagian dada:		
	• semasa mengalami selesema?		
	• semasa tidak mengalami selesema?		
Jika YA untuk Soalan 1			
2.	Sudah berapa lamakah anak anda mengalami masalah dada berbunyi ini?		tahun
3.	Pernahkah anak anda mengalami serangan dada berbunyi sehingga menyebabkan dia mengalami masalah sesak nafas?		
4.	Adakah anak anda pernah mengalami masalah ini setelah anak anda melakukan aktiviti seperti senaman atau latihan kecergasan?		

C4. KESAKITAN DADA

		YA	TIDAK
1.	Sejak 3 tahun lepas, adakah anak anda pernah mengalami kesesakan bahagian dada yang menghalang anak anda daripada melakukan aktiviti biasa selama 3 hari? **Jika YA sila jawab soalan seterusnya.**		
2.	Adakah anak anda mengeluarkan kahak atau mengalami kesesakan nafas yang lain daripada keadaan biasa selain mengalami penyakit ini?		
3.	Adakah anak anda pernah dimasukkan ke hospital kerana mengalami masalah jangkitan di dada yang serius sebelum berumur 2 tahun?		

C5. ALERGI/ALAHAN

		YA	TIDAK
1.	Adakah doktor pernah mengatakan bahawa anak anda mengalami alahan terhadap debu?		
2.	Adakah doktor pernah mengatakan bahawa kulit anak anda mengalami alahan terhadap detergen atau bahan kimia tertentu?		
3.	Adakah anak anda mengambil suntikan untuk mengurangkan masalah alahan tersebut?		

C6. PENYAKIT-PENYAKIT LAIN

Adakah anak anda mempunyai penyakit-penyakit seperti di bawah? **Jika YA**, pada umur berapakah penyakit itu dikesan?	YA	TIDAK	Umur dikesan
a) Campak			
b) Bronkitis (radang paru-paru)			
c) Emfisema (paru-paru mengembang dan rosak)			
d) Asma (lelah/semput)			
e) Pneumonia (jangkitan paru-paru)			
f) Ekzema (gatal kulit)			

BAHAGIAN D: PENDEDAHAN KEPADA ASAP ROKOK

		YA	TIDAK
1.	Adakah ahli keluarga yang tinggal di rumah anda/pengasuh anak anda seorang perokok? **Jika YA**, sila nyatakan siapa: _____		

2. Dalam tempoh 7 hari lepas, di manakah lokasi pendedahan asap rokok kepada anak anda?

a) Di dalam/luar rumah sendiri	
b) Di dalam kereta sendiri	
c) Di dalam kenderaan awam/orang lain	
d) Di kawasan luar	
e) Di rumah saudara/kenalan	
f) Di rumah pengasuh/pusat jagaan kanak-kanak	
g) Lain-lain. Sila nyatakan: _____	
h) Anak saya tidak terdedah kepada asap rokok	

BAHAGIAN E: PERSEKITARAN RUMAH

1. Apakah jenis rumah yang didiami sekarang?
   a) Teres ☐ d) Flat ☐ g) Kampung ☐
   b) Berkembar (*Semi-D*) ☐ e) Pangsapuri ☐ h) Lain-lain. Nyatakan: ☐
   c) Banglo ☐ f) Kondominium ☐ _____

2. Di manakah lokasi perumahan anda?
   a) Bandar ☐ b) Sub-bandar ☐ c) Luar Bandar ☐

3. Adakah anak anda tinggal di kediaman yang sama sejak lahir? Ya ☐ Tidak ☐
   Jika TIDAK, pada tahun berapakah anda berpindah ke kediaman sekarang? _____

4. Berapakah anggaran keluasan tempat kediaman anda? _____

5. Bahan apakah yang digunakan dalam pembinaan rumah anda?
   a) Batu-bata ☐ c) Konkrit ☐ d) Lain-lain. Nyatakan: ☐
   b) Papan ☐ _____

6. Adakah sebahagian daripada rumah anda dicat dalam tempoh 12 bulan yang lepas? Ya ☐ Tidak ☐

7. Adakah lantai rumah anda baru ditukar sejak 12 bulan yang lepas? Ya ☐ Tidak ☐

8. Adakah terdapat binatang peliharaan di dalam kediaman anda? Ya ☐ Tidak ☐
Jika YA, apakah binatang itu? Nyatakan: _____

