Increased Chromosomal Damage among Children in Proximity to an Industrial Zone

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ABSTRACT

This study aimed to investigate air pollutant exposure at five primary schools in Terengganu, Malaysia, and to assess the chromosomal damage among the students by evaluating 176 school children aged 10–11 years. Members of the exposed group lived close to the industrial zone, whereas those of the comparative group lived far from it. The parameters for the indoor air monitoring included suspended particulate matter, gaseous pollutants (NO₂ and SO₂), and physical variables (temperature, relative humidity, and air velocity). Respiratory symptoms were assessed through questionnaires ($n = 176$), and a micronucleus assay was conducted on the buccal epithelial cells of 91 children. The findings showed that the air pollutant levels at the schools of the exposed group were significantly higher ($p < 0.05$) than those of the comparative group. The highest concentrations of PM₁, PM_{2.5}, and PM₁₀ recorded at the exposed schools were 43.30, 44.83, and 60.83 $\mu\text{g m}^{-3}$, respectively. Coughing was the most significant recurring respiratory symptom among the children with a 2.52 odds ratio ($p < 0.05$). An average micronucleus frequency of 5.02 ± 3.43 MN per 1000 cells was displayed by children in the exposed group vs. 2.00 ± 1.56 MN per 1000 cells for the comparative group. After controlling for all possible confounding factors, these results strongly suggest that exposure to industrial air pollutants significantly influences the formation of micronuclei and increases the prevalence of respiratory symptoms among children living in proximity to an industrial area. Our study provides baseline data for genotoxic damage among such children in South East Asia specifically.

Keywords: Air pollutants; Chromosomal damage; Respiratory symptom; Children.

INTRODUCTION

The ever-increasing levels of air pollutants in many urban and industrial areas can result in adverse health impacts on people especially the young, the old, and the infirm. The young are especially susceptible due to their still developing physique as well as immature lungs and immune systems (Branco *et al.*, 2014; Esposito *et al.*, 2014; Korten *et al.*, 2017). Prolonged exposure to high levels of air pollutants may increase the risk of developing cancer especially for those living near to heavy industries (Tsai *et al.*, 2009; Yuan *et al.*, 2018; Seifi *et al.*, 2019). Many epidemiological studies have convincingly demonstrated the deterioration of air quality in schools in the proximity of big industrial areas, which

has led to significant respiratory health impacts especially of the acute nature (de Moraes *et al.*, 2010; Godoi *et al.*, 2013; Demirel *et al.*, 2014; Rovira *et al.*, 2014; Suhaimi *et al.*, 2017). The proximity to industrial zone has been associated with a higher prevalence of respiratory symptoms, a high incidence of asthma attack, and reduction in lung function as compared to the population in non-industrial areas (Loyo-Berrios *et al.*, 2007; Smargiassi *et al.*, 2009; Wichmann *et al.*, 2009; Ayuni *et al.*, 2014). On the other hand, epidemiological studies on chronic health effects are scarcely documented among susceptible population especially children. Nevertheless, some studies have shown that children living in proximity to petrochemical installations in Taiwan experienced high mortality rate due to bone, brain, and blood cancer (Pan *et al.*, 1994; Yu *et al.*, 2005; Liu *et al.*, 2008).

Chronic exposure to air pollutants has been frequently associated with genetic defects among children (Mielzynska-svach *et al.*, 2013). Prolonged exposure to small or high concentrations of air pollutants may induce genetic toxicity and subsequently may increase the risk of cancer. Thus,

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biomarkers of genomic damage or chromosomal damage, such as micronucleus frequency, are widely used in cytogenetic epidemiological studies to predict the risk of cancer when exposed to polluted air (Pedersen *et al.*, 2009; Mergener *et al.*, 2014; de Oliveira Galvão *et al.*, 2017; Sopian *et al.*, 2017; Tan *et al.*, 2017; Muhamad Daud *et al.*, 2018). The micronucleus is derived from acentric chromosome fragments or whole chromosomes which have failed to migrate to spindle poles during the anaphase stage (Fenech, 2000). The presence of a small nucleus in a dividing cell provides a convenient indicator of genotoxicity due to chromosomal breakage and loss, known as chromosomal aberration. It is frequently assessed in human lymphocytes, erythrocytes, and exfoliated epithelial cells with different micronucleus frequency of similar exposure (de Oliveira Galvão *et al.*, 2017; Tan *et al.*, 2017; Muhamad Daud *et al.*, 2018). The exfoliation of epithelial cells, such as buccal cells, is reported to be a minimally invasive method (proposed since 1983) as compared to lymphocytes and erythrocytes in indicating and determining the level of genetic damage due to inhalation and ingestion of xenobiotic substances (Holland *et al.*, 2008; Thomas *et al.*, 2011).

