



***IN VITRO* OXIDATIVE POTENTIAL (OP) ASSESSMENT
FOR INDOOR AIRBORNE PARTICULATE MATTERS
(PM) AT SELECTED PRIMARY SCHOOLS IN PAHANG**

BY

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ABSTRACT

The inadequacy of Indoor Air Quality (IAQ) inside the classrooms especially in tropical countries has been highlighted in previous research, and the increased respiratory symptoms among the schoolchildren were associated with this inadequacy of IAQ issue. Therefore, this study aimed to assess the selected IAQ parameters (temperature, relative humidity (RH), air movement and particulate matter (PM)) at different backgrounds of sampling locations (industrial, residential and rural areas) and monsoonal variations during occupied and non-occupied periods. The next objective was to determine the prevalence of respiratory symptoms among the schoolchildren and its association with IAQ parameters. The last objective was to assess the PM-induced oxidative activity by using an *in vitro* synthetic respiratory tract lining fluid (synRTLFL) model. Initially, the IAQ monitoring was conducted at the selected primary school buildings in different background areas (industrial, residential and rural). This study was conducted twice during the southwest monsoon (from May to October 2015) and the northeast monsoon (from November 2015 to April 2016). Then, the association between respiratory symptoms and indoor PM mass concentration was determined using a chi-squared test. Prior to that, the prevalence of chronic respiratory symptoms; non-specific respiratory disease (NSRD) and persistent cough and phlegm (PCP) was assessed using questionnaires adapted from a well-established questionnaires (ATS-DLD-78-C). A further assessment of indoor PM toxicity or oxidative activity was conducted using a novel method, namely synRTLFL in order to obtain the PM OP metrics. From the IAQ assessment, thermal comfort parameters (temperature, RH and air movement) were influenced by the Malaysian hot and humid climate. A two-way ANOVA analysis showed that thermal comfort parameters were significantly different between two monsoons ($p < 0.05$). Based on the two-way ANOVA analysis, there was a significant effect of monsoonal variations on the TSP, PM₁₀, PM_{2.5} and PM₁ concentration. During SW season, the TSP, PM₁₀, PM_{2.5}, and PM₁ concentrations were significantly higher than NE season ($p < 0.001$). Meanwhile, the prevalence of NSRD and PCP was low. For SW monsoon, the highest prevalence of persistent phlegm (15.7%), wheezing (12.0%), and chronic bronchitis (3.9%) was recorded in SKIM, while the highest prevalence of persistent cough (15.0%) and bronchial asthma (12.2%) was recorded in SKT. In comparison with NE monsoon, the highest percentages of bronchial asthma (9.8%) and persistent phlegm (1.2%) were recorded in SKT, persistent cough (4.8%) in SKIM, while wheezing (1.9%) and chronic bronchitis (0.7%) in SKBB. Bronchial asthma and persistent cough were the most common symptoms significantly associated with coarse PM mass concentration. Furthermore, from the independent *t*-test analysis, there was no significant difference for indoor and outdoor PM OP metrics ($p > 0.05$). A higher PM OP metrics was recorded at the schools located nearer to the main roads. In conclusion, the inadequacy of IAQ status at the classrooms was mainly influenced by monsoonal varieties and outdoor ambient background location. A low prevalence of respiratory symptoms was recorded, and from the PM OP metrics result, PM toxicity was observed especially in PM collected at the schools' proximity to main roads.

