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Chemical and biological characterization of air particulate matter 2.5, collected from five cities in China

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ABSTRACT

Fifteen polycyclic aromatic hydrocarbons (PAHs) in $PM_{2.5}$ samples collected in five different cities (Hong Kong (HK), Guangzhou (GZ), Xiamen (XM), Xi'an (XA) and Beijing (BJ)) in China in the winter 2012–12 were analyzed by gas chromatography-mass spectrometry. The biological effects of organic extracts were assayed using the human bronchial epithelial cells BEAS-2B. All sixteen priority PAHs can be found in the $PM_{2.5}$ samples of XA and BJ, but not in HK, GZ and XM, demonstrating the differential spatial source and distribution of PAHs. Our results showed that the total PAHs ranged from 3.35 to 80.45 ng/m^3 air, leading by BJ, followed by XA, XM, GZ and HK. In the cell culture study, transcript levels of pro-inflammatory cytokine interleukin-6 (IL-6), CYP1A1 and CYP1B1 were found to be induced in the treatment. The cells exposed to extracts from XA and BJ demonstrated significant migratory activities, indicating a sign of increase of tumorigenicity.

1. Introduction

Air pollution problem in Northern China has reached alarming levels. In the winter of 2012–13, transboundary air pollution from China was reported and became a global concern. A remarkable increase in the prevalence of lung cancers in the country in the past decades was found to be related to particulate matter (PM) with an aerodynamic diameter of less than 2.5 μm (Chen et al., 2013). The latest report from World Health Organization (WHO) stated that air pollution has become the world's single biggest environmental health risk, linked to around 7 million – or nearly one in eight deaths in 2012. The figures suggested that outdoor pollution from traffic fumes, coal and wood burning may kill more people than smoking and diabetes combined. Outdoor air pollution came as a result of stroke, cardiovascular and pulmonary disease and the vast majority was found to be in Asia and America. The situation worsens with the exponential growth in population together with the industrialization in this area.

It has been well known that ambient PM suspended in the atmosphere as aerosol are adversely affecting human health, especially to the respiratory and cardiovascular systems (Brunekreef et al., 2009; Langrish et al., 2012; Lee et al., 2007; Park et al., 2011). PM_{10} and $PM_{2.5}$ which are differentiated by the particulate diameters (μm) are commonly used in the air quality monitoring scheme all over the world (USEPA). Of which, $PM_{2.5}$, as compare to PM_{10}, can penetrate into the deeper area of the lung, directly affect the respiratory surfaces and dissolve into blood which may cause systemic toxic effects. According to the Environmental Protection Agency (USEPA), the ambient concentration of $PM_{2.5}$ was set as 35 μg/m^3 (24 h mean) and 12 μg/m^3 (annual mean) for national ambient air quality standard (USEPA, 2013). The pollutants which attached on the $PM_{2.5}$, which can pass all along the respiratory tract to the deeper alveolar sac, are the disease-causing reason of air pollution. Organic and inorganic pollutants were adsorbed on $PM_{2.5}$ found globally (Cachon et al., 2014; Chang et al., 2006).

Polycyclic aromatic compounds (PAHs) had been accepted as a class of ubiquitous environmental pollutants which are mostly products of energy production such as coal and petroleum, natural

