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Effects of Ambient $PM_{2.5}$ Collected Using Cyclonic Separator from Asian Cities on Human Airway Epithelial Cells

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ABSTRACT

Recent studies have shown that air pollution is intense and hazardous in Asia compared to other parts of the world due to the late and poor implementation of updated technology in automobiles and industry as well as to the high population density. Respiratory disease, including asthma, is exacerbated by air pollution. However, the effects of $PM_{2.5}$, especially on respiratory allergies in Asian cities, have not yet been examined in detail. In this study, airway epithelial cells were exposed to crude $PM_{2.5}$ particles collected by cyclonic separation from three different Asian cities, namely, Sakai, Bangkok, and Taipei. We compared the cytotoxicity and inflammatory potential of the $PM_{2.5}$ from these cities by measuring IL-6 and IL-8. The samples from Sakai and Bangkok caused cytotoxic effects at a dose of 75 $\mu g\ mL^{-1}$ and, moreover, induced the release of IL-6 and IL-8 even at low doses. The release of these two interleukins was highly associated with fluoranthene derivatives, microbial factors (endotoxin and β-glucan), metals (e.g., Ti), and organic (OC2 and OC3) and elemental carbon (EC1) in the $PM_{2.5}$. Thus, these components potentially contribute to cellular damage and a pro-inflammatory response in the airway epithelial cells, and the effect depends on $PM_{2.5}$ sources in the locations.

Keywords: Crude $PM_{2.5}$; Cyclone sampler; Cytotoxicity; Pro-inflammatory response.

INTRODUCTION

Asian countries suffer from the worse air quality mostly because of the poor implementation of regulations and time lag in introducing updated vehicle technology (Gautam *et al.*, 2016). Air pollution is more prominent in the most densely populated areas of Asia (Cohen *et al.*, 2017). The rapid growth of industries dramatically increased coal consumption in parts of Asia, reportedly in China, which is the major emitter of polycyclic aromatic hydrocarbons (PAHs) and other particulate matter with aerodynamic dias. $\leq 2.5\ \mu m$ ($PM_{2.5}$) (Wang *et al.*, 2014), raising $PM_{2.5}$ and secondary organic aerosol (SOA) generation locally and distantly. A study in Taiwan (Chen *et al.*, 2016) showed that $PM_{2.5}$ components, including PAHs, showed differences owing to various reasons such as high temperatures, humidity and high solar radiation. Moreover, the low mixing layer and thermal inversion in the winter can reduce atmospheric dispersion, and thereby trap air pollutants that lead to higher pollutant density than usual which cause various health effects. In additional to anthropogenic pollutants, Asian dust generated from China is an important factor that affects air quality in Asian countries, such as Japan, Korea, and Taiwan, especially in the spring (Park *et al.*, 2005; Watanabe *et al.*, 2011; Chien *et al.*, 2012). Asian dust contains large amounts of PM_{10}, a small amount of $PM_{2.5}$, and associated EC, OC, total carbon (TC), sulfate, nitrate, black carbon, and PAHs (Liang *et al.*, 2013).

Previous epidemiological studies have shown that air pollution is associated with adverse cardiovascular effects in Taiwan (Liu *et al.*, 2015). In Japan, respiratory symptoms were more prevalent in individuals living near busy roads than in those whose places of residence were exposed to less traffic, suggesting that traffic-related air pollution could be a risk factor for respiratory symptoms and lung function (Nakai *et al.*, 1999). However, the effects of $PM_{2.5}$ on respiratory allergy in Asian cities have not yet been studied adequately. As $PM_{2.5}$ is composed of a complex mixture of

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carbon nuclei associated with metals, ions, and organic, inorganic, and microbial components, the determination of the contributory factors and elucidation of pathophysiological mechanisms that exacerbate respiratory diseases are also necessary (Chowdhury *et al.*, 2017). It has been shown that metals and PAHs are important factors that cause respiratory allergy (Borgie *et al.*, 2015; Bowatte *et al.*, 2017).

A large amount of $PM_{2.5}$ is required to evaluate respiratory health in *in vitro* assays. However, it is difficult to collect large amounts of $PM_{2.5}$ by the conventional method of collecting on a filter. In addition, it is not possible to expose the crude particle when it is attached to the filter. Thus, the conventional method requires the extraction of $PM_{2.5}$ from the filter to evaluate the effect of $PM_{2.5}$ on respiratory health, which may lead to loss of components of $PM_{2.5}$ and differences in extraction efficiency among samples. The cyclonic technique is a promising alternative for collecting sufficient PM within a limited time to obtain particles of the required size. The crude particle can be directly exposed without extraction, and therefore the risk of loss of components during the extraction process can be eliminated. Researchers are currently investigating ways to improve the performance of cyclonic technique (Avci *et al.*, 2003; Zhao *et al.*, 2003; Fu *et al.*, 2016).

To best of our knowledge this study for the first time focused on the response of airway epithelial cells to crude $PM_{2.5}$ collected from three different Asian cities, namely Sakai in Japan, Bangkok in Thailand, and Taipei in Taiwan, using cyclonic technique. We compared the pro-inflammatory potential of $PM_{2.5}$ among the three cities and aimed to identify the components of $PM_{2.5}$ that contribute to respiratory and allergic diseases.

METHODS

Cell Preparation

The BEAS-2B cell line, which is derived from human bronchial epithelial cells transformed by an adenovirus (12-SV40 hybrid virus), was purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). To initiate cell culture, the vial containing cells was taken out from liquid nitrogen and added to serum-free LHC-9 medium (Life Technologies, Carlsbad, California, USA). LHC-9 medium is already supplemented with retinoic acid, epinephrine, gentamicin etc. The subculture was maintained in LHC-9 medium in an incubator in a 5% CO_2 atmosphere at 37°C. For particular experiments, the cells were seeded in 96- and 12-well collagen I-coated plates and incubated for 72 h to reach semiconfluence at the same conditions as those used for the subculture.

Collection and Characterization of $PM_{2.5}$

$PM_{2.5}$ was collected by using a cyclone sampler placed on the 4th-floor rooftop of a building in a residential area in Sakai. In Bangkok, the cyclone sampler was placed on the 7th-floor rooftop of a building on the roadside in a commercial district. In Taipei, the sampling locations were near the main road in a commercial area, with a river nearby. The collection sites are shown in Fig. 1. $PM_{2.5}$ was collected during different seasons within a span of 1 year as follows: in Sakai, between May 6, 2016, and May 24, 2016 (spring); in Bangkok, between August 1, 2016, and August 12, 2016, as well as August 15, 2016, and September 4, 2016 (rainy season); and in Taipei, between October 31, 2016, and January 10, 2017 (winter). A schematic diagram and image of the cyclone sampler used for the present study are shown in Fig. 2.