According to an *in vivo* genotoxicity study, Han *et al.* (2013) demonstrated the increasing inhalation dose of nitrogen dioxide, NO₂, significantly induced higher micronuclei frequencies. The mechanism involved might be contributed by metabolite reaction of nitrite and nitrate as it can damage cellular DNA and result in mutation or cancer. Inhalation of highly soluble sulfur dioxide, SO₂, will be metabolized into sulfites, which can hinder DNA synthesis and induce chromosomal aberration. Besides that, oxidation of SO₂ metabolite to sulfate may generate abundance of free radicals that can bind to DNA. The binding to DNA can cause crosslinking and DNA breaks (ATSDR, 1998). Meanwhile, the inhalation of particulate matter can induce the generation of reactive oxygen species, which break the phosphodiester backbone of DNA leading to DNA damage (Fenech *et al.*, 2016).

To date, there have been limited study on the association between industrial air pollutant exposure and genotoxic outcome conducted among children in South East Asia. This study aims to evaluate the impact of industrial air pollutants on potential genotoxic outcome as indicated by the micronucleus frequency among primary school children living in the vicinity of industries.

METHODS

Study Location

Sampling was carried out from January to May 2017 at five selected schools and residential areas in Terengganu, a state in Peninsular Malaysia. A huge petrochemical complex in Kertih and Paka lies within the district of Dungun and Kemaman. The rapid development and economic growth in these districts since the 1980s have been primarily due to activities related to the oil and gas, and manufacturing industries. These activities boosted the human population in these districts with increasing numbers of immigrants moving into Terengganu to meet the job demand here.

According to the Department of Statistics, Malaysia (2010), Kemaman has a population of 161,959, while Dungun has a population of 149,851, of which 95% are Malays.

Despite experiencing rapid economic growth, both districts are facing the threats of air pollution due to emissions from industrial activities and the ever-increasing vehicles on the roads. This study was designed to assess the impact of air pollutant exposure among two groups of children in the two areas. The exposed group comprised children from primary schools located about 5 km from the industries, as correspond to the previous study (de Moraes *et al.*, 2010). They lived nearby and attended three schools referred to as *exposed schools*, viz., S1, S2, and S3. The comparative group comprised children from primary schools located in Dungun, about 30 km from the industries. The comparative area was represented by two schools referred to as *comparative schools*, viz., C1 and C2, as shown in the map (Fig. 1). All the selected schools were located within 1 km from the main road. Meanwhile, 73 respondents' residences located nearby the exposed and comparative area were selected for home indoor air quality assessment.

Respondent Recruitment

This study involved 176 children aged between 10 and 11 years old. The number of respondents required was determined by a sample size calculation of two population means (Lwanga and Lemeshow, 1991). The selection of the respondents was based on stratified random sampling as was used in previous studies (Ayuni *et al.*, 2014; Suhaimi *et al.*, 2015). Validated questionnaires, adopted from Juliana *et al.* (2001) and Jalaludin *et al.* (2014), on socio-demographic patterns, respiratory symptoms, and home exposure assessment were randomly distributed to the primary school children. The section of home exposure assessment comprised questions on indoor smoking; building material finishes (walls, and floor); open burning; and window opening during cooking. Those children who met the inclusion and exclusion criteria (free from history of doctor-diagnosed chronic disease, radiotherapy or chemotherapy in the previous 12 months, or X-rays in the previous 3 months) were recruited for buccal cell collection to determine the frequency of micronuclei (MN).

Exposure Assessment

The selection of air pollutant parameters is corresponding to six criteria air pollutants in the Air Pollutant Index (API) which has been used since 1989 in Malaysia. An indoor air assessment was conducted in the classrooms of the five selected schools during school hours from 8 a.m. to 1 p.m. (5 h). The classrooms have a typical design with two doors, louvre windows, ceiling fans for general ventilation, and a surface area of 64.3–67.2 m². The parameters for the indoor air monitoring included suspended particulate matter (PM₁, PM_{2.5}, and PM₁₀), gaseous pollutants (NO₂ and SO₂), and physical variables (temperature, relative humidity, and air velocity). The measurements of suspended particulate matter in the classrooms were carried out using a handheld instrument DustTrak™ DRX Aerosol Monitor 8532 (TSI Incorporated, USA). This lightweight instrument is equipped with a scattering laser photometer and can provide real-time

Fig. 1. Terengganu located in the east coast of Peninsular Malaysia (insert). Location of sampling site (primary schools) mark as S1, S2, S3, C1 and C2. Residential's sampling sites are symbolize as triangle, located nearby the schools.

aerosol mass readings. This device is capable of measuring aerosol concentrations of multi-size fraction ranging from 0.001 to 150 mg m^{-3} .