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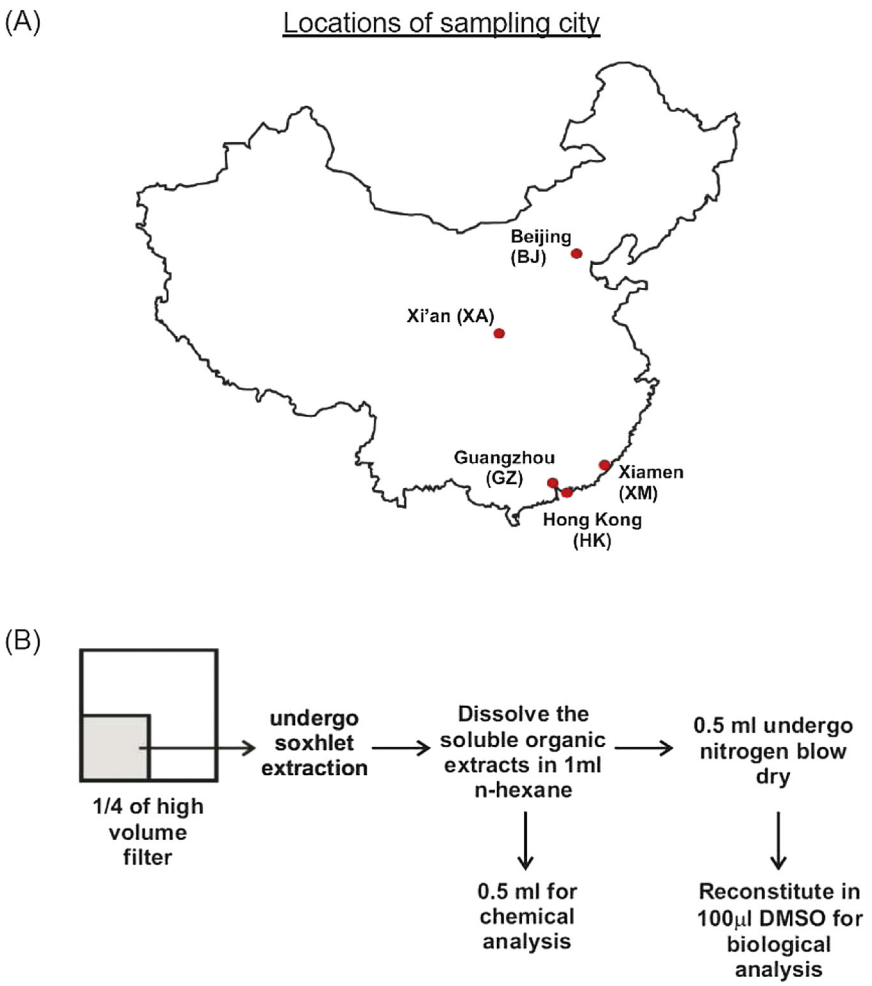

Fig. 1. Experimental set up of present study. (A) Location of five sampling cities on a China map. (B) The workflow of handling and processing the air filters. One fourth of the air filter we collected underwent soxhlet extraction and the organic extract was dissolved in n-hexane. Half of the dissolved extracts performed chemical analysis by GC–MS while the other half underwent nitrogen dry and reconstituted in 1 mL DMSO for biological analysis using cell line.

gas and fossil fuels, as well as cigarette smoking and waste incineration (Bostrom et al., 2002; Kong et al., 2010). The sources of PAHs production may varies from countries to countries; in Sweden, phenanthrene is the dominant PAHs found in 1994–2000 (Bostrom et al., 2002); while fluoranthene being the most abundant PAHs detected in Beijing, China (Wang et al., 2008). The USEPA has classified 7 PAHs as probable human carcinogens: benz(a)anthracene, benz(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene.

Due to its small size, $PM_{2.5}$ is able to penetrate to the lower respiratory tract to alveolar sacs and induce cell damage. There are two major routes of inhaled PAHs to the blood circulations. The majority of the PAHs deposits on the thinner alveolar epithelium where they readily absorbed and enter the circulation; while about 10–20% of PAHs inhaled will deposit on the thicker bronchial epithelium, slowly absorbed and transport through the epithelium. Due to the lipophilic properties of most PAHs, a fraction of compounds can be retained in the bronchial tissues and accumulated to attain a high local dose even at low environmental exposure levels (Bostrom et al., 2002). Industrial development and exponential growth in population in China raises the needs of energy and subsequent different kind of pollutions to the environment. On Feb 2014, Beijing recorded dangerously high level of suspended $PM_{2.5}$ for over 15 times of the recommended levels of WHO limits (25 μg/m^3 24 h mean $PM_{2.5}$) for consecutive 6 days (Chen et al., 2013). This leads to our concern to the air quality monitoring and its potential health effects to lung, which is the first organ encountered with the suspended pollution in air. Thus, in this study, human bronchial epithelial Beas-2b cell line was used to evaluate the cytotoxicity of $PM_{2.5}$ collected from the five cities in China.