The collected $PM_{2.5}$ was characterized by ion exchange chromatography for ions, thermal/optical reflectance for organic and elemental carbon, inductively coupled plasma mass spectrometry (ICP-MS) for metals, and high-performance liquid chromatography (HPLC) for PAH. Microbial materials such as endotoxin and β-glucan were measured by Japan Pharmacopeia test.

Ions

The collected $PM_{2.5}$ was measured gravimetrically and extracted with 10 mL of ultrapure water. After 30 min of sonication and occasional stirring the filtered extract was collected as final volume of 6 mL and anion species (Cl^-, NO_3^-, and SO_4^{2-}) and cation species (Na^+, NH^{4+}, K^+, Mg^{2+}, and Ca^{2+}) were measured by ion chromatography.

Organic and Elemental Carbon

Thermal/Optical Carbon Analyzer (Atmoslytic Inc., Calabasas, CA, USA) produces four OC fractions (OC1, OC2, OC3, and OC4 at 120, 250, 450, and 550°C, respectively) in the He atmosphere. Elemental carbon (EC1, EC2, and EC3 fractions) was generated at 550, 700, and 800°C respectively at the 2% O_2, 98% He atmosphere. Samples were loaded in carbon analyzer using quartz fiber filter.

Metals

Particles were measured gravimetrically and went through microwave-assisted digestion using diluted nitric acid and hydrogen fluoride (3.5:1 respectively). Final volume was achieved as 50 mL and Na, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Mo, Sb, Cs, Ba, La, Ce, Hf, W, Pb and Cd were measured by ICP-MS.

PAHs

Particle mass was measured gravimetrically and extracted by 3 mL of dichloromethane. After 30 min of sonication and occasional stirring the extract was filtrated as a final volume of 5 mL. The extract was pressurized by nitrogen flow to remove dichloromethane and 1 mL of acetonitrile was added before fluoranthene, pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene were measured in HPLC.

Microbial Materials

Microbial materials such as endotoxin and β-glucan were measured by Japan Pharmacopeia test. As biological components of the $PM_{2.5}$ extract, we measured an endotoxin and a β-glucan by kinetic-turbidimetric method using Limulus Amebocyte Lysate.

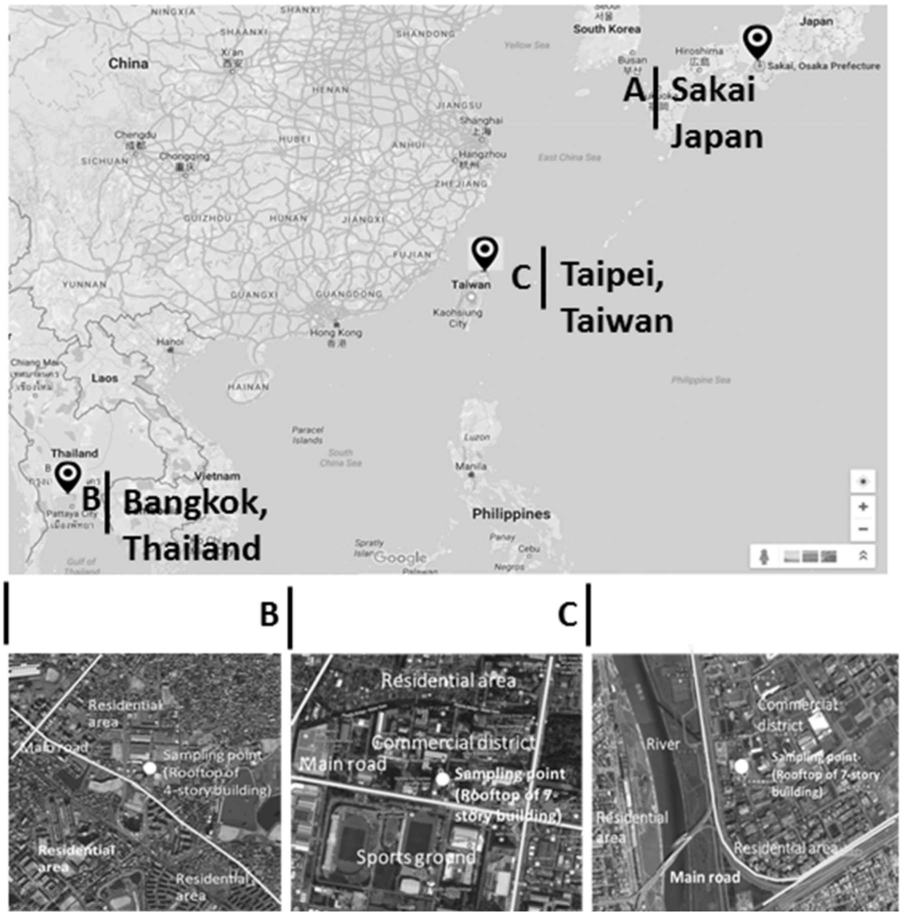


Fig. 1. Location of cities and consecutively the sampling point of each city (google map, 2017).

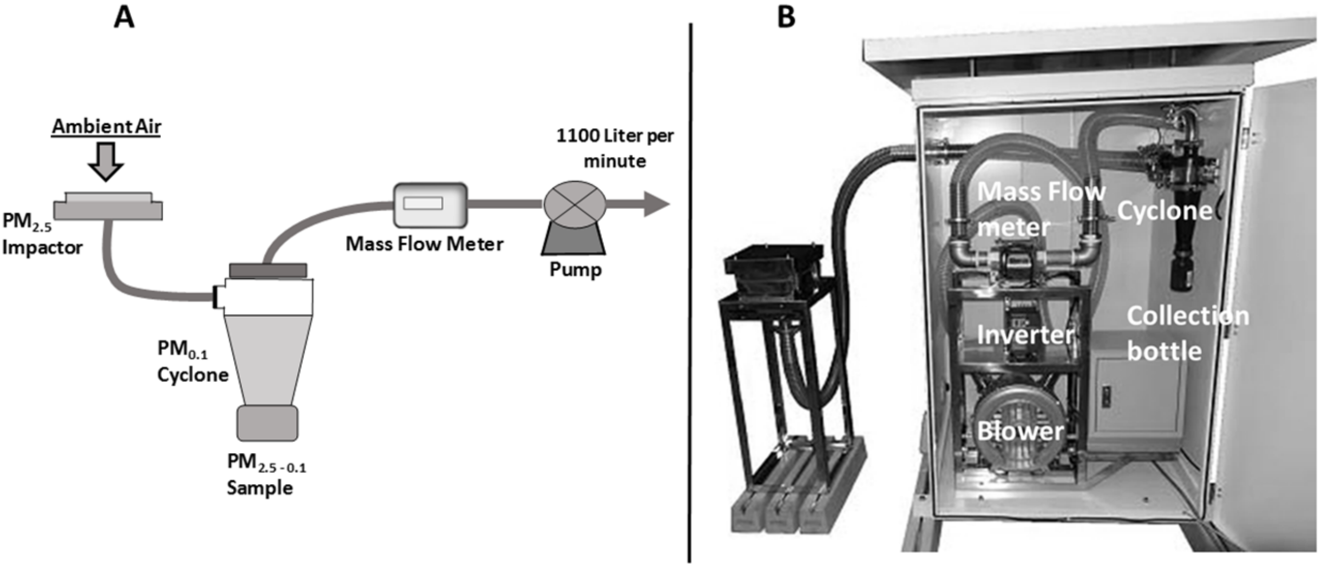


Fig. 2. A cyclone sampler used to collect $PM_{2.5}$ for this study. (A) Schematic diagram and (B) Photographs of the system (Okuda *et al*., 2015).