A handheld VelociCalc® Air Velocity Meter 9565 (TSI Incorporated, USA), equipped with multiple sensors, was used to monitor the air velocity, temperature, and relative humidity. This device with 0.01 m s^{-1} resolution is capable of detecting up to 78.7 m s^{-1} . The gaseous pollutants were measured using the LaMotte Nitrogen Dioxide and Sulfur Dioxide in Air test kit (LaMotte, USA), in which the air was slowly aspirated into a 10 mL mixture of absorbing solution at a flow rate of 0.2 and 1.0 L min^{-1} for NO_2 and SO_2 respectively. The principle of detection is colorimetric, in which the intensity of the resulting color of each gas is

compared with the LaMotte reference color chart. The measurements were carried out for about an hour each at three scheduled times during the school hours. All the equipment was placed at the back of the classroom at the height of 0.8 m from the floor to represent the breathing zone of the seated children. The air monitoring was conducted over 4 consecutive days.

Home Assessments

Home assessments were conducted to determine the micro-environment exposure of the respondents. Consent and approval were obtained from the guardians of the children prior to the home assessment. It involved 40 children's houses located nearby the exposed schools and 33 homes

situated nearby the comparative schools. The main assessment involved the collection of PM₁₀ using an Escort ELF Personal Air Sampling Pump (Zefon International, USA) at a flow rate of 1.7 L min⁻¹, equipped with a pre-weighed 37 mm diameter size filter paper. The filter was dried in an oven and then placed in a desiccator for at least 24 h prior to the measurement. It was then placed for 24 h (at breathing zone's height) at the location where the respondents spent most of their time when at home, e.g., in the bedroom and living room. The sampled filter was then dried in a desiccator for at least a day before its final weight was taken. The concentration of PM₁₀ was determined gravimetrically by the difference in weight of the filter paper before and after sampling.

Collection of Exfoliated Buccal Mucosa and MN Assay

The buccal mucosa cells were taken by gently scraping both the inner side of the cheeks with a sterile cytology brush. The brush was then dipped into a microcentrifuge tube containing 0.1 M phosphate buffer solution (pH 7.5) and stored in a -20°C freezer (Holland *et al.*, 2008). Next, the cells were smeared onto a glass slide followed by a fixation process with a cold solution of 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.5) for 20 min. The slide was then stained with a Feulgen solution using a modified procedure by Belien *et al.* (1995) and counterstained with 0.1% Fast Green for 20 seconds. Finally, the slide was observed under a light microscope at 40× magnification. The results were then expressed as frequency of MN in 1000 cells (Fig. 2).

Statistical Analysis

All the data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 22.0. A normality test was performed to assess the data distribution among the exposed and comparative groups. The information gathered would then determine the selection of subsequent statistical analysis, i.e., either parametric or non-parametric testing. The analysis comprised univariate, bivariate, and multivariate statistical testing in proving the relationship between air pollutant exposure and the occurrence of respiratory symptoms and micronuclei formation. The univariate analysis comprised

descriptive statistics for socio-demographic information, air pollutant concentration, and micronuclei frequency. The bivariate analysis involved mean or median comparison of the studied variables, specifically for continuous data. Meanwhile, categorical data such as respiratory symptoms and homes' distances were assessed through chi-square analysis. In addition, it was performed to evaluate whether the differences in micronuclei frequency have a relationship with air pollutants' concentration. Multiple linear regression was performed to assess the contributing factors of micronuclei frequency among the respondents.

RESULTS AND DISCUSSION

Socio-demographic

176 respondents were involved in this study. For the exposed group, 48 (47.5%) respondents were boys and another 53 (52.5%) were girls. For the comparative group, there were 34 boys (45.3%) and 41 girls (54.7%). Most of the children lived rather close to the main road, i.e., 37 (38.9%) for the exposed group and 39 (54.2%) for the comparative group. Proximity to a road can lead to higher levels of indoor air pollutants in the house due to emissions from the road vehicles being so much closer. A study conducted in Australia found that houses nearby busy roads exhibited both higher indoor and outdoor air pollutant concentrations compared to houses further away from the road (Cheng *et al.*, 2010). Most of the children in the exposed group lived more than 1.5 km from the industries, i.e., 53 (58.9%) children. Meanwhile, most of the children in the comparative group lived more than 5 km from the industries, i.e., 53 (77.9%) children.

Air Quality in Indoor Environment: Schools

The monitored results pertaining to suspended particulate matter (PM₁, PM_{2.5} and PM₁₀), gaseous pollutants (NO₂, SO₂) and physical parameters (temperature, relative humidity, air velocity) in the classrooms are tabulated in Table 1. The classroom of the exposed school S1, which is located less than 5 km from the industrial zone, recorded the highest PM₁ concentration at 43.33 µg m⁻³ as compared to other schools. Meanwhile, the average concentration of PM_{2.5} and PM₁₀

Fig. 2. Buccal epithelial cell with presence of micronucleus under 40× magnification (arrow indicates of micronucleus).