2. Materials and methods

2.1. Air sample collection

Two northern (BJ and XA) and three southern (GZ, XM and HK) Chinese cities were selected in the present study (Fig 1A). The samples were taken for six to eight days during the air pollution episode of haze from the end of January to the beginning of February in 2013. The $PM_{2.5}$ samples were collected on quartz fiber filter (8 inch × 10 inch) using a high-volume sampler at a flow rate of 1.05–1.16 m^3 min^{-1}. Seven to eight air filters were collected from each sampling

Table 1
List of primers used in this study.

Primer	Forward primer sequences	Reverse primer sequences
CYP1A1	AGCAGCTGGATGAGAACGCC	GCCGTGACCTGCCAATCACT
CYP1B1	TTGTGCCTGTCACTATTCCTC	ATCAAAGTTCTCCGGGTTAGG
TNF-α	GGGCCTGTACCTCATCTACT	TAGATGGGCTCATACCAGGG
IL-6	AGCCCACCGGGAACGAAAGA	TGTGTGGGGCGGCTACATCT
IL-8	AAGCCACCGGAGCACTCCAT	CACGGCCAGCTTGGAAGTCA
h-Actin	GACTACCTCATGAAGATCCTCACC	TCTCCTTAATGTCACGCACGATT

sites. A sampling period of ~24 h was adopted at all sampling sites. The filters were then wrapped in aluminum foils for further analysis in our laboratory.

City	Location	Longitude	Latitude	Site description
Beijing (BJ)	Institute of Atmosphere Physics	116°23′09.25″	39°59′10.78″	Urban residential
Xi'an (XA)	Institute of Earth Environment	108°52′58.59″	34°13′49.36″	Commercial and residential
Guangzhou (GZ)	South China Institute of Environmental Sciences	113°21′18.14″	23°07′26.53″	Urban residential
Xiamen (XM)	Hu Li District Power Supply Bureau	118°06′08.04″	24°29′11.20″	Coastal
Hong Kong (HK)	Hong Kong Polytechnic Univ.	114°11′00.17″	22°18′11.49″	Roadside & coastal

The weights of filters were measured before and after the sampling procedure and were stored in desiccator cabinet at room temperature. Field blanks were also collected at each sampling site by putting the filters in the sampler without drawing air through. The workflow of the experiment was shown in Fig 1B.

2.2. Measurement of PAHs in air filters

One fourth of the air filters were extracted for 16–18 h at 68 °C with 60 ml of the solution mix (acetone: dichloromethane (DCM), n-hexane 1:1:1) in a soxhlet extractor, as described in the standard method 3540C (USEPA Method 3540C, 1996). The extracted solution was concentrated to 1 ml by a rotary evaporator, and was then followed by florisil cleanup (USEPA Method 3620B, 1996). The cleaned extract was dried under nitrogen gas and resuspended in 1 ml of n-hexane. Deuterated PAH internal standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}) were added into the extracts at the concentration of 320 ng/g prior to GC-MSD analysis (Hewlett-Packard (HP) 6890 N gas chromatograph (GC) coupled with an HP-5973 mass selective detector (MSD) and a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J&W Scientific Co. Ltd., Folsom, CA), as described in the Standard method 8270C (USEPA Method 8270C, 1996). The native PAH standards (naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorine (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b+k)fluoranthene (BbkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (Ind), dibenzo(a,h)anthracene (DbA), benzo(g,h,i)pyrelene (BghiP)) were used to obtain the standard curve with the concentrations of 0, 2, 5, 10, 20, 50, 100 and 200 ng/g. ***QA/QC analysis.*** The limit of detection (LOD) was determined as the concentration of an individual PAH detected in a sample with a signal-to-noise ratio of 3, ranging from 0.05 to 0.15 ng/g. The recoveries of individual PAHs were from 74 to 108%. For each batch of samples, a method blank (solvent), a spiked blank, a sample duplicate and a standard reference material were analyzed. The variation of coefficients of concentrations of PAHs of duplicate samples was less than 10%. The concentrations of PAHs in the method blank were less than the LOD. All the results were expressed as per one fourth of air filter.