9. Berapakah bilangan bilik yang terdapat di dalam rumah anda? ____ bilik

10. Berapakah bilangan orang yang tinggal di dalam rumah anda? ____ orang

11. Kanak-kanak ini tidur/tinggal di dalam bilik
a) sendiri ☐ c) berkongsi dengan 2 orang ☐
b) berkongsi dengan 1 orang ☐ d) berkongsi dengan ≥ 3 orang ☐

12. Apakah bahan api yang digunakan untuk memasak di dalam rumah anda?
a) Elektrik ☐ c) Minyak Tanah ☐ e) Arang ☐
b) Gas ☐ d) Kayu Api ☐ f) Lain-lain. Nyatakan: _____ ☐

13. Berapa kali dalam sehari anda menggunakan bahan api di atas untuk memasak? ____ kali

14. Adakah anda menggunakan alat penyedut asap di dapur? Ya ☐ Tidak ☐

15. Semasa memasak, adakah anda membuka tingkap atau pintu untuk membenarkan pengaliran udara di dalam rumah? Ya ☐ Tidak ☐

16. Apakah alat yang digunakan untuk menyejukkan udara di dalam rumah?
a) Penyaman Udara ☐ b) Kipas ☐ c) Lain-lain. Nyatakan: _____ ☐

17. Adakah anda menggunakan bahan tertentu untuk mengelakkan serangan nyamuk? Ya ☐ Tidak ☐

18. **Jika YA**, apakah jenis yang selalu digunakan?
a) Lingkaran biasa ☐ c) Semburan aerosol ☐
b) Elektrik ☐ d) Lain-lain. Nyatakan _____ ☐

19. Berapa banyakkah kuantiti yang digunakan untuk setiap malam? ____

20. Di manakah bahan penghalau nyamuk digunakan di dalam rumah?
a) Ruang tamu ☐ b) Bilik tidur ☐ c) Lain-lain. Nyatakan: _____ ☐

21. Apakah alat yang digunakan untuk membersihkan rumah anda? _____

22. Berapa kerapkah anda membersihkan rumah anda dalam seminggu? ____ kali

23. Adakah anda menggunakan karpet di rumah anda? Ya ☐ Tidak ☐

24. Adakah anda menggunakan sebarang wangian di rumah anda? Ya ☐ Tidak ☐

25. **Jika YA**, nyatakan jenis pewangi:
a) Semburan ☐ b) Cairan ☐ c) Ketulan ☐

26. Nyatakan di mana anda menggunakan pewangi tersebut di rumah anda. _____

27. Nyatakan kekerapan penggunaan pewangi tersebut dalam seminggu. ____ kali

28. Adakah anda meletakkan ubat gegat di dalam almari pakaian anak anda? Ya ☐ Tidak ☐

29. Lokasi rumah dari kilang:
a) < 2.5 km dari kilang ☐
b) 2.5 – 4.9 km dari kilang ☐
c) ≥ 5 km dari kilang ☐

30. Lokasi rumah dari stesen janakuasa elektrik:
a) < 2.5 km dari stesen janakuasa ☐
b) 2.5 – 4.9 km dari stesen janakuasa ☐
c) ≥ 5 km dari stesen janakuasa ☐

31. Apakah pendapat anda mengenai persekitaran di rumah anda?
a) Sangat berhabuk ☐ b) Sederhana berhabuk ☐ c) Kurang berhabuk ☐

32. Adakah anda kerap melakukan pembakaran terbuka di luar rumah seperti membakar daun-daun kering? Ya ☐ Tidak ☐

33. Jika YA, berapa kerapkah anda melakukan aktiviti pembakaran tersebut?
a) Hampir setiap hari ☐ b) Sekali seminggu ☐ c) Sekali sebulan ☐