Exposure and Measurement

Cell Viability by WST-1

Cell viability was assessed by WST-1 assay. The BEAS-2B cell suspension containing 7.5×10^4 cells mL^{-1} was seeded at a density of 70 µL $well^{-1}$ in collagen I-coated 96-well plates and cultured for 3 days. On Day 3, the medium was discarded and cells were exposed to the sample solution at an equal volume (70 µL $well^{-1}$). Crude $PM_{2.5}$

was suspended in phosphate-buffered saline (PBS) and subjected to ultrasonication at a power of 40 for 3 min. BEAS-2B cells were exposed to $PM_{2.5}$ at doses of 0, 7.5, 22.5, or 75 µg mL^{-1}. WST-1 reagent was added after 21 h of exposure. The doses were selected based on our prior studies (Honda *et al.*, 2017; Chowdhury *et al.*, 2018). The amount of WST-1 reagent should be 1/10 of the sample volume (7 µL). After 3 h the absorbance of the plate was measured at 450 nm using a microplate reader (reference wavelength: 630 nm).

Quantification of Cytokines in Culture Supernatant by ELISA

Interleukin 6 (IL-6) is produced at the site of inflammation and plays a key role in various acute- and chronic-phase response via different signal-transduction pathways for instance, protein kinase C, cAMP/protein kinase A, and calcium ionophore (Gabay, 2006; Alfaro-Moreno *et al.*, 2009). Moreover, IL-6 exerts stimulatory effects on T- and B-cells which favors inflammatory responses (Gabay, 2006). IL-8 is an inflammatory chemokine, able to affects the function and recruitment of various inflammatory cells and fibroblasts (Moyer *et al.*, 2002). These molecules related to inflammation can affect exacerbation of asthma. IL-13 works as a central mediator of asthma though a cascade of biochemical pathway including regulation of immunoglobulin E (IgE) production, promoting migration of eosinophils into the lung, and upregulation of adhesion molecules which bind to eosinophils, increased flexibility of airway epithelial cells, higher mucus production, production of nitric oxide synthase by airway epithelial cells, collagen deposition in airway, proliferation of airway smooth muscle, and stimulation of airways hyperresponsiveness (Corren, 2013).

After exposure to crude $PM_{2.5}$, BEAS-2B cells were incubated for 24 h and centrifuged at 300 × *g* for 5 min before the supernatant was collected. The supernatant was stored at –80°C. The levels of IL-6, IL-8, and IL-13 released from BEAS-2B cells were measured by Quantikine ELISA kits (IL-6 and IL-8 from ThermoFisher Scientific Human ELISA kit). The detection limits for IL-6, IL-8, and IL-13 were < 2.00, < 6.44, and 30.57 pg mL^{-1}, respectively.

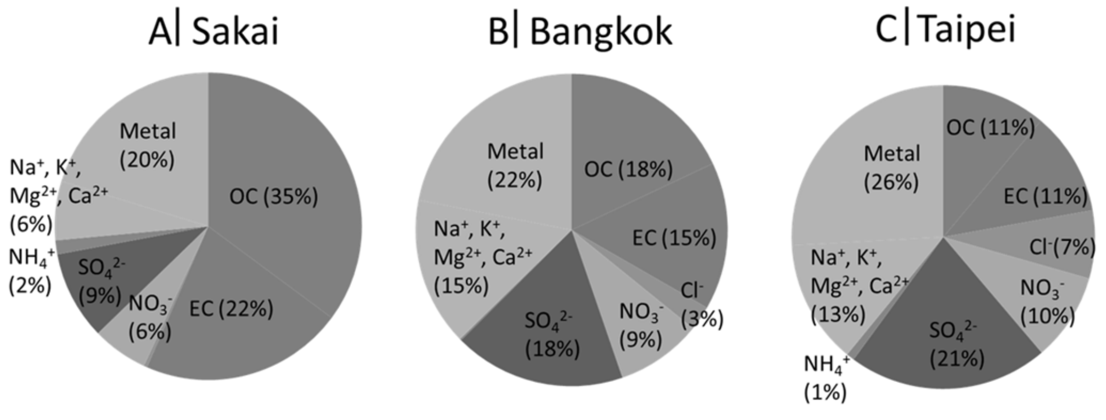
Statistical Analysis

The experiments for cytotoxicity and cytokine release were performed with multiple samples (n = 3–4). The average values ± standard error of the mean were calculated for all statistical analyses. The statistical significance was examined by Dunnett's multiple-comparison tests. $p < 0.05$ and $p < 0.01$ were considered significant, indicated in figures as "*" and "**," respectively. The correlation between $PM_{2.5}$ components and cytotoxicity and IL release were determined by using Pearson's correlation coefficient with two-tailed significance by using IBM SPSS software. The correlation analysis was conducted by pooling the samples. A source appointment study was also performed based on previous literature.

RESULTS

Characterization of $PM_{2.5}$

Characterization results showed the detail composition of $PM_{2.5}$ collected from the three cities Sakai, Bangkok, and Taipei. The mean mass concentration of $PM_{2.5}$ was 18.1, 16.1, and 15.6 µg m^{-3}, respectively. The components identified were OC1, OC2, OC3, EC1, EC2, EC3, EC4, inorganic ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Mg^+, Ca^{2+}), heavy metals, trace elements, and microbial elements (Fig. 3, Table 1). PAHs have been included in OC and shown separately in Table 2. The most abundant component of $PM_{2.5}$ was OC (35%) in Sakai, while that in Bangkok and Taipei was metals (22 and 26%, respectively). The OC/EC ratio in Sakai, Bangkok, and Taipei was 1.64, 1.18, and 0.99, respectively.