2.3. Risk assessment

The risk assessment methodology advocated by WHO was used for assessing the excess lifetime cancer risk from exposure to PAHs. The unit risk of lung cancer suggested by WHO air quality guidelines is 87×10^{-6} per ng BaP per m^3 air. The lifetime cancer risk for lung cancer is then calculated by the equation below. The cancer risk was expressed as extra cancer cases occur in 100,000 exposed individuals.

$$\text{Cancer risk} = \text{BaP equivalents (ng/m}^3) \times 87 \times 10^{-6}$$

2.4. Cell culture and treatment

The human normal bronchial epithelium cell line, Beas-2b was grown in DMEM/F12 (HAM's) medium supplemented with 10% FBS (HyClone, Perbio, Thermo Fisher Scientific, Carmlington, UK) and antibiotics (50U/ml penicillin and 50 μg/ml streptomycin) (Invitrogen, CA, USA). For the soluble organic extractable matter in the extracts for biological assay, half of the extracts in n-hexane (0.5 ml) were dried under nitrogen and reconstituted with dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA.). Filter extracts from same region were combined to form a mixture for each region and approximately 500 μg of extracts were used for cell assay (DMSO <0.005%).

2.5. MTT assay

Effects of filter extracts on cell viability were determined by MTT assay. Human Beas-2b cells were plated into 96-well plates (Iwaki, Tokyo, Japan) at a density reaching 70–80% confluence by the time of adding 500 μg of extracts. After incubation for 24 h with extracts in 5% CO2 at 37C, the cells were incubated with 100 μl of 10% MTT solution (Sigma Aldrich) for another 4 h at 37 °C for color development. The medium was then removed and 100 μl of DMSO was added to dissolve the intracellular blue crystalline formazan product for 10 min at room temperature. Optical density was measured at 540 nm by absorbance microplate reader (BioTek ELx800). The results were presented as a percentage of the absorbance of control cells.

2.6. Cell migration assay

Cell migration was performed by using transwell chambers with 8 μm pore size (Costar) in a 24-well plate (Iwaki, Tokyo, Japan). Cells were seeded at 2×10^4 in 0.2 ml in the top chambers while the bottom chambers contained 0.7 ml of completed medium with 500 μg of extracts from different locations. Migration assay was done in duplicate. After incubation at 37 °C and 5% CO2 for 24 h, the migrated cells were fixed with ice-cold absolute methanol for 10 min and stained with crystal violet solution for another 10 min before mounted on glass slide. More than 10 views were analyzed under light microscope (100×) and number of migrated cells was scored. The mean value was expressed as percentage from two or three independent experiments as compared to the blank filter controls.

2.7. Real-time PCR

Beas-2b cells were plated at a density of 7 x 104 in a 12-well plate (Iwaki, Tokyo, Japan) and incubated at 37◦C, 5% CO2 for 24 h until the cells had reached 70–80% confluence. 500 μg of extracts were then added and incubated for 24 h. Total RNA was then isolated and measured. Total RNA with A260:A280 ratio between 1.8 and 1.9 was used for real-time PCR analysis. Complementary DNA was synthesized from 100 ng of total RNA using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, CA). Primers which are gene-specific were designed from published sequences. Real-time PCR was carried out with programmed time and temperature: 3min at 95◦C, 40 cycles of 95◦C for 15 s, 56◦C for 20 s and 72◦C for 30 s cDNAs from samples were quantified by StepOne Real-Time PCR system using SYBR Green Master mix (Applied Biosystems). Cycle numbers were then calculated and normalized by the transcript level of human actin. Sequences of primers were listed in Table 1.

2.8. Statistical analysis

Statistical evaluations were conducted by SigmaStat 3.5. All data were tested to be normally distributed and independent by using the Normal Plots in SigmaStat significance were 0.05. Differences among groups were tested for statistical significance by analysis of variance (ANOVA) followed by Duncan's Multiple Range test (significance at $p < 0.05$). Results are presented as the mean + SD. Groups were considered significantly different if $p < 0.05$.