Table 1. Summary of indoor air pollutants concentrations in the classrooms.

School	Statistic	Exposed School			Comparative School	
		S1	S2	S3	C1	C2
PM ₁ (µg m ⁻³)	Min	25.00	16.25	20.00	14.33	14.00
	Max	120.83	140.00	191.00	106.44	80.50
	Avg	43.30	30.75	39.25	29.33	24.75
PM _{2.5} (µg m ⁻³)	Min	25.50	16.50	20.75	14.33	14.00
	Max	121.33	152.00	202.75	113.00	81.75
	Avg	44.83	32.00	41.00	29.67	25.50
PM ₁₀ (µg m ⁻³)	Min	28.00	17.25	22.75	14.67	14.75
	Max	241.67	296.00	425.25	215.00	116.75
	Avg	60.83	39.50	60.50	35.00	31.25
NO ₂ (µg m ⁻³)	Min	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Max	87.75				
	Avg	21.94				
SO ₂ (µg m ⁻³)	Min	130.93	78.56	340.44	< 0.01	78.56
	Max	130.93	130.93	340.43		78.56
	Avg	130.93	104.75	340.43		78.56
T (°C)	Min	28.00	28.50	28.94	27.50	27.80
	Max	31.20	31.40	28.94	33.00	31.80
	Avg	30.10	30.10	28.94	30.20	30.20
RH (%)	Min	66.6	71.20	77.70	58.50	65.30
	Max	72.4	81.70	77.70	84.40	81.00
	Avg	69.4	74.80	77.70	71.60	72.20
V (m s ⁻¹)	Min	0.37	0.16	0.25	0.28	0.033
	Max	0.78	0.17	0.45	0.36	0.36
	Avg	0.49	0.17	0.30	0.31	0.33

for S1 were 44.83 and 60.83 µg m⁻³, respectively. High concentrations of fine particles are usually contributed by outdoor infiltration of the pollutants into the indoor space (Almeida *et al.*, 2011). In addition, the outdoor pollutants easily penetrate the indoor environment due to a greater air exchange rate through the larger window area (Yang *et al.*, 2017). A study by Yang *et al.* (2017) demonstrated the high concentration of indoor particles in the classroom was confidently contributed by the outdoor source, with 0.9217 infiltration coefficient.

Two comparative schools (C1 and C2), located over 20 km from the industries, recorded the lowest particulate matter concentrations, ranging from 24.75 to 35.00 µg m⁻³ (Table 1). The concentrations in the comparative schools seemed to be relatively lower than the concentration of PM_{2.5} and PM₁₀ in Northern Iran (PM_{2.5}: 46.9 µg m⁻³ and PM₁₀: 397.2 µg m⁻³) due to seasonal differences (Mohammadyan *et al.*, 2017). Statistical analysis depicted a significant difference of particulate matter concentrations between the exposed and comparative schools. As shown in Table 2, the average concentration of particulate matter was found to be higher than those reported in the previous study (Yang Razali *et al.*, 2015). The mean particulate matter concentrations of those schools in Bandar Baru Bangi and Putrajaya, Malaysia, recorded during school hours were 16, 18, and 31 µg m⁻³ for PM₁, PM_{2.5}, and PM₁₀, respectively.

Furthermore, indoor gas measurement demonstrated the highest NO₂ concentration at the exposed school S1 (21.94 µg m⁻³), while the other schools exhibited low NO₂ value and under the limit of detection. The indoor NO₂

concentration recorded in this study did not exceed the recommended value by the WHO Guidelines for Indoor Air Quality of 200 µg m⁻³ and the Malaysian New Ambient Air Quality Standard of 320 µg m⁻³ for 1-h averaging time (DOE, 2020; WHO, 2006). Similarly, the NO₂ concentration in this study achieved the excellent criteria of the Hong Kong IAQ Objectives for Offices and Public Places, as the recommended NO₂ concentration is 150 µg m⁻³ (EMSD, 2003). In the absence of indoor sources, the NO₂ concentration found in this study indicated the infiltration of outdoor sources through windows and door openings (Wichmann *et al.*, 2010). Moreover, the location of S1 is adjacent to a high school and it is close to the main road, which also justified the high NO₂ concentration in the indoor classroom (Demirel *et al.*, 2014). In a recent study, a similar concentration of NO₂ was obtained (25 µg m⁻³) from the sampling site at the closest roadside to the cleaner industrial city, which was detected on working days (Azhari *et al.*, 2018). The highest indoor concentration of SO₂ was recorded at S3 (340 µg m⁻³), followed by S1 (130.93 µg m⁻³), S2 (104.75 µg m⁻³), and C2 (78.56 µg m⁻³). Generally, the SO₂ concentrations in this study, especially at the exposed schools, were lower than the concentration recorded in Suhaimi *et al.* (2015), with a median concentration of 332.57 µg m⁻³, as the pollutant source from that study was mainly generated by multisource industrial combustion such as bitumen and steel manufacturing. The proximity to the industry strongly explained the high concentration of colorless SO₂ at the exposed area was predominantly associated with industrial emission (Ielpo *et al.*, 2019).