- Average $PM_{2.5}$ mass concentration: 18.1 µg m^{-3}
- Endotoxin and β-glucan: 14.20 EU mg^{-1}

- Average $PM_{2.5}$ mass concentration: 16.1 µg m^{-3}
- Endotoxin and β-glucan: 12.16 EU mg^{-1}

- Average $PM_{2.5}$ mass concentration: 15.6 µg m^{-3}
- Endotoxin and β-glucan: 0.6273 EU mg^{-1}

Fig. 3. Components of $PM_{2.5}$ collected from (A) Sakai, (B) Bangkok, and (C) Taipei.

Table 1. Density of organic and elemental carbon in ng mg^{-1}.

	OC1	OC2	OC3	OC4	EC1	EC2	EC3
Sakai	520	25,000	100,000	18,000	74,000	11,000	2300
Bangkok	0	12,000	52,000	21,000	65,000	6200	960
Taipei	UDL	6400	30,000	15,000	27,000	22,000	2800

UDL: under detection level.

Table 2. PAHs detected in $PM_{2.5}$.

PAH (ng mg^{-1})	Sakai	Bangkok	Taipei
Fluoranthene	1.1	0.79	0.54
Pyrene	0.62	0.98	0.39
Benzo[*b*]fluoranthene	2.7	2.3	0.67
Benzo[*k*]fluoranthene	0.70	0.82	0.17
Benzo[*a*]pyrene	0.47	0.88	0.14
Benzo[*ghi*]perylene	0.86	2.8	0.30
Total	6.45	8.57	2.21

We designated Ca, Mg, Na, K, Al, Fe, and Ti as crustal elements and V, Cr, Ni, Zn, Cd, Pb, and Cu as anthropogenic tracers. In this study, the concentrations of crustal elements were highest in Taipei (179.08 µg mg^{-1}) and lowest in Sakai (104.1 µg mg^{-1}). However, the ratio of crustal elements to anthropogenic elements was highest in Taipei (52.17), followed by Sakai (40.87) and Bangkok (20.82).

Effects on Airway Epithelial Cells

$PM_{2.5}$ collected from Sakai and Bangkok did not show adequate difference in cell viability, except at a concentration of 75 µg mL^{-1}. At this concentration, the samples collected from Sakai and Bangkok reduced cell activity by 11.82 and 4.36%, respectively (Fig. 4). $PM_{2.5}$ collected from all cities significantly increased IL-6 release at doses of 22.5 and 75 µg mL^{-1} ($p < 0.05$) (Fig. 5). Moreover, $PM_{2.5}$ collected in Bangkok significantly increased IL-6 levels even at a concentration of 7.5 µg mL^{-1}. In contrast, exposure to all doses of $PM_{2.5}$ samples collected from Sakai and Bangkok elevated IL-8 release (Fig. 6). However, exposure to Taipei sample increased IL-8 release only at a concentration of 75 µg mL^{-1} (Fig. 6). IL-13 levels in the cells remained unaffected after exposure to the three samples at all concentrations (data not shown).

Correlation Study Results

Pearson's correlation between cell viability, IL-6, and IL-8 with the components of $PM_{2.5}$ is shown in Table 3. Cell viability was negatively correlated with microbial factors, such as endotoxin and β-glucan, OC2, and OC3 ($p < 0.01$, Fig. 7). IL-6 and IL-8 release showed a positive correlation with multiple components, including PAHs, inorganic and organic carbon, microbial elements, and metals. Benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), EC1, microbial elements, and Ti demonstrated the highest correlation (> 0.9) with IL-6 release (Fig. 8). BbF, OC2, OC3, microbial factors, Ti, and EC1 showed the highest correlation with the IL-8 release (Fig. 9).

DISCUSSION

We performed similar sets of experiments and obtained comparable results in widely separated locations. In the present study, $PM_{2.5}$ collected at Sakai and Bangkok had cytotoxic effects only at the highest dose (75 µg mL^{-1}). In addition, this dose increased IL-6 and IL-8 release from airway epithelial cells. However, $PM_{2.5}$ collected from Taipei had comparatively lower potential to initiate respiratory inflammation via IL-6 and IL-8 release. The characterization

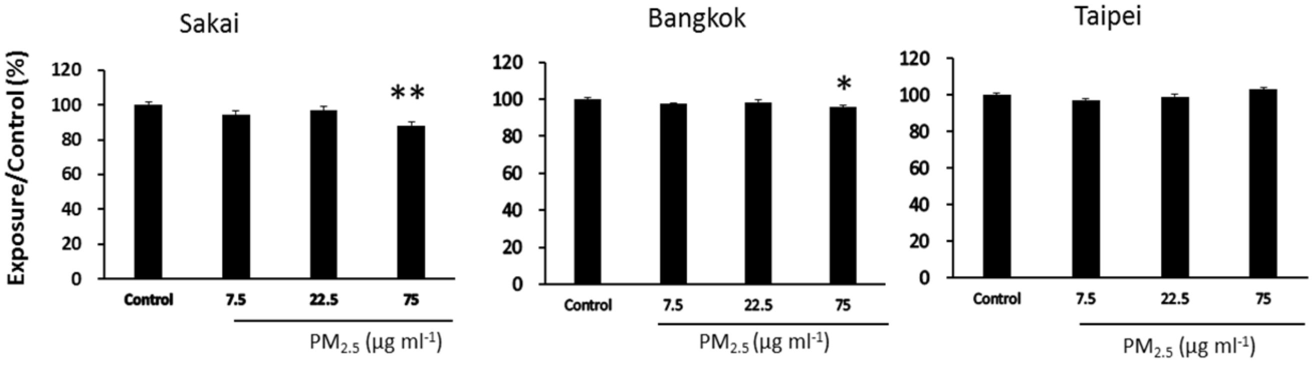


Fig. 4. Cell viability of BEAS-2B cells exposed to crude $PM_{2.5}$ of Sakai, Bangkok, and Taipei. *$p < 0.05$ vs. corresponding control, **$p < 0.01$ vs. corresponding control.

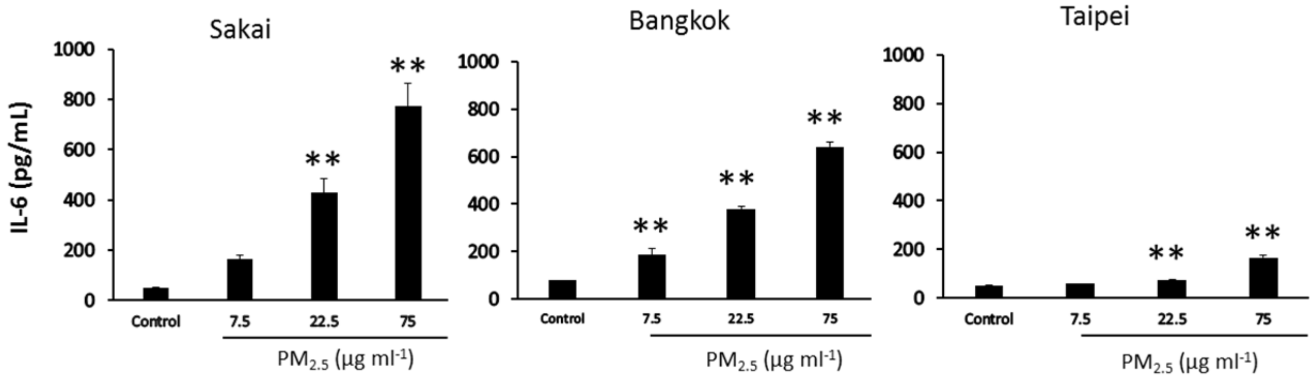


Fig. 5. Levels of IL-6 produced by BEAS-2B cells exposed to crude $PM_{2.5}$ of Sakai, Bangkok, and Taipei. **p < 0.01 vs. corresponding control.