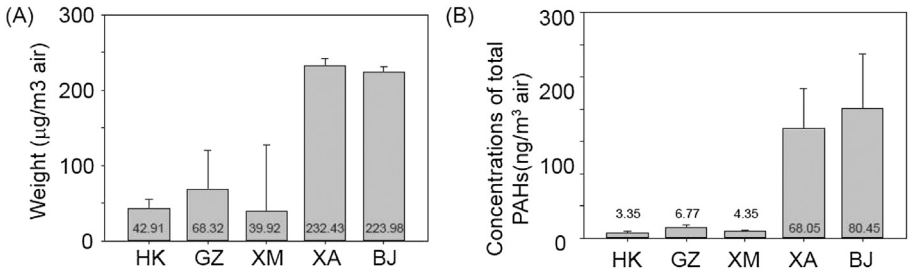

Fig. 2. Weights of $PM_{2.5}$ dust in air filters and the mean concentrations of total PAHs in each sampling site.

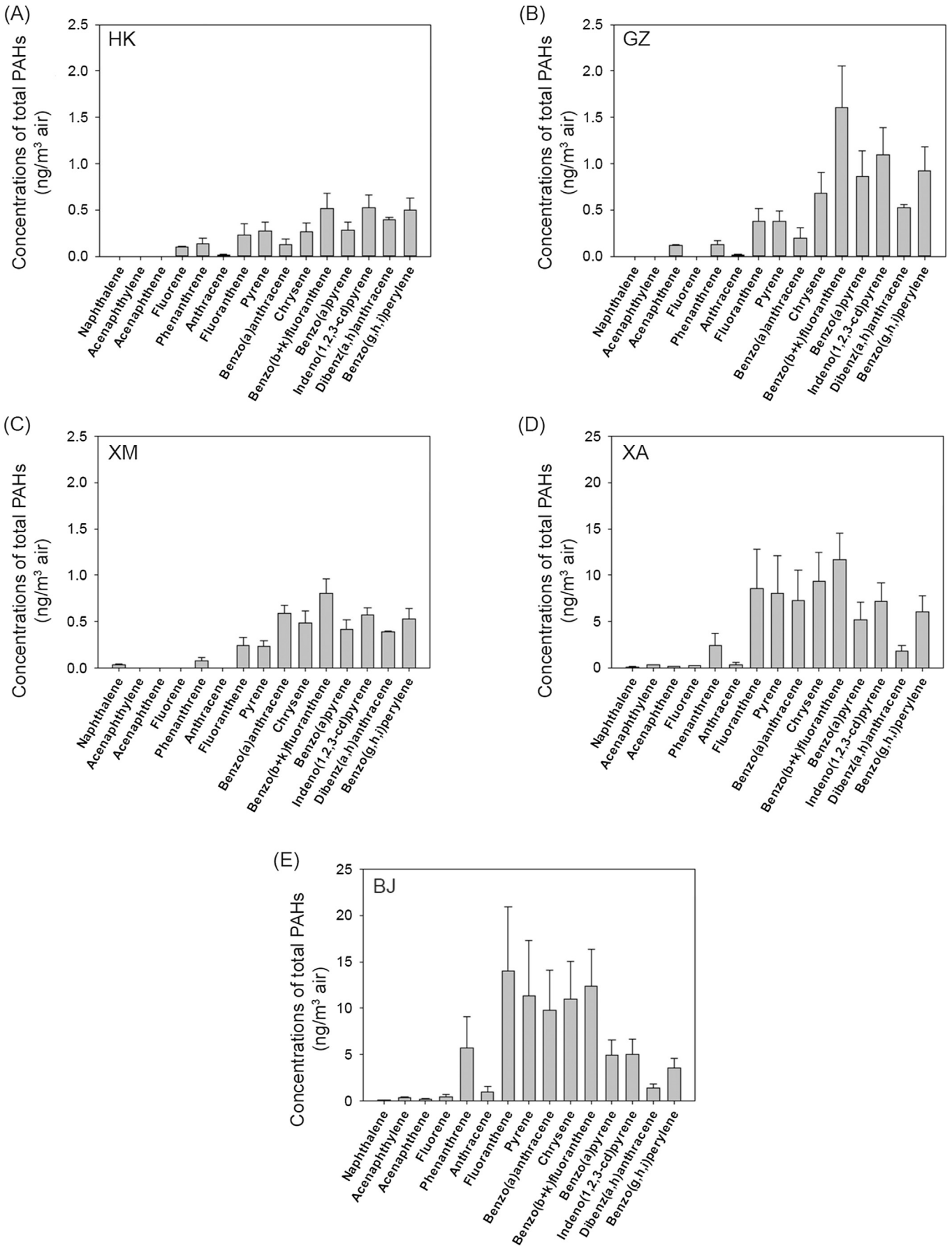

Fig. 3. Concentrations and distributions of PAH congeners in each sampling site.