BAHAGIAN F: TABIAT PEMAKANAN ANAK ANDA SEKARANG

Kekerapan (Isikan dengan 1x, 2x, 3x, >3x)	Sekali (1x), 2 Kali (2x), 3 Kali (3x), Lebih 3 Kali (>3x)			
	Tidak Pernah	Sehari	Seminggu	Sebulan
1. Berapa kerapkah anak anda makan daging?				
2. Berapa kerapkah anak anda makan ikan? Nyatakan jenis ikan._____				
3. Berapa kerapkah anak anda makan makanan laut? Nyatakan jenis makanan laut._____				
4. Berapa kerapkah anak anda makan buah-buahan? Nyatakan jenis buah. _____				
5. Berapa kerapkah anak anda makan ulam-ulaman?				
6. Berapa kerapkah anak anda makan sayur yang dimasak? Nyatakan jenis sayur. _____				
7. Berapa kerapkah anak anda minum susu?				
8. Berapa kerapkah anak anda mengambil makanan tenusu? (yogurt, keju, dan lain-lain)				
9. Berapa kerapkah anak anda makan makanan segera? (burger, pizza, hotdog, nugget, dan lain-lain)				
10. Berapa kerapkah anak anda minum jus buah-buahan?				
11. Berapa kerapkah anak anda minum air bergas?				
12. Berapa kerapkah anak anda mengambil makanan tambahan seperti vitamin atau jus kesihatan? Nyatakan. _____				
13. Berapa kerapkah anak anda makan masakan panggang yang menggunakan arang? (ayam, daging, dan lain-lain)				

14. Apakah jenis minyak masak yang sering digunakan di rumah anda?
a) Minyak kelapa sawit ☐ c) Minyak jagung ☐ e) Mentega ☐
b) Minyak sayuran ☐ d) Marjerin ☐ f) Lain-lain. Nyatakan: ☐

BAHAGIAN G: PENDEDAHAN TERHADAP PENCEMARAN UDARA TRAFIK DI KAWASAN RUMAH

1. Apakah kenderaan yang paling kerap digunakan oleh anak anda untuk ke sekolah?

a) Kereta ☐ c) Basikal ☐ e) Van ☐
b) Bas ☐ d) Motosikal ☐ f) Berjalan kaki ☐

2. Berapa lamakah masa diambil untuk anak anda pergi ke sekolah? _____ minit

3. Lokasi rumah dari jalan utama:

a) < 100 m dari jalan utama ☐ d) 300 – 399 m dari jalan utama ☐
b) 100 – 199 m dari jalan utama ☐ e) 400 – 499 m dari jalan utama ☐
c) 200 – 299 m dari jalan utama ☐ f) ≥ 500 m dari jalan utama ☐

4. Lokasi rumah dari lebuhraya:

a) < 100 m dari lebuhraya ☐ d) 300 – 399 m dari lebuhraya ☐
b) 100 – 199 m dari lebuhraya ☐ e) 400 – 499 m dari lebuhraya ☐
c) 200 – 299 m dari lebuhraya ☐ f) ≥ 500 m dari lebuhraya ☐

Sila tandakan yang berkenaan.	Tidak Pernah	Jarang	Kerap	Berterusan
5. Sekiranya anak anda ke sekolah menaiki kereta, bas atau van, adakah tingkap kenderaan tersebut dibuka semasa kenderaan bergerak?				
6. Adakah kawasan tempat tinggal anda mengalami kesesakan lalu lintas kereta?				
7. Adakah kawasan tempat tinggal anda mengalami kesesakan lalu lintas lori?				
8. Adakah kawasan tempat tinggal anda mengalami pencemaran bunyi disebabkan kesesakan lalu lintas?				
9. Adakah asap hitam daripada kenderaan dapat dilihat di kawasan rumah anda?				
10. Adakah anda menghidu bau asap daripada kenderaan di kawasan rumah anda?				
11. Adakah pencemaran udara trafik yang dilepaskan daripada kenderaan di kawasan rumah anda memberi kesan kesihatan kepada anak anda?				

Kekerapan (Isikan dengan < 50 / 50 - 99 / 100 - 499 / ≥ 500)	< 50 / 50 - 99 / 100 - 499 / ≥ 500	
	Hari bekerja	Hari minggu
12. Berapakah anggaran bilangan kereta yang lalu di kawasan tempat tinggal anda?		
13. Berapakah anggaran bilangan bas yang lalu di kawasan tempat tinggal anda?		
14. Berapakah anggaran bilangan lori yang lalu di kawasan tempat tinggal anda?		
15. Berapakah anggaran bilangan motosikal yang lalu di kawasan tempat tinggal anda?		

DIARI HARIAN

Sila isikan jadual di bawah dengan aktiviti yang paling kerap dilakukan oleh anak (sekurang-kurangnya 1 jam) beserta lokasi dan kenderaan. Contoh disediakan pada baris pertama.