Table 2. Comparison of the indoor air pollutants concentrations between the schools of the exposed and comparative schools.

Variable	Exposed schools		Comparative schools		<i>p</i> -value
	Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	Median (IQR)	
PM ₁ ($\mu\text{g m}^{-3}$)	46.90 $\pm$ 15.64	40.00 (28.00)	28.50 $\pm$ 4.47	26.00 (7.75)	0.006*
PM _{2.5} ($\mu\text{g m}^{-3}$)	48.82 $\pm$ 15.89	41.00 (28.00)	29.75 $\pm$ 5.36	27 (6.75)	0.005*
PM ₁₀ ($\mu\text{g m}^{-3}$)	69.73 $\pm$ 19.72	77.00 (35.00)	36.00 $\pm$ 6.44	34 (8.50)	< 0.001**
NO ₂ ($\mu\text{g m}^{-3}$)	31.91 $\pm$ 44.27	0.00 (0.14)	< 0.01	< 0.01	0.075
SO ₂ ($\mu\text{g m}^{-3}$)	197.59 $\pm$ 98.93	130.93 (209.5)	72.01 $\pm$ 49.98	78.56 (98.20)	0.002*
T (°C)	28.88 $\pm$ 0.86	28.91 (0.91)	29.06 $\pm$ 0.98	29.15 (1.66)	0.534
RH (%)	76.83 $\pm$ 3.43	77.70 (3.43)	75.69 $\pm$ 5.89	75.28 (9.51)	0.484
V (m s^{-1})	0.32 $\pm$ 0.19	0.26 (0.26)	0.54 $\pm$ 0.40	0.33 (0.61)	0.163

N = 20, Mann-Whitney *U* test, * significant at *p* < 0.05, ** significant at *p* < 0.00.

The physical parameters of the indoor air quality for each school are depicted in Table 1. The indoor temperature indicated a similar value range value from 27.50 to 33.00°C, while the indoor relative humidity recorded readings between 58.50% and 84.40% during the school hour. Both parameters statistically appear to indicate no significant differences between the two groups; however, the relative humidity and temperature in the classrooms generally exceeded the maximum comfort range value of temperature (23–26°C) and relative humidity (40–70%) as recommended in the DOSH guidelines (DOSH, 2010). In addition, the values also did not comply with the international standard, American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) Standard 55, as the recommended comfortability's temperature in summer ranges from 22.8 to 26.1°C (ASHRAE, 2013). Meanwhile, the indoor relative humidity levels should be maintained between 30% and 65% for optimum comfort as suggested by ASHRAE. The measured indoor air velocity for both exposed and comparative schools met the recommended air velocity in the DOSH guidelines, which is 0.15–0.50 m s^{-1} .

Home Assessments

The concentrations of the air pollutants observed in the homes (*n* = 73) of the children in the exposed and comparative groups are shown in Table 3. The mean concentration of the pollutants was observed to be higher for the exposed group, 108.39 $\pm$ 94.11 $\mu\text{g m}^{-3}$, than the comparative group, 46.15 $\pm$ 41.29 $\mu\text{g m}^{-3}$. The PM₁₀ concentrations, however, were still within the Malaysian Ambient Air Quality Standards (150 $\mu\text{g m}^{-3}$ for 24 h). The higher levels of pollutants observed in the homes of the exposed group could indicate high penetration of outdoor air pollutants into their homes through the windows and doors, which were normally left open during the daytime for better ventilation. The result of

Mann-Whitney *U* disclosed a significant difference between the two groups, with the exposed area recording higher indoor PM₁₀ concentration than the homes in comparative area. Their values, however, were lower than those observed in another study by Suhaimi *et al.* (2015). In that study, the location was near to heavy industries such as metal processing plants, an oil refinery and a gas processing plant. Vanker *et al.* (2015) demonstrated the daily PM₁₀ measured in the 592 homes in a peri-urban area of Cape Town, South Africa, was 33.1 (interquartile range, IQR 13–62.2) $\mu\text{g m}^{-3}$, which is lower than the PM₁₀ measured in this study. About 4 homes in this study had low particles concentrations of less than 46.15 $\mu\text{g m}^{-3}$, and this might be closely related to frequent cleaning as it may reduce dust accumulation on the floor and furniture surface.