Table 2
Levels of PAHs and TEQ (ppt) measured in the air samples from the five cities in China.

PAH Species	TEF	HK		GZ		XM		XA		BJ	
		PAH (ng/m^3)	TEQ (ppt)	PAH (ng/m^3)	TEQ (ppt)	PAH (ng/m^3)	TEQ (ppt)	PAH (ng/m^3)	TEQ (ppt)	PAH (ng/m^3)	TEQ (ppt)
Naphthalene		n.d		n.d		0.03		0.09		0.08	
Acenaphthylene		n.d		n.d		n.d		0.32		0.34	
Acenaphthene		n.d		0.12		n.d		0.12		0.20	
Fluorene		0.1		n.d		n.d		0.21		0.43	
Phenanthrene		0.13		0.12		0.07		2.45		5.74	
Anthracene		0.01		0.02		n.d		0.36		0.93	
Fluoranthene		0.23		0.37		0.25		8.56		13.97	
Pyrene		0.28		0.38		0.24		8.00		11.35	
Benz(a)anthracene	0.1	0.12	0.01	0.19	0.02	0.58	0.06	7.27	0.73	9.74	0.97
Chrysene	0.001	0.26	0.00	0.68	0.001	0.48	0.00	9.37	0.01	11.00	0.01
Benzo(b + k)fluoranthene	0.1	0.52	0.05	1.61	0.16	0.81	0.08	11.63	1.16	12.40	1.24
Benzo(a)pyrene	1	0.28	0.28	0.86	0.86	0.42	0.42	5.16	5.16	4.91	4.91
Indeno(1,2,3-cd)pyrene	0.1	0.53	0.05	1.10	0.11	0.57	0.06	7.13	0.71	5.04	0.50
Dibenz(a,h)anthracene	1	0.39	0.39	0.52	0.52	0.39	0.39	1.84	1.84	1.40	1.40
Benzo(g,h,i)perylene		0.49		0.92		0.53		6.06		3.54	
Total PAHs		3.35		6.77		4.35		68.05		80.45	
Total BaP equivalents(BaPeq)			0.79		1.68		1.00		9.61		9.04
Lifetime excess cancer risk (cancer cases per million exposed individuals)			6.8		14.6		8.7		83.6		78.6

All 16 PAHs were detected in the samples from XA and BJ, while PAHs with shorter carbon chains were undetectable in HK, GZ and XM samples (Fig. 3A–E). Total PAH concentrations were calculated by the summation of all 16 PAHs congeners, the data ranging from 3.35 to 80.45 ng/m^3, leading by BJ, followed by XA, GZ, XM and HK. Our results were relatively lower than previous studies on PAHs contamination in ambient air in China (Okuda et al., 2010; Sin et al., 2005; Wang et al., 2008).

3. Results and discussion

3.1. 16 USEPA PAHs were detected in $PM_{2.5}$ of samples from China

Air filters were collected from the five cities in southern (HK, GZ and XM) and northern (XA and BJ) China. The one fourth of each air filters collected was undergo standardized soxhlet extraction for PAHs and performed chemical and biological analysis. The weights of $PM_{2.5}$ collected on the filters were measured (Fig 2A). The weights of $PM_{2.5}$ per m^3 air collected in the northern cities of China were approximately 3 times higher than that of the southern cities, weighted in average of 228 μg and 50 μg respectively. The concentrations of 16 USEPA PAHs in $PM_{2.5}$ per m^3 air flow of the five selected cities were shown in Fig 2B and details were listed in Table 2.