Hari / Masa	Isnin	Selasa	Rabu	Khamis	Jumaat	Sabtu	Ahad
CONTOH *6 a.m. – 11.59 a.m*	*Aktiviti: Sekolah* *Lokasi: SK Sentul 2* *Kenderaan: Kereta*	*Aktiviti: Sekolah* *Lokasi: SK Sentul 2* *Kenderaan: Kereta*	*Aktiviti: Sekolah* *Lokasi: SK Sentul 2* *Kenderaan: Kereta*	*Aktiviti: Sekolah* *Lokasi: SK Sentul 2* *Kenderaan: Kereta*	*Aktiviti: Sekolah* *Lokasi: SK Sentul 2* *Kenderaan: Kereta*	*Aktiviti: Berenang* *Lokasi: Pusat Akuatik Nasional* *Kenderaan: Kereta*	*Aktiviti: Tuisyen* *Lokasi: Rumah* *Kenderaan: Tiada*
6 a.m. – 11.59 a.m.							
12 p.m. – 2.59 p.m.							
3 p.m. – 6.59 p.m.							
7 p.m. – 10.59 p.m.							
11 p.m. – 5.59 a.m.							

BIODATA OF STUDENT

Nur Faseeha Binti Suhaimi was born on 1st August 1991 in Kuala Lumpur Hospital. She received her primary education at Sekolah Kebangsaan Serdang, Selangor from 1998 to 2002. She passed Level One Evaluation (PTS) in 2000, so she was offered to skip Standard 4 and went straight to Standard 5. In 2002, she obtained 5A in Primary School Evaluation Test (UPSR) but continued her lower secondary education in a co-ed daily school of Sekolah Menengah Kebangsaan Seri Serdang, Selangor. Later in 2005, she obtained 9A in Lower Secondary Evaluation (PMR) and was offered to further her upper secondary education in an all-girls boarding school of Tunku Kurshiah College, Negeri Sembilan, from 2006 to 2007. In 2008, she passed the Malaysian Certificate of Education (SPM) with 8A1 and 2A2 and was offered a full government scholarship by Public Service Department (JPA) to further her first degree in the United States of America under the American Degree Foundation Programme. She obtained her Bachelor of Science in Molecular Bioscience and Biotechnology from Rochester Institute of Technology, USA, in 2013. In 2014, she received scholarships from the Ministry of Higher Education (MOHE) Malaysia and the UPM Graduate Research Fund (GRF) to pursue her master's degree. She was conferred a Master of Science degree in Environmental Health from Universiti Putra Malaysia in 2016.

Faseeha is married and is blessed with a daughter and a son. She is currently furthering her study in Doctor of Philosophy in Environmental Health from Universiti Putra Malaysia. She is fully sponsored by Ministry of Higher Education (MOHE) Malaysia under *Skim Latihan Akademik Bumiputera* (SLAB) and UPM under *Tenaga Akademik Muda* (TAM).

LIST OF PUBLICATIONS

Journals

Nur Faseeha Suhaimi, Juliana Jalaludin and Suhaili Abu Bakar (2021). The Influence of Traffic-Related Air Pollution (TRAP) in Primary Schools and Residential Proximity to Traffic Sources on Histone H3 Level in Selected Malaysian Children. International Journal of Environmental Research and Public Health, 18 (15): 7995. https://doi.org/10.3390/ijerph18157995. IF 3.390

Nur Faseeha Suhaimi, Juliana Jalaludin and Suhaili Abu Bakar (2021). Deoxyribonucleic Acid (DNA) Methylation in Children Exposed to Air Pollution: A Possible Mechanism Underlying Respiratory Health Effects Development. Reviews on Environmental Health, 36(1): 77-93. https://doi.org/10.1515/reveh-2020-0065. IF 3.458

Nur Faseeha Suhaimi, Juliana Jalaludin and Muhammad Afif Mohd Juhari (2020). The Impact of Traffic-Related Air Pollution on Lung Function Status and Respiratory Symptoms among Children in Klang Valley, Malaysia. International Journal of Environmental Health Research. (Published ahead of print) https://doi.org/10.1080/09603123.2020.1784397. IF 3.411