خلاصة البحث

لقد تم إبراز عدم كفاية جودة الهواء الداخلي (IAQ) داخل الفصول الدراسية وخاصة في البلدان المدارية في أبحاث سابقة، وارتبطت الأعراض التنفسية المتزايدة بين أطفال المدارس بهذا القصور في مشكلة IAQ. لذلك، تهدف هذه الدراسة إلى تقييم معالم IAQ المحددة (درجة الحرارة والرطوبة النسبية (RH) وحركة الهواء والجسيمات (PM) في خلفيات مختلفة لمواقع أخذ العينات (المناطق الصناعية والسكنية والريفية) والتغيرات الموسمية خلال التغيرات المشغولة والفترات غير المشغولة. كان الهدف الثاني هو تحديد مدى انتشار الأعراض التنفسية بين أطفال المدارس وارتباطها بمعلمات IAQ. كان الهدف الثالث هو تحديد مدى انتشار الأعراض التنفسية بين أطفال المدارس وارتباطها بمعلم IAQ. كان الهدف الأخير هو تقييم النشاط المؤكسد الناجم عن حركة الهواء والجسيمات (PM) باستخدام نموذج سائل بطانة الجهاز التنفسي الاصطناعي المختبر (synRTLF). في البداية، أجري رصد IAQ في مباني المدارس الابتدائية المختارة في مناطق مختلفة (صناعية وسكنية وريفية). وأجريت هذه الدراسة مرتين خلال الرياح الموسمية الجنوبية الغربية (من مايو إلى أكتوبر 2015) والرياح الموسمية الشمالية الشرقية (من نوفمبر 2015 إلى أبريل 2016). ثم تم تحديد العلاقة بين أعراض الجهاز التنفسي وتركيز الكتلة PM الداخلي باستخدام اختبار خي المربع. قبل ذلك، انتشار أعراض الجهاز التنفسي المزمنة. تم تقييم مرض الجهاز التنفسي غير محددة (NSRD) والسعال والبلغم المستمر (PCP) باستخدام استبيانات مقتبسة من استبيانات راسخة (ATS-DLD-78-C). تم إجراء تقييم إضافي لسمية PM الداخلية أو النشاط المؤكسد باستخدام طريقة جديدة، وهي synRTLF من أجل الحصول على مقاييس PM OP. من تقييم IAQ، تأثرت معالم الراحة الحرارية (درجة الحرارة والرطوبة النسبية (RH) وحركة الهواء والجسيمات) بالمناخ الحار والرطب الماليزي. وأظهر تحليل ANOVA ثنائي الاتجاه أن معايير الراحة الحرارية مختلفة بشكل كبير بين الاثنين من الرياح الموسمية (P < 0.05). بناءً على تحليل ANOVA ثنائي الاتجاه. كان هناك تأثير كبير للتغيرات الموسمية على تركيز TSP و PM10 و PM2.5 و PM1. خلال موسم SW، كانت تركيزات TSP PM10 و PM2.5 و PM1 أعلى بكثير من موسم NE (P < 0.001). وفي الوقت نفسه، كان معدل انتشار NSRD و PCP منخفضًا. بالنسبة للرياح الموسمية SW، سجلت أعلى نسبة انتشار للبلغم المستمر (15.7٪)، والصفير (12.0٪)، والتهاب الشعب الهوائية المزمن (3.9٪) في SKIM، في حين سجلت أعلى نسبة انتشار للسعال المستمر (15.0٪) والربو الشعبي (تم تسجيله في SKT). وبالمقارنة مع الرياح الموسمية NE، تم تسجيل أعلى النسب المئوية للربو القصبي (9.8٪) والبلغم المستمر (1.2٪) في SKT والسعال المستمر (4.8٪) في SKIM، بينما الصفير (1.9٪) والتهاب الشعب الهوائية المزمن (0.7٪) في SKBB. وكانت الربو القصبي والسعال المستمر الأعراض الأكثر شيوعًا المرتبطة بشكل كبير مع تركيز الكتلة الخشنة PM. علاوة على ذلك، من تحليل الاختبار المستقل، لم يكن هناك اختلاف كبير في مقاييس PM OP الداخلية والخارجية (p > 0.05). تم تسجيل مقاييس PM OP أعلى في المدارس الواقعة بالقرب من الطرق الرئيسية. وفي الختام، تأثر عدم كفاية وضع IAQ في الفصول الدراسية بشكل أساسي بالأصناف الموسمية وموقع الخلفية المحيط الخارجي. تم تسجيل انخفاض في معدل انتشار أعراض الجهاز التنفسي، ومن نتيجة مقاييس PM OP، لوحظ تسمم PM خاصة في المدارس الثانوية التي تم جمعها عند قرب المدارس من الطرق الرئيسية.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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In the name of Allah, the Most Gracious and the Most Merciful. And of His signs is that He sends the winds as bringers of good tidings and to let you taste His mercy and so the ships may sail at His command and so you may seek of His bounty, and perhaps you will be grateful (Ar-Rum: 46).