Benzo(b + k)fluoranthene (BbkF) was the most abundance species among 15 PAHs measured with the highest concentrations detected in most sampling sites except HK and BJ, ranging from 0.52 to 12.40 ng/m^3. However, its levels were found to be noticeable higher (>12.40 ng/m^3) in the northern cities (XA and BJ) versus in all the three southern cites (<2 ng/m^3 air). Fluoranthrene is the most abundance species found in BJ with the mean concentration of 13.97 ng/m^3. Similar to a study conducted in 2006, the most abundant quantified PAHs in $PM_{2.5}$ in BJ city was Fla, at which this species of PAHs is closely associated with the increase uses of natural gas (Wang et al., 2008).

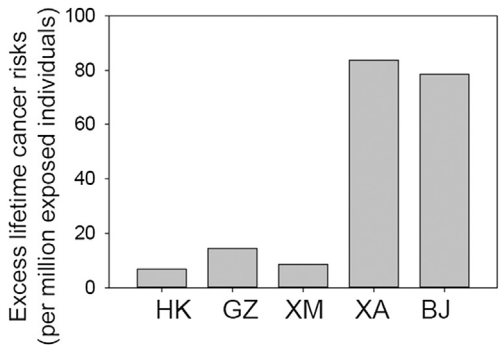

Fig. 4. Calculated excess lifetime cancer risks (per million exposed individuals) of the five sampling sites.

PAHs concentrations of samples from northern China were at least 10 times higher than that from southern China, showing less severe pollution in the southern cities. The higher PAHs contaminations in northern cities may be due to more coal and wood combustions for heat production in the winter, comparing to the lesser demand on heating in the relatively warmer southern cities. In addition, contaminations in the southern cities which are closer to the coastal area are readily dispersed by the sea wind verses inland northern cities which tends to accumulate the polluted air masses to local areas. Topography and climate differences may also contributed to the difference in levels of PAHs contaminations in mainland China. The average temperature and precipitation in January was much lower in BJ and XA (average temperature below 0 °C and 7 inches for average precipitation) than in the three southern cities (average temperature above 12 °C and precipitation above 24 inches). Higher rainfall in southern China brings ambient air particulate matter to the ground, may contribute to the lower PAH concentrations in these cities. Furthermore, BJ was geographically surrounded by three mountains, polluted air mass produced was difficult to be dispersed and led to a higher chance of smog accumulations, thus more $PM_{2.5}$ collected and higher PAHs concentration in our samples.

PAHs in ambient air can be in both gaseous and particulates forms. PAHs in particulates were of higher molecular weight and greater carcinogenicity (Chang et al., 2006). PAH congeners with carbon chain shorter than 14, noted naphthalene, acenaphthylene, acenaphthene and fluorene, were found in much lower concentrations than that with longer carbon chain (highest concentration with 0.43 ng fluorine/m^3 air, with most of them were in undetectable level). However, PAHs with longer carbon chain or higher molecular weight were found in higher concentrations in all five sampling sites.

3.2. Cancer risks via inhalation of PAHs

Data from a number of health studies suggested that there is an association between lung cancer and exposure to PAH compounds

Table 3
Diagnostic ratios of PM-bounded PAHs in different cities of China.

Diagnostic ratio	Ind/(Ind + BghiP)	BaP/BghiP	Flu/(Flu + Pyr)	Ant/(Ant + Phe)	BaA/(BaA + Chr)
Gasoline engine	>0.18	0.5–0.6	<0.5	>0.5	>0.49
Diesel engine	0.35–0.7	0.3–0.4	>0.5	>0.35	>0.68
Coal burning	>0.56	0.9–6.6	>0.57	>0.24	>0.46
Non-burned fossil fuels inputs	–	>0.58	–	<0.1	–
Natural gas combustion	>0.32	–	>0.49	>0.12	>0.39
HK	0.52 ± 0.009	0.55 ± 0.039	0.30 ± 0.093	0.098 ± 0.013	0.31 ± 0.029
GZ	0.54 ± 0.008	0.95 ± 0.078	–	0.13 ± 0.027	0.19 ± 0.08
XM	0.52 ± 0.025	0.78 ± 0.080	–	–	0.55 ± 0.026
XA	0.54 ± 0.011	0.87 ± 0.134	0.03 ± 0.01	0.13 ± 0.022	0.42 ± 0.036
BJ	0.59 ± 0.011	1.37 ± 0.060	0.04 ± 0.005	0.14 ± 0.031	0.46 ± 0.042