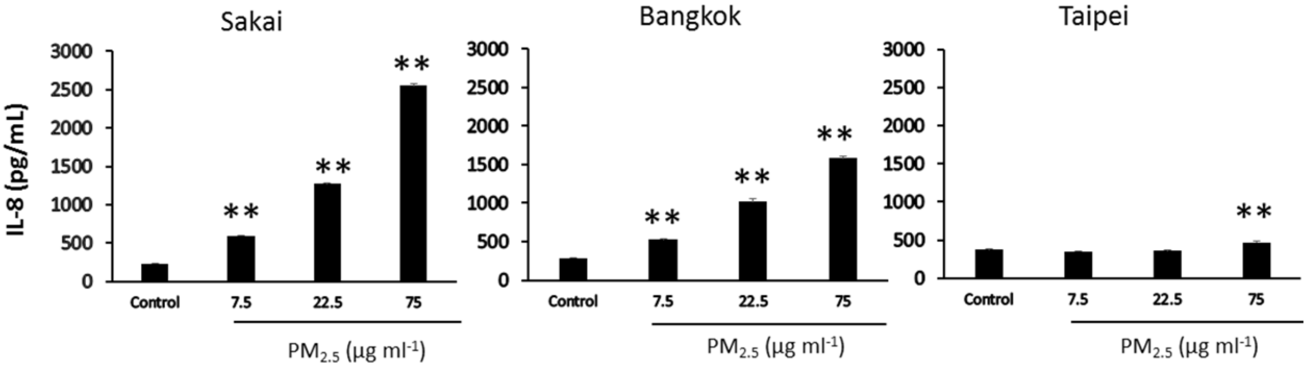


Fig. 6. Levels of IL-8 produced by BEAS-2B cells exposed to crude $PM_{2.5}$ from Sakai, Bangkok, and Taipei. **p < 0.01 vs. corresponding control.

of $PM_{2.5}$ revealed the following components: OC1, OC2, OC3, EC1, EC2, EC3, EC4, inorganic ions, PAHs, metals, trace elements, and microbial elements. From correlation studies, it was observed that cytotoxicity is correlated with microbial elements, OC2, and OC3. In contrast, IL-6 and IL-8 release were highly correlated with PAHs, e.g., benzene derivatives of fluoranthene, EC1, OC2, OC3, metals such as Ti, and microbial elements.

In the present study, we used crude $PM_{2.5}$ collected by cyclone method. Hence, the risk of contamination on the filter and during the process of extraction was avoided. *In vitro* experiments and characterization require adequate amounts of $PM_{2.5}$ for repeated exposure at different doses. Cyclone method is efficient for obtaining adequate $PM_{2.5}$ (Okuda *et al.*, 2015). Comparing the results of this study with our previous study (Chowdhury *et al.*, 2018), we concluded that crude $PM_{2.5}$ shows more profound biological effects than aqueous as well as organic extracts of $PM_{2.5}$. Aqueous extract of $PM_{2.5}$ exhibited cytotoxicity; however, both the extracts failed to show any pro-inflammatory response through IL-6 and IL-8 release. In the present experiment, crude $PM_{2.5}$ showed enhanced effects on cytokine release especially in higher doses, because particles have a marked effect on cellular events compared to extracts. An image of the cyclone sampler and representative diagram illustrating the process of cyclone separation described by Okuda (2015) are shown in Fig. 2. A study showed that $PM_{2.5}$ collected by the same cyclone model induced allergic airway inflammation (Ogino *et al.*, 2017).

Sakai is a city located in Osaka Prefecture of Japan, with a population of more than 800,000. It is a suburban area near Osaka city and one of the important seaports. Bangkok is the capital city of Thailand and has a population of over 8.28 million, almost 10 times more than Sakai. Taipei, the capital of Taiwan, has a population of 2.7 million, which is almost one third of the Bangkok's population. Thus, our study includes three cities with different population levels and geographical distribution. Some studies (Chuersuwan *et al.*, 2008; Wimolwattanapun *et al.*, 2011) reported that automobile (32%) and biomass burning (26%) are the two major sources of $PM_{2.5}$ in traffic sites of Bangkok, and biomass burning alone is the main contributor of $PM_{2.5}$ at residential sites. Although no source appointment studies have prominently shown the contributors of $PM_{2.5}$ in Taipei, a study (Hsu *et al.*, 2016) conducted in central Taiwan identified traffic and industry emissions as the major sources of $PM_{2.5}$. Japan and Taiwan suffered from Asian sand dust (ASD) in spring (Liang *et al.*, 2013; Lee *et al.*, 2015). A previous study reported that total suspended particles (TSP), secondary aerosols formed from SO_2, NO_x, and hydrocarbons from diesel exhausts, and small aerosols generated from factories are the sources of air pollutants in Sakai (Mizohata *et al.*, 1980).

The theoretic reconstruction of components from the

Table 3. Components of $PM_{2.5}$ with Pearson's correlation (highest to lowest) and statistical significance.