Winds is the movement of air, and is a crucial element in air pollutions (to dilute the air pollutants) and is applied to dilute the indoor air pollutants as well. Good tidings in this verse (Ar-Rum: 46) were referred to the rain that play important role to wash away the ambient air pollutants, providing us a better air quality. SubhannAllah and Alhamdulillah, for these bounties and may we always be thankful

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LIST OF ABBREVIATIONS

API	Air Pollutant Index
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
ATS	American Thoracic Society
BRI	Building Related Illnesses
Cd	Cadmium
CH ₂ O	Formaldehyde
CH ₄	Methane
CO	Carbon monoxide
DNA	Deoxyribonucleic acid
DOE	Malaysian Department of Environment
DOSH	Department of Safety and Health
ELISA	Enzyme-Linked Immunosorbent Assay
FeNO	Fraction of Exhaled Nitric Oxide
GC-MS	Gas Chromatography-Mass Spectrometry
GN	Hong Kong's Guidance Notes
GSH	Glutathione
H ₂ S	Hydrogen sulfide
HEI	Health Effects Institute
HPLC-MS	High performance liquid chromatography-mass spectrometry
HVAC	Heating, ventilation, and air conditioning
IAQ	Indoor Air Quality
ICOP on IAQ 2010	Industrial Code of Practice on Indoor Air Quality 2010
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IL-6	Interleukin-6
ISAAC	International Study of Asthma and Allergies in Childhood
MCE	Mixed Cellulose Ester
mΩ	Milli-Q
NE	North East
NO	Nitrogen oxides
NO ₂	Nitrogen dioxides
NSRD	Non-Specific Respiratory Disease
O ₃	Ozone
OAPEC	Organisation of Arab Petroleum Exporting Countries

OP	Oxidative potential
PAHs	Polyaromatic hydrocarbons
Pb	Lead
PCP	Persistent cough and phlegm
PCR	Polymerase chain reaction
PM	Particulate matter
PM OP	Particulate matter oxidative potential
RH	Relative Humidity
ROS	Reactive oxygen species
RSP	Respirable Suspended Particulate Matter
RTLFL	Respiratory Tract Lining Fluid
SBS	Sick Building Syndrome
SO ₂	Sulphur dioxide
SW	South West
synRTLFL	Synthetic Respiratory Tract Lining Fluid
TSP	Total suspended particle
TVOC	Total Volatile Organic Compound
USA	United States of America
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

In a recent decade, studies have found that humans spend 90% of their life indoors and thus are exposed to the indoor air pollutants (de Gennaro et al., 2014; Pegas et al., 2010). In fact, an earlier study, The National Human Activity Pattern Survey (NHAPS) conducted by Klepeis et al. (2001) found that 87% of the respondents spent their time indoors, and about 6% of the time was spent in enclosed vehicles. The high percentage of time humans spend indoors indicate a high exposure to air pollutants. For that reason, the exposure to indoor air pollutants is becoming a key concern nowadays as humans are frequently in contact with the air pollutants that originate from the mixture of off-gassing from the end users products and the building materials.

Living indoors does not mean humans are protected from harmful air pollutants. In fact, the study by the United States Environmental Protection Agency (U.S. EPA) on human exposure to air pollutants indicated that the indoor levels of air pollutants may be two to five times and occasionally more than 100 times higher than the outdoor levels. In fact, the decreased indoor air quality (IAQ) has been ranked as one of the highest risks to human health (U.S. EPA, 2017).

Furthermore, World Health Organisation (WHO) estimated that 3.3 million deaths were linked to indoor air pollution, and 2.6 million deaths were related to outdoor air pollution (WHO, 2014). These estimates reflect the increased concentration of indoor air pollutants, the large number of people exposed to air

pollution, and the time spent indoors (Yang & Omaye, 2009). Those figures of death estimates reveal the growing numbers of industries, especially in China and India, along with continuing deep poverty in rural areas (Vidal, 2014). The indoor air pollution in low-income and middle-income countries was mostly attributed to biomass smoke from burning of wood and crop residues for heating and cooking necessities. Biomass smoke contains thousands of health-harmful substances including small particulates, especially those of less than 10 microns in diameter, which are among the most dangerous substances (WHO, 2014). These small particulates are able to penetrate deep into the lungs and are an important factor in the development of illnesses including acute lower respiratory disease, chronic obstructive pulmonary disease, and cancers (Gordon, Mackay, & Rehfuss, 2004; Mackey et al., 2014).