(Zhang and Smith, 2007; Zhang et al., 2009). The most important exposure route for lung cancer would appear to be via inhalation. Most of the PAHs are known to be associated with airborne particles. According to USEPA, some of the PAHs are classified as human carcinogens such as BaP, BaA, BbkF, Chr, InP, and DbA. BaP, a probable human carcinogen found in appreciable concentrations in the atmosphere, can be used as a marker of the carcinogenic risk of airborne PAH. A previous study suggested that an average of 6.5 million people in China was diagnosis with lung cancer due to inhalation of ambient PAHs (Zhang et al., 2009). Total BaP equivalent measures the relative toxicity of PAHs to BaP. The total BaP equivalents (BaPeq) determined by toxic equivalent factors (TEF) and the concentrations of individual PAHs was listed in Table 2. The data from XA (9.61 ppt) show the highest calculated BaPeq among five cities, followed by BJ (9.04 ppt), GZ (1.68 ppt), XM (1.00 ppt) and HK (0.79 ppt) with the lowest. Excess lifetime cancer risk calculated based on total BaPeq of each city was shown in Fig 4. For XA and BJ, the estimated lifetime cancer risk were the highest, reaching 83.6 and 78.6 extra cancer cases per 100,000 exposed individuals; while HK, GZ and XM were with much lower exposure risk at 6.8, 14.6 and 8.7 extra cancer cases in 100,000 exposed individuals respectively. The guideline value for ambient BaP was set to be 0.1 ng/m^3, thus the acceptable risk of exposure to PAHs via inhalation was 1.0e-05. Our result suggested if the risk is higher than the maximum acceptable risk level of 1 per 100,000 exposed individuals, more legislation on the air quality control on PAHs-bound PM emission is needed to control the pollution.

3.3. Coal combustion and vehicle emission were the common sources of PAHs in PM 2.5

Analyses of the diagnostic ratio were widely used to give an idea on the possible emission sources of PAHs in environmental monitoring (Akyuz and Cabuk, 2008; Vasilakos et al., 2007). Five diagnostic ratios were calculated and shown in Table 3. For Hong Kong, the ratio BaP/BghiP (0.55) and Ant/(Ant + Phe) (0.098) indicated gasoline engine and non-burned fossil fuels contributed to the major sources of emission. On the other hand, the sources of PAHs in other cities were mainly due to the burning of coal and natural gas combustion. Diagnostic ratios shown in Fig. 5 illustrated the source of PAHs in XM, XA and BJ are mainly from grass, wood and coal combustion; whereas the source in HK and GZ are mainly from mixed sources of both combustion and petroleum for vehicles.

3.4. Gene expression levels of pro-inflammatory cytokine and xenobiotic metabolizing enzymes were altered by $PM_{2.5}$ exposure

Human bronchial epithelial cells which were usually used in *in vitro study* to determine the pro-inflammatory and cytotoxic effects, were exposed to 500 μg of $PM_{2.5}$ extracts for 24 h, gene expression levels of pro-inflammatory cytokines and xenobiotic metabolizing enzymes were investigated. Samples from all locations were found to significantly induce the gene expression level of interleukin-6 (IL-6), but not IL-8 and tumor necrosis factor-alpha (TNFα) (Fig. 6A). In addition, gene expression levels of xenobiotic metabolic enzymes (CYP1A1 and CYP1B1) were also up-regulated by $PM_{2.5}$ extract treatment for 24 h (Fig. 6B). Recent studies demonstrated the PAHs adsorbed on particulate matter can lead to cell damage, induce cytotoxicity and pro-inflammatory responses (Cachon et al., 2014; Osornio-Vargas et al., 2011).

Initiation of inflammatory response involves in the induction of various cytokines and chemokine. Other studies reported the evaluation of the relationship between PM exposure and cytokines induction (Cachon et al., 2014; Dergham et al., 2012; Michael et al., 2013). IL-6 and IL-8 were both critical pro-inflammatory cytokine produced by epithelial cells to stimulate inflammatory response. In the present study, IL-6 was significantly induced by $PM_{2.5}$ extracts,