The gas pollutant measurement depicted significant differences of NO₂ and SO₂ concentration between the residences in exposed and comparative areas. The NO₂ concentration of the exposed area were highly detected in those homes located in the vicinity of main road, which is less than 100 m. This is corresponded to anthropogenic source of NO₂ which mainly originated from vehicle combustion and secondarily from industrial emission (Ielpo *et al.*, 2019). However, the indoor NO₂ concentration in this study (20.78 $\pm$ 37.81 $\mu\text{g m}^{-3}$) were found to be extremely lower than in the study by Suhaimi *et al.* (2015) where the obtained value was between 58.29 and 165.48 $\mu\text{g m}^{-3}$. This could be due to the active emission of by-products of high-temperature combustion from the nearby industry in the Kemaman industrial area. Nevertheless, SO₂ concentration in the residences of the exposed area were found to be higher than concentration measured in previous study (Suhaimi *et al.*, 2015). The Mann-Whitney *U* analysis disclosed a significantly higher SO₂ concentration in the exposed area (141.96 $\pm$ 59.85 $\mu\text{g m}^{-3}$) as compared to those in the comparative area (130.93 $\mu\text{g m}^{-3}$).

Table 3. Comparison of indoor air pollutants concentrations between the homes of the respondents in the exposed and comparative groups.

Variable	Exposed Area (<i>N</i> = 40)		Comparative Area (<i>N</i> = 33)		<i>z</i> -value	<i>p</i> -value
	Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	Median (IQR)		
PM ₁₀ ($\mu\text{g m}^{-3}$)	108.39 $\pm$ 94.11	84.22(60.79)	46.15 $\pm$ 41.29	42.22(64.13)	−2.694	0.007*
NO ₂ ($\mu\text{g m}^{-3}$)	20.78 $\pm$ 37.81	0.00 (21.94)	< 0.01	< 0.01	−2.930	0.003*
SO ₂ ($\mu\text{g m}^{-3}$)	141.96 $\pm$ 59.85	130.93(0.00)	6.89 $\pm$ 30.04	0.00(0.00)	−5.233	< 0.001**

N = 73, Man Whitney *U* test, * Significant at *p* < 0.05, ** Significant at *p* < 0.001.

This study also considered other variables in their homes that might have possible effects on the indoor air quality. This part was assessed in the questionnaires. These variables included family members who smoke; types of materials for the walls, floors etc.; fuels for cooking; whether windows were opened during cooking; and whether there were carpets in the homes. Generally, all the possible indoor air pollutants

confounders did not exhibit any significant difference between the exposed and comparative groups.

Time Series for Particulate Matter Concentrations

The results of the 5-h sampling for particulate matter in the five different schools are shown in Fig. 3. Several observations could be made regarding the particulate trend

Fig. 3. 5 h time series on indoor PM₁, PM_{2.5} and PM₁₀ in classroom of 5 different schools.

Fig. 3. (continued).

during the sampling period. Relatively high concentrations were observed during the first 15–20 min when the children entered the classrooms, possibly due to a lot of movements to get to their seats and to get their books from the bookshelves, as well as the daily routine of sweeping the floor clean. The concentration of particulate matter was relatively low prior to recess time due to lower levels of physical activities as the children were more engrossed in their schoolwork. This finding is consistent to those observed by Yang *et al.* (2015) in that higher pollutants levels in the classrooms were contributed by the number of occupants inside and their activities, such as walking and running, which caused the particulate matter to be churned up into the air (Yang *et al.*, 2015). High levels of particulate were also observed during the break time due to the re-suspension of the particles caused by the movement of the children. This is consistent with the trend observed in previous studies (Ismail *et al.*, 2010; Yang Razali *et al.*, 2015). In addition, the active movement of the children also tended to increase the air turbulence, leading to high uplifting of indoor particles (Madureira *et al.*, 2015).

Comparison of Reported Respiratory Health Symptoms

The prevalence of respiratory health symptoms (cough, chest tightness, wheezing, and phlegm) among the respondents ($n = 176$) in the exposed and comparative groups was assessed using a set of standardized questionnaires. A significant difference in the prevalence of cough was observed when comparing the two groups, with prevalence ratio 2.52 (95% confidence interval [CI] 1.13–1.56). The prevalence ratio for chest tightness and phlegm were 1.80 (95% CI 0.53–6.07) and 1.15 (95% CI 0.45–2.98), respectively. Children in the exposed group with higher exposure to air pollutants were three times more likely to develop respiratory symptoms than those children in the comparative area, as parallel with an earlier study by Suhaimi *et al.* (2015). It was suggested that their proximity to the heavy industries could have worsened their respiratory health (de Moraes *et al.*, 2010; Rusconi *et al.*, 2011; Sopian *et al.*, 2016). A study by Ayuni *et al.* (2014) also found significantly high respiratory health symptoms among children living in proximity to a