Due to the adverse health effects of particulates, a particulate matter with an aerodynamics diameter of less than 10 μm (PM_{10}) and a particulate matter with an aerodynamics diameter of less than 2.5 μm ($\text{PM}_{2.5}$) are categorised by the U.S. EPA as two common air pollutants, or known as “criteria air pollutants”. Other than PM_{10} and $\text{PM}_{2.5}$, also included as criteria air pollutants are ozone (O_3), carbon monoxide (CO), nitrogen oxides (NO), and lead (Pb) that can harm the human health and the environment, along with causing property damage (U.S. EPA, 2016). Similarly, in Malaysia, PM_{10} , O_3 , CO, sulphur dioxide (SO_2), and nitrogen dioxide (NO_2) are measured to determine their classification of air pollutant index (API). Just recently in August 2018, $\text{PM}_{2.5}$ reading is included in the API to improve the Malaysian API standard, as also practised by the developed countries. Used by the Malaysian Department of Environment (DOE) to determine the status of air quality in this country, this index is important for the public to understand the quality of air for their

own health and precautions. The range of API is from zero to more than 300 and classified as “good (0–50), moderate (51–100), unhealthy (101–200), very unhealthy (201–300), and hazardous (301 and above)” (Abd Rahman, Lee, Latif, & Suhartono, 2013).

However, API can only be applied to an outdoor environment. On the other hand, for an indoor environment, there is a standard to determine its quality or indoor air quality (IAQ) status. This IAQ standard, which is Industrial Code of Practice on Indoor Air Quality 2010 (ICOP on IAQ 2010), is applied for the industry. This standard is endorsed by the Department of Safety and Health (DOSH), Malaysia, and the application is limited to the occupational health and safety. This standard is used to control the air quality in the indoor environment to prevent occupational diseases in industrial buildings. This is different from the Hong Kong’s Guidance Notes (GN) on IAQ for offices and public spaces, whereby the standard is aimed to ensure the public, which consist of vulnerable groups such as children and pregnant ladies, to have good IAQ (IAQMG, 2003).

Indoor air quality had received new attention after the energy crisis due to the oil embargo of the Organisation of Arab Petroleum Exporting Countries (OAPEC) to the United States of America in 1973. In fact, IAQ researches only emerged in the late 1960’s and early 1970’s. Initially, the concern of IAQ was only for industrial buildings. Later on, the poor IAQ in industrial workplaces led to growing concern about the IAQ in the public spaces or non-industrial buildings too. In Malaysia, the ICOP on IAQ 2010 is the only IAQ standard available, and the scope of IAQ under this code of practice is limited and does not include public spaces. Therefore, a new IAQ guideline for the public spaces such as childcare centres and schools should be established to protect the vulnerable groups.

Another noteworthy information is that, IAQ studies in Malaysian kindergarten, preschool, and school are very limited (Kamaruzzaman & Razak, 2011). Although a number of IAQ studies have been conducted in preschools and schools in this country, the areas covered were only Kuala Lumpur, Selangor, Negeri Sembilan, and Terengganu (Latif et al., 2014; Sofian & Ismail, 2011; Zainal Abidin, Semple, Rasdi, Ismail, & Ayres, 2014). Moreover, according to Ismail, Sofian, & Abdullah (2010), the studies related to IAQ in school buildings in Malaysia's east coast are inadequate. Specifically, to the best of the author's knowledge, only few IAQ studies have been conducted in the state of Pahang. Therefore, there is an urge to conduct a study on IAQ in schools especially in this state.

Generally, in Malaysia, primary schoolchildren spend about 6 to 8 hours in the school in different locations such as classroom, canteen, and playgrounds. These locations, which have different environments, expose the schoolchildren to different air pollutants. Along the hours of schooling, these schoolchildren spend most of the time at the classroom. A poor IAQ at the classroom could affect their health status in long and short terms (Fromme et al., 2008; Mostafae, 2010). Because of this matter, schoolchildren deserve to get a good IAQ for their optimum development and ideal learning process.