Components	Pearson correlation (viability)	P value	Components	Pearson correlation (IL-6)	P value	Components	Pearson correlation (IL-8)	P value
Endotoxin, β-glucan	–0.724*	0.027	Benzo[*b*]fluoranthene	0.952**	0.000	Benzo[*b*]fluoranthene	0.936**	0.000
OC2	–0.706*	0.034	Benzo[*k*]fluoranthene	0.930**	0.000	OC2	0.947**	0.000
OC3	–0.673*	0.047	EC1	0.926**	0.000	OC3	0.938**	0.000
Cl^-	0.649	0.059	Endotoxin, β-glucan	0.97**	0.000	Endotoxin, β-glucan	0.956**	0.000
Benzo[*b*]fluoranthene	–0.636	0.065	Ti	0.914**	0.001	Ti	0.901**	0.001
EC1	–0.566	0.112	Fe	0.893**	0.001	EC1	0.904**	0.001
Ti	–0.558	0.119	OC2	0.892**	0.001	Fluoranthene	0.872**	0.002
Benzo[*k*]fluoranthene	–0.551	0.124	OC3	0.892**	0.001	Benzo[*k*]fluoranthene	0.869**	0.002
W	0.521	0.150	Fluoranthene	0.867**	0.002	Fe	0.857**	0.003
Fluoranthene	–0.515	0.156	Rb	0.855**	0.003	Ce	0.861**	0.003
Na^+	0.512	0.159	Ce	0.861**	0.003	Mn	0.845**	0.004
Ce	–0.494	0.176	Mn	0.841**	0.005	La	0.793*	0.011
Mg^{2+}	0.493	0.177	Cs	0.838**	0.005	Hf	0.784*	0.012
Fe	–0.481	0.190	Benzo[*a*]pyrene	0.829**	0.006	Rb	0.779*	0.013
Mn	–0.481	0.199	Co	0.819**	0.007	Cs	0.776*	0.014
Na	0.430	0.248	K	0.809**	0.008	Co	0.748*	0.021
La	–0.414	0.268	Hf	0.813**	0.008	Ba	0.743*	0.022
Al	0.396	0.292	Pyrene	0.808**	0.008	K	0.735*	0.024
Benzo[*a*]pyrene	–0.381	0.311	Ba	0.802**	0.009	Ni	0.732*	0.025
Rb	–0.38	0.313	La	0.774*	0.014	Benzo[*a*]pyrene	0.714*	0.031
Hf	–0.367	0.331	Ni	0.764*	0.016	Pyrene	0.705*	0.034
NH_4^+	–0.365	0.334	OC4	0.755*	0.019	OC4	0.690*	0.040
Cs	–0.360	0.341	Cu	0.713*	0.031	Cu	0.620	0.075
Co	–0.322	0.398	Benzo[*ghi*]perylene	0.701*	0.035	NH_4^+	0.617	0.077
SO_4^{2-}	0.315	0.408	Ca	0.687*	0.041	V	0.602	0.086
Ba	–0.308	0.420	Ca^{2+}	0.681*	0.043	Mo	0.571	0.108
K	–0.304	0.426	Mo	0.682*	0.043	Benzo[*ghi*]perylene	0.550	0.125
Pyrene	–0.298	0.436	V	0.645	0.061	Ca	0.535	0.138
Ni	–0.296	0.439	Pb	0.600	0.088	Ca^{2+}	0.530	0.142
Se	0.295	0.441	Zn	0.575	0.105	Pb	0.460	0.213
EC2	0.272	0.478	As	0.517	0.154	As	0.414	0.268
OC4	–0.237	0.539	NH_4^+	0.508	0.163	Zn	0.409	0.274
Benzo[*ghi*]perylene	–0.232	0.549	K^+	0.496	0.174	EC3	0.394	0.294
Cr	0.202	0.602	Cd	0.895	0.175	Cd	0.384	0.307
NO_3^-	0.173	0.656	Sb	0.479	0.192	Sb	0.384	0.308
Ca	–0.171	0.659	NO_3^-	0.478	0.251	K^+	0.374	0.321
Cu	–0.156	0.688	EC3	0.352	0.353	NO_3^-	0.329	0.388
Ca^{2+}	–0.154	0.692	Cr	0.343	0.366	Cr	0.285	0.457
V	–0.134	0.732	Se	0.299	0.434	Cl^-	–0.251	0.515

Table 3. *(continued)*.

Components	Pearson correlation (viability)	P value	Components	Pearson correlation (IL-6)	P value	Components	Pearson correlation (IL-8)	P value
K^+	0.119	0.760	SO_4^{2-}	0.287	0.453	Se	0.202	0.602
Sb	0.117	0.765	Cl^-	–0.179	0.645	SO_4^{2-}	0.181	0.642
Cd	0.114	0.771	W	–0.140	0.720	W	–0.152	0.695
Mo	–0.106	0.786	Na	0.132	0.735	EC2	0.130	0.739
As	0.084	0.830	EC2	0.120	0.758	Na^+	–0.051	0.897
EC3	–0.008	0.983	Al	0.088	0.821	Al	0.048	0.902
Zn	–0.006	0.987	Mg^{2+}	0.056	0.886	Na	0.045	0.909
Pb	–0.002	0.996	Na^+	0.046	0.907	Mg^{2+}	–0.032	0.936

** and *: significant difference (**$p < 0.01$ and *$p < 0.05$) between components and cell responses through Pearson's correlation test.

characterization data of $PM_{2.5}$ and primary sources of pollution has been reported previously (Behera *et al.*, 2012). We used a similar method to identify the source of pollutants found in the three cities considered in our study. Our results showed that the concentration of crustal elements (Ca, Mg, Na, K, Al, Fe, and Ti) were higher than that of anthropogenic elements (V, Cr, Ni, Zn, Cd, Pb, and Cu) by 52.17, 40.87, and 20.82 times in Taipei, Sakai, and Bangkok, respectively. As the ratio of crustal elements to anthropogenic elements is the lowest in Bangkok, it is assumed that anthropogenic activity is high owing to high traffic. In contrast, the reason for the lowest concentration of crustal elements at Sakai (104.1 µg mg^{-1}) may be because of the advanced road structure and compact city planning in Japan. Taipei has the highest concentration of crustal elements as well as highest crustal to anthropogenic elements ratio possibly because of the dry and windy weather in winter. EC is generally emitted from primary combustion sources and stay in the atmosphere in the particulate form. As EC undergoes a limited secondary transformation, it is considered a good tracer for primary carbonaceous aerosols of combustion origin. In contrast, OC can be emitted from combustion as well as evaporation of fuels and solvents and often undergo secondary transformation (Turpin *et al.*, 1991). As the OC/EC ratio was 1.64 and 1.17 at Sakai and Bangkok, respectively, we can assume that the contribution from non-combustion origin (i.e., biogenic, soil and road re-suspension, long-range transport, and evaporation of fuel and solvents) is higher at Sakai and Bangkok than EC, which indicates the contribution from urban sources (i.e., vehicles and industry). The results of samples from Taipei showed the almost equal contribution from both non-combustion and combustion origin.

Furthermore, Liu *et al.* (2017) recently identified the sources of different types of OC and EC to determine the sources of aerosol components in Haikou, China. They assigned OC1 to biomass burning, OC2, OC3, OC4, and EC1 to gasoline-fueled vehicles, while EC2 was the most abundant species in the exhaust of diesel-fueled vehicles. OC2 is mainly derived from coal combustion. Based on these criteria, we can predict that biomass burning is negligible in Bangkok and Taipei. In addition, biomass burning is not a contributor to carbon emission in Sakai. By comparing EC1 and EC2, we suggest that gasoline exhaust contributes more than diesel exhaust in Sakai (EC1: 32%, EC2: 5%) and Bangkok (EC1: 41%, EC2: 4%). In Taipei, the contributions from both the sources were almost similar (EC1: 26%, EC2: 21%).