petrochemical industry with the prevalence ratio for cough, phlegm, chest tightness and wheezing being 5.09 (95% CI 2.23–11.65), 9.66 (95% CI 2.10–44.46), 9.08 (95% CI 1.09–75.0) and 9.07 (95% CI 1.89–25.2), respectively. In addition, the incremental increase in air pollutant levels in the air have also been convincingly associated with the high occurrence of respiratory illness among non-asthmatic children, especially for 7-day average exposures to PM_{2.5}, PM₁₀, O₃, NO₂, and SO₂ (Prieto-parra *et al.*, 2017). A recent study by Luong *et al.* (2017) also observed an association between elevated levels of particulate matter and the occurrence of respiratory illness among children resulting in incremental respiratory hospital admission. One can thus infer that exposure to high concentrations of air pollutants from traffic or industrial emissions could worsen the respiratory health among children.

Micronucleus Assay

A micronucleus assay was carried out on 91 children who fulfilled the inclusive criteria. Children in the exposed group had an average micronuclei frequency of 5.02 ± 3.43 per 1000 cells, while those in the comparative group had 2.00 ± 1.56 . The Mann-Whitney U analysis found a significant difference in the median of the exposed group ($p < 0.001$). The results showed that the exposed group had a micronuclei frequency two times higher than a normal baseline value (a mean of 2.32 ± 0.28 per 1000 cells) of a similar age group as indicated in Gajski *et al.* (2013). Similar findings were also observed in 74 children aged 5–15 years old in a highly polluted province in Poland, with a mean micronuclei frequency of 6.56 ± 5.00 MN per 1000 cells (Mielzynska-Svach *et al.*, 2014). High exposure to biomass burning had also induced a significant increase in micronuclei frequency among a similar age group of children with a mean of 15.7 ± 8.3 MN per 1000 cells (Sisenando *et al.*, 2012). On the other hand, the comparative group had a relatively low micronuclei frequency, similar to the normal value of healthy children in a clean town in Brazil, i.e., 3 ± 4.1 per 1000 cells (Silva da Silva *et al.*, 2015).

To date, there is no specific cut-off point of micronuclei frequency that can be used as a reference to justify the genotoxicity at the microcellular level. As such, the

genotoxicity of buccal mucosa (micronuclei frequency) was categorized based on the median value of frequency measured, which was 3. The values that were higher than or equal to the median (≥ 3) were categorized as *likely present*, while the values lower than the median (< 3) were categorized as *unlikely present* (Table 4). The chi-square analysis indicated that there was a significant positive correlation between indoor air pollutants and micronuclei frequency. This finding supported the results of other studies regarding exposure to high particulate matter and high micronuclei frequency counted on buccal epithelial cells (Sisenando et al., 2012; Ceretti et al., 2014; Tan et al., 2017). One could thus infer that prolonged exposure to high levels of air pollutants could induce cumulative chromosomal damages and increase the risk of cancer among children living in the vicinity of polluting industries.

Multiple linear regression was performed to assess the contributing factors of micronuclei frequency among the respondents. The model contained five main indoor air pollutants (PM_{10} , $PM_{2.5}$, PM_{10} , SO_2 , and NO_2) that were monitored in this study. Statistically, there was an association between the indoor air pollutants (PM_{10} , SO_2 , and NO_2) and micronuclei frequency. Based on regression analysis, we found that NO_2 was the most significant air pollutant predictor to induce micronuclei formation with the adjusted odds ratio of 6.82 (95% CI 35.72–105.18) and R^2 value of 0.498, after controlling all possible confounders such as distance of the homes from the roads and industries, as well as indoor exposure from smoking and carpets. It has been suggested that NO_2 caused induction of micronuclei frequency or permanent DNA impairment on human nasal epithelial cell at 0.2 mg m^{-3} after being exposed for 3 h (Koehler et al., 2011). The high exposure to air pollutants, such as PM_{10} and SO_2 , had likely contributed to the high formation of micronuclei among the respondents, with adjusted odds ratio of 2.31 (95% CI 0.06–0.73) and 4.03 (95% CI 9.70–17.67), respectively.

Synergistic and additive effects of other air pollutants such as volatile organic compounds (VOCs), polyaromatic hydrocarbon (PAHs), and heavy metals emitted from the industry should be considered in this study. Inhalation of metal can interfere with catalytic site of DNA synthesis and may result in disrepair of DNA strand break. On the other hand, inhalation of PAHs can inhibit DNA methylation and induce DNA adduct (e.g., benzo[a]pyrene diol epoxide).

The failure of the cell to repair the DNA strand break, lesion and adduct can lead to induction of micronuclei (Fenech et al., 2016). This could be one limitation of the present study as the measurement of other pollutants were not investigated. Apart from that, the smaller sample size in this study may also limit the findings. Future studies with larger sample size, and additional application of genotoxic biomarker are warranted to authorize the findings.