According to Sofian & Ismail (2011), the indoor PM mass concentrations at the classrooms in Terengganu were higher than the outdoor PM mass concentrations. Similarly, high indoor PM mass concentrations have also been observed in other studies (Braniš, Řezáčová, & Domasová, 2005; Heudorf, Neitzert, & Spark, 2009; Yang, Sohn, Kim, Son, & Park, 2009). As stated by Fromme et al. (2008), these high indoor PM mass concentrations could be due to several factors such as the ventilation systems in the school and the hygiene practice at the classroom. However, the most

highlighted factor is a large number of students against room area and volume, with constant resuspension of particles from room surfaces.

In addition, high indoor PM mass concentrations are also influenced by the ambient meteorological condition through the infiltration of outdoor PM that increases the indoor PM mass concentrations especially at naturally ventilated classrooms (Chen, Gall, & Chang, 2016). Studies have found the ambient's total suspended particulates (TSP), PM₁₀, and PM_{2.5} are influenced by the monsoonal variety in Malaysia (Hamid, Rahmat, & Sapani, 2018; Juneng, Latif, & Tangang, 2011; Md Yusof et al., 2010; Yassen & Md. Jahi, 2007). In particular, TSP mass concentration is higher during the southwest (SW) monsoon compared to the northeast (NE) monsoon (Yassen & Md. Jahi, 2007). Other than PM mass concentrations, ambient temperature and relative humidity (RH) also influence IAQ at the the naturally ventilated classrooms in the tropical countries such as Singapore and Thailand (Klinmalee, Srimongkol, & Kim Oanh, 2009; Tippayawong, Khuntong, Nitatwichit, Khunatorn, & Tantakitti, 2009; Wong & Khoo, 2003). Since the ambient condition influences the IAQ at the classrooms, the effects of monsoonal changes to the IAQ at the classrooms should be thoroughly determined especially for indoor PM mass concentration, considering the fact that the exposure of PM towards schoolchildren can be harmful (Kelly & Fussell, 2012).

The relationship between PM mass concentration and respiratory symptoms among the schoolchildren has been well-established by recent studies (Ayuni, Juliana, & Ibrahim, 2014; Mohd Nor Rawi, Jalaludin, & Chua, 2015; Mark & Heath, 2005; Nazariah, Juliana, & Abdah, 2013). Their findings are consistent with a past study by Pope, Ezzati, and Dockery (2009), who found that the exposure to high PM₁₀ mass concentrations is associated with increased risk of death due to cardiovascular or

respiratory symptoms among the schoolchildren. Plus, the schoolchildren are among the vulnerable groups to the air pollutants since their immune system has not fully developed yet (Salvi, 2007). Besides respiratory symptoms, a poor IAQ also leads to sick building syndrome (SBS) (Asmi, Putra, & Rahman, 2012). Thus, this study aims to determine the relationship between IAQ and prevalence of chronic respiratory symptoms (non-specific respiratory disease (NSRD) and persistent cough and phlegm (PCP)) using a slightly modified recommended respiratory disease questionnaire for children by the American Thoracic Society (ATS-DLD-78-C).

Other than using a questionnaire as a tool to determine the relationship between IAQ and respiratory symptoms, PM-induced oxidative stress has been proposed as having a key role in examining health effects, particularly in humans (Boogaard et al., 2011). Oxidative stress caused by PM is known as PM oxidative potential (PM OP), which is increasingly studied as a relevant metric for health impact. This is because PM mass concentration does not contribute to PM toxicity as much as the ambient particle mass (Calas et al., 2017). PM OP has been suggested as an important underlying factor of PM exposure mechanism, by which exposure to PM may lead to adverse health effects (Nel, 2005). This is because, PM OP integrates several biologically relevant PM properties such as size, surface, and chemical composition. Hence, PM OP may provide a better health-based exposure measure than PM mass concentration alone (Borm, Kelly, Künzli, Schins, & Donaldson, 2007).

This present study involving *in vitro* analysis of synthetic respiratory tract lining fluid (synRTLFL) model was conducted to determine the oxidative potential of the PM collected inside and outside of the classrooms. This *in vitro* study is more preferable because, compared to human or animal exposure studies, *in vitro* cell studies are simpler, inexpensive, and less time-consuming, a large number of