We observed that $PM_{2.5}$ collected at Sakai and Bangkok showed high cytotoxic potential possibly due to the high density of microbial elements and organic carbons. However, the amount of organic carbon that contains PAHs in $PM_{2.5}$ was the highest (35%) at Sakai among the three cities, whereas that at Taipei showed the lowest concentration (11%). The same pattern was observed for endotoxin and β-glucan concentration, i.e., 14.20 and 0.6273 EU mg^{-1} for Sakai and Taipei, respectively (Fig. 2). It has been reported that $PM_{2.5}$ with higher PAHs has cytotoxic potential (Kang *et al.*, 2010). It is evident that endotoxin and β-glucan can

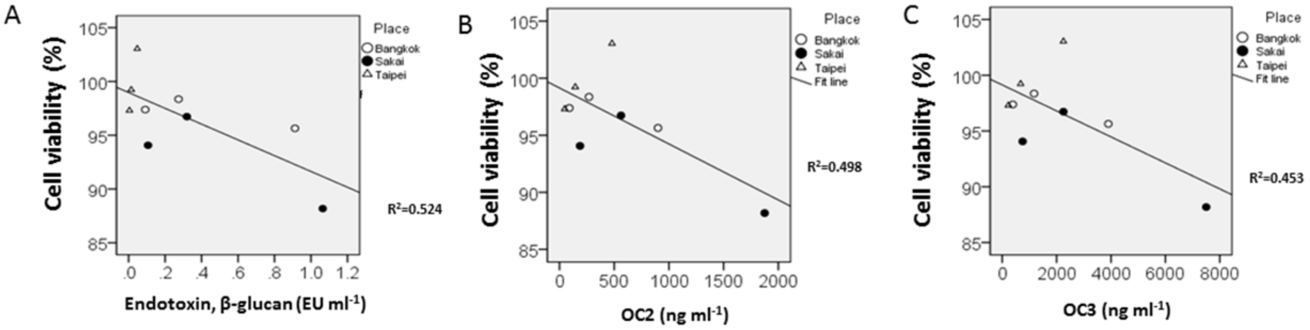


Fig. 7. A fit line representation of the correlation of cell viability and $PM_{2.5}$ components.

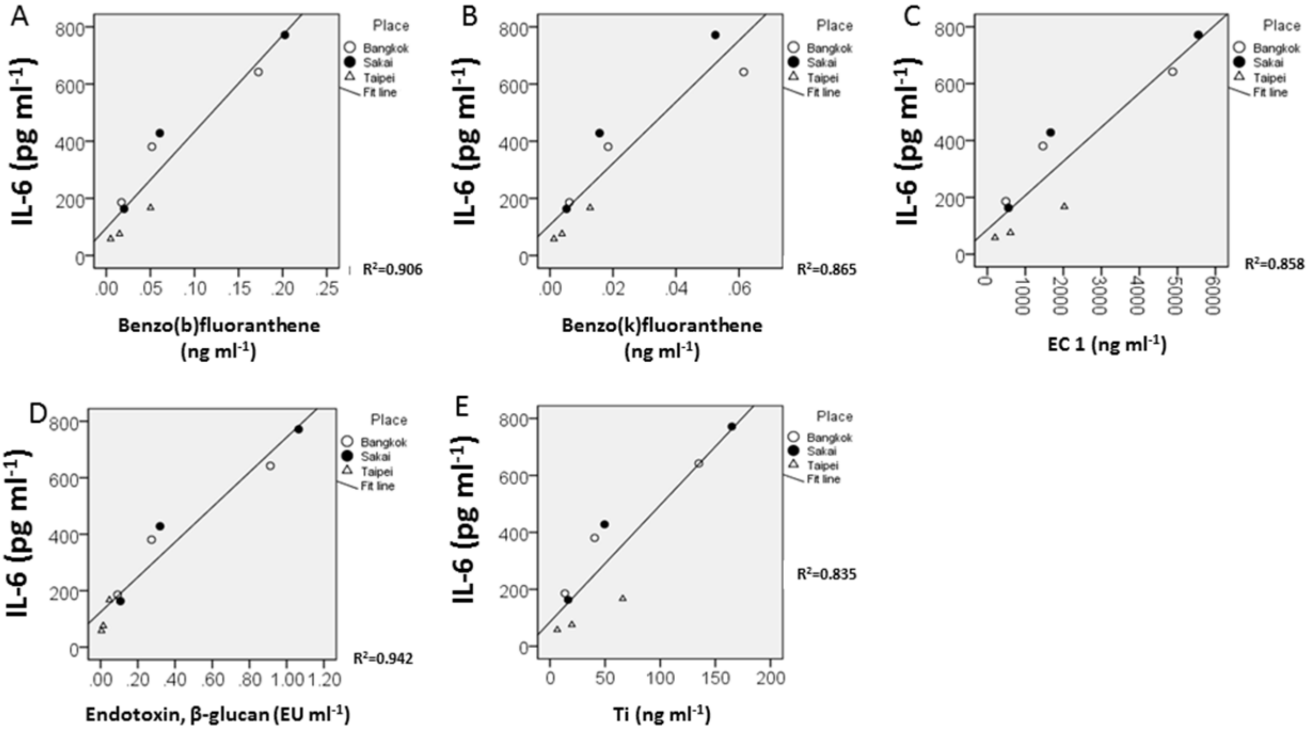


Fig. 8. A fit line representation of the correlation of IL-6 release and $PM_{2.5}$ components.

induce apoptosis via inflammatory events in macrophages (Murphy *et al.*, 2017); however, their effect on epithelial cells showed varied results (Lamkanfi *et al.*, 2010). Endotoxin interferes with histone-mediated cell death mechanism, especially in mammalian cells (Chen *et al.*, 2014; Burris *et al.*, 2015). β-glucan is known to be a powerful immune stimulant (Akramiene *et al.*, 2007). The combination of β-glucan and endotoxin resulted in hypersensitivity and inflammatory responses in airways (Fogelmark *et al.*, 1994). Thus, the conjugated mechanism of β-glucan and endotoxin may explain the significant release of IL-6 and IL-8 from the samples collected in Sakai and Bangkok.

The concentrations of PAHs were the lowest in Taipei among the three cities (Table 2). Jung *et al.* (2014) reported that non-volatile PAHs did not correlate with asthma; however, semi-volatile PAHs, such as pyrene, exacerbated asthma in children. We identified fluoranthene and pyrene as semi-volatile PAHs in all $PM_{2.5}$ samples, and they significantly correlated with IL-6 and IL-8 release (Table 3).

In this study, BbF and BkF correlated highly with IL-6 and IL-8 release. Previous studies have shown that BbF and BkF are the important PAHs present in diesel exhaust particles (DEP) and ambient $PM_{2.5}$ (Boland *et al.*, 1999; Yang *et al.*, 2013; Guo *et al.*, 2017). They are included in the 16 PAH priority pollutants listed by U.S. Environmental Protection Agency owing to their abundance in the air, as well as toxicity. Hence, it can be suggested that BbF alone or synergistically with other $PM_{2.5}$ components causes the pro-inflammatory response. BkF and fluoranthene showed high correlation with the expression of cytokines associated with pro-inflammation. Therefore, further studies are warranted to understand the effect of fluoranthene and its derivatives alone and synergistically.

EC1 was highly correlated with cytokine release. A previous study reported that carbon nuclei might induce IL-6 expression (Totlandsdal *et al.*, 2009) via involvement

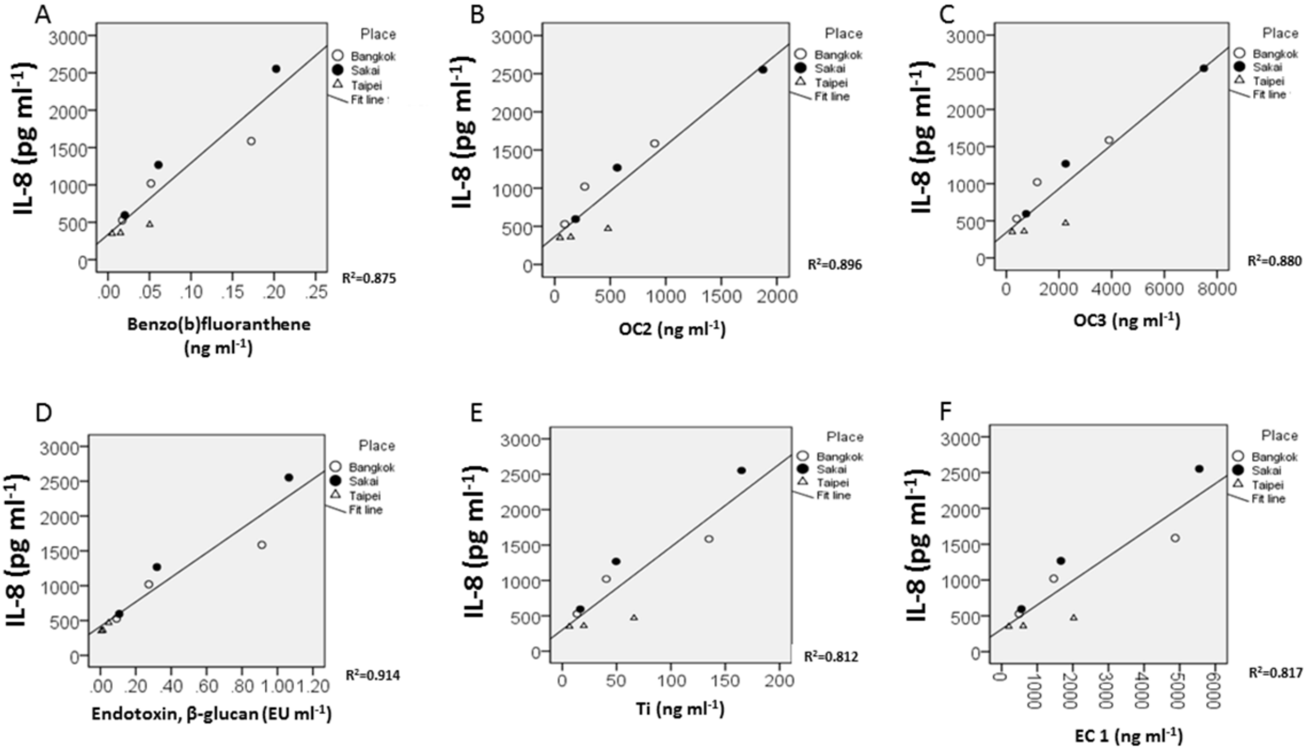


Fig. 9. A fit line representation of the correlation of IL-8 release and $PM_{2.5}$ components.

of NF-κB or mitogen-activated protein kinase (MAPK). Several studies have reported that IL-6 and IL-8 expression was elevated when exposed to DEP (Kim *et al.*, 2016). $PM_{2.5}$ was found to increase IL-6 and IL-8 release in BEAS-2B cells probably via regulation of n405968 gene on chromosome 4 in humans (Huang *et al.*, 2017).

From the present results, it can be suggested that besides the microbial factors and PAHs, organic and inorganic carbons, and metals can contribute to pro-inflammatory response. $PM_{2.5}$ from Taipei increased IL-6 release at doses of 22.5 and 75 µg mL^{-1}, while IL-8 release was increased at a dose of 75 µg mL^{-1}. Compared to microbial factors, ions and metals were higher in concentration in $PM_{2.5}$ collected from Taipei. The correlation result (Table 3) showed that Ti, Fe, Ce, and Mn were positively correlated ($p < 0.01$) with IL-6 and IL-8 release. Oxides of Ti (TiO_2) induced pro-inflammation in asthmatic mice (Jonasson *et al.*, 2013). Fe alone has not been documented to be responsible for direct inflammation; however, Dunea *et al.* (2016) concluded that $PM_{2.5}$ with a high content of heavy metals, including Fe, and its long-term exposure could exacerbate existing respiratory diseases. A recent study suggested that airborne Mn might affect respiratory health, thereby causing wheezing and asthma (Rosa *et al.*, 2016). Apart from Ti and Fe, Ce and Mn also correlate highly ($p < 0.01$) with IL-8 release. Vehicle emission is a source of Ce (Dale *et al.*, 2017); however, it has not been documented to pose health risks in humans.

This study compared $PM_{2.5}$ collected from different cities in different seasons. PM collection was not feasible simultaneously in same season in three different cities and so this study cannot compare the ambient $PM_{2.5}$ quality in same time of the year.

CONCLUSION

Overall, $PM_{2.5}$ collected from these three Asian cities caused cytotoxicity or a pro-inflammatory response in airway epithelial cells, with the effects differing between the cities. Cyclonic separation is an efficient technique for collecting crude $PM_{2.5}$ for exposure studies. It is possible that the release of IL-6 and IL-8 observed in this study was caused by fluoranthene derivatives, microbial factors, metal ions, OC2, OC3, and EC1 in the $PM_{2.5}$. These components may exacerbate respiratory disease, such as asthma. To confirm these conclusions, we suggest identifying the components correlated with carbon nuclei in airway epithelial cells through further exposure studies.

DISCLOSURE

The authors declare no competing financial interest.

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