CONCLUSION

This study reveals significant differences in the concentrations of indoor air pollutants (especially PM_{10} , $PM_{2.5}$, PM_{10} , and SO_2) between schools in the vicinity of an industrial park (the schools for the exposed group) and those situated farther away (the schools for the comparative group). The pollutant levels were significantly higher at former, particularly for PM_{10} (69.73 ± 19.72 vs. $36.00 \pm 6.44 \text{ } \mu\text{g m}^{-3}$; $p < 0.001$). Additionally, the mean PM_{10} concentrations at the homes of the exposed and comparative groups were 108.39 ± 94.11 and $46.15 \pm 41.29 \text{ } \mu\text{g m}^{-3}$, respectively. Poor air quality conditions increased the average micronucleus frequency among the children (5.02 ± 3.43 MN per 1000 cells for the exposed group vs. 2.00 ± 1.56 MN per 1000 cells for the comparative group), indicating a higher genotoxic risk, as well as the prevalence of respiratory symptoms (children in the exposed group were three times more likely to develop symptoms, especially coughing).

These results, after controlling for confounding factors (e.g., demographic, socio-economic, and lifestyle factors, and exposure to tobacco smoke), strongly suggest that exposure to industrial air pollutants (NO_2 , SO_2 , and PM_{10}) increases the risk of chromosomal damage for children residing in proximity to an industrial area. Specifically, statistical analysis reveals a significant association between micronucleus frequency and indoor concentrations of NO_2 (adjusted odds ratio of 6.82; 35.72–105.18, 95% CI), SO_2 (adjusted odds ratio of 4.03; 9.70–17.67, 95% CI), and PM_{10} (adjusted odds ratio of 2.31; 0.06–0.73, 95% CI). Furthermore, the pollutants we measured (both gaseous and solid) are proxy indicators of pollution in the area, as demonstrated by ambient data from continuous air quality monitoring stations and previous local studies.

This study fills the knowledge gap in epidemiological studies regarding the relationship between industrial air

Table 4. Association of school indoor air pollutants with frequency of MN among primary school children

Variable	Likely present (MN ≥ 3) Total (%)	Unlikely present (MN < 3) Total (%)	χ^2	p-value	PR	95% CI
High PM_{10} ($\geq 33 \text{ } \mu\text{g m}^{-3}$)	33 (78.6)	9 (21.4)	17.588	< 0.001*	2.265	1.495–3.431
Low PM_{10} ($< 33 \text{ } \mu\text{g m}^{-3}$)	17 (34.7)	32 (65.3)				
High $PM_{2.5}$ ($\geq 36 \text{ } \mu\text{g m}^{-3}$)	36 (73.5)	13 (26.5)	14.716	< 0.001*	2.204	1.392–3.490
Low $PM_{2.5}$ ($< 36 \text{ } \mu\text{g m}^{-3}$)	14 (33.3)	28 (66.7)				
High PM_{10} ($\geq 51 \text{ } \mu\text{g m}^{-3}$)	38 (74.5)	13 (25.5)	17.940	< 0.001*	2.484	1.507–4.094
Low PM_{10} ($< 51 \text{ } \mu\text{g m}^{-3}$)	12 (30.0)	28 (70.0)				
High SO_2 ($\geq 130 \text{ } \mu\text{g m}^{-3}$)	43 (68.3)	20 (31.7)	14.650	< 0.001*	2.730	1.406–5.300
Low SO_2 ($< 130 \text{ } \mu\text{g m}^{-3}$)	7 (25.0)	21 (75.0)				

$N = 91$, Chi-square test, * Significant at $p < 0.001$, Significant PR at 95% CI > 1 .

pollutants and genotoxicity among children in South East Asia. However, certain limitations must be noted, including the small number of respondents and our inability to investigate personal air pollutant exposure. Moreover, toxic pollutants that were not addressed in this study, such as PAHs and benzene, potentially influence the formation of micronuclei in children through additive and synergistic effects. Future research should quantify the carcinogenic air pollutants in the study area in order to evaluate the combined effects of exposure to industrial air pollutants on children. A comprehensive air quality assessment can be achieved by actively sampling a larger air volume, increasing the sampling duration (up to 24 h), determining the particulate matter composition (i.e., identifying heavy metals and inorganic and organic compounds), measuring toxic gases, and conducting source apportionment.

ACKNOWLEDGEMENT

The authors acknowledge the great support and assistance from the Environmental Health Lab, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia and Laboratory 2146, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. This study was funded by Universiti Putra Malaysia (project code: GP-IPS/2016/9504000).

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Received for review, December 25, 2019

Revised, February 25, 2020

Accepted, February 28, 2020