Nur Faseeha Suhaimi, Juliana Jalaludin and Mohd Talib Latif (2020). Demystifying A Possible Relationship between COVID-19, Air Quality and Meteorological Factors: Evidence from Kuala Lumpur, Malaysia. Aerosol and Air Quality Research, 20: 1520-1529. https://doi.org/10.4209/aaqr.2020.05.0218. IF 3.063

Ili Nabila Ismail, Juliana Jalaludin, Suhaili Abu Bakar, Nur Hazirah Hisamuddin, Nur Faseeha Suhaimi (2019). Association of Traffic-Related Air Pollution (TRAP) with DNA Damage and Respiratory Health Symptoms among Primary School Children in Selangor. Asian Journal of Atmospheric Environment, 13(2): 106-116. https://doi.org/10.5572/ajae.2019.13.2.106

Conferences

Nur Faseeha Suhaimi, Juliana Jalaludin and Muhammad Afif Mohd Juhari. Children's Respiratory Health and Indoor Air Pollutants (IAP) in Selected Malaysian Primary Schools. The 16th Conference of the International Society of Indoor Air Quality & Climate on 1 November 2020 held online and organised by International Society of Indoor Air Quality (ISIAQ)

Nur Faseeha Suhaimi, Juliana Jalaludin, Ili Nabila Ismail and Suhaili Abu Bakar. Association of Traffic-Related Air Pollution (TRAP) with DNA Damage and Respiratory Symptoms among Primary School Children in Selangor. The 10th Better Air Quality Conference on 14-16 November 2018 at Borneo Convention Centre Kuching, Sarawak. – Best Poster Presenter (5th Place)

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : FIRST SEMESTER 2021/2022

TITLE OF THESIS / PROJECT REPORT :

INFLUENCE OF TRAFFIC-RELATED AIR POLLUTION EXPOSURE ON RESPIRATORY HEALTH, TNFα AND CYP1A1 GENE AND HISTONE MODIFICATIONS AMONG SCHOOL CHILDREN IN THE KLANG VALLEY, MALAYSIA

NAME OF STUDENT : NUR FASEEHA BINTI SUHAIMI

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☑	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ (date) until _____ (date)

Approved by:

(Signature of Student)
New IC No/ ~~Passport No.~~: 910801-14-6118

Date : 11/11/2021

(Signature of Chairman of Supervisory Committee)
Name: PROF. DR JULIANA JALALUDIN

Date : 11/11/2021

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]

Traffic-related air pollution (TRAP) is a complex mixture of many pollutants, which has adverse health impacts, especially on children who live near heavily-travelled roads. This cross-sectional comparative study was conducted at eight primary schools in high traffic (HT) and low traffic (LT) areas to investigate the potential risks from TRAP exposure to respiratory health among children with the incorporation of histone H3 level and deoxyribonucleic acid methylation (DNAm) status of *Tumour Necrosis Factor Alpha (TNFα)* and *Cytochrome P450 Family 1 Subfamily A Member 1 (CYP1A1)*. Respondents' background information, personal exposure to TRAP, and respiratory symptoms were obtained from validated questionnaires. Real-time monitoring instruments were used for 6-h measurements of PM_{10}, $PM_{2.5}$, PM_1, NO_2, SO_2, O_3, CO, and TVOC. Meanwhile, 24-h measurements of $PM_{2.5}$-bound BC in schools and particulate matters in residences were performed using air sampling pumps. A lung function test was conducted using a spirometer. Histone H3 modification was captured using an enzyme-linked immunosorbent assay (ELISA) kit, whereas DNAm was quantified using a methylation-specific polymerase chain reaction (MS-PCR) kit on bisulphite-treated DNA; both from saliva samples. The results indicate that HT area had significantly higher concentrations of air pollutants than LT area. Findings from multiple logistic regression show that methylated *TNFα* and *CYP1A1* were mostly influenced by exposure to NO_2 and BC, respectively. Meanwhile, results from multiple linear regression revealed that BC and NO_2 were the most significant factors influencing the FVC% among children. FEV_1% were mostly influenced by BC, PM_1 and $PM_{2.5}$, whereas NO_2 was the most significant factor that influenced the histone H3 level among children. In conclusion, epigenetic mechanisms may govern the relationships between TRAP exposures and respiratory health by acting as mediators. This study also provides the groundwork for future preventive interventions, particularly developing mitigation plans to reduce TRAP exposure in Malaysia.

School of Graduate Studies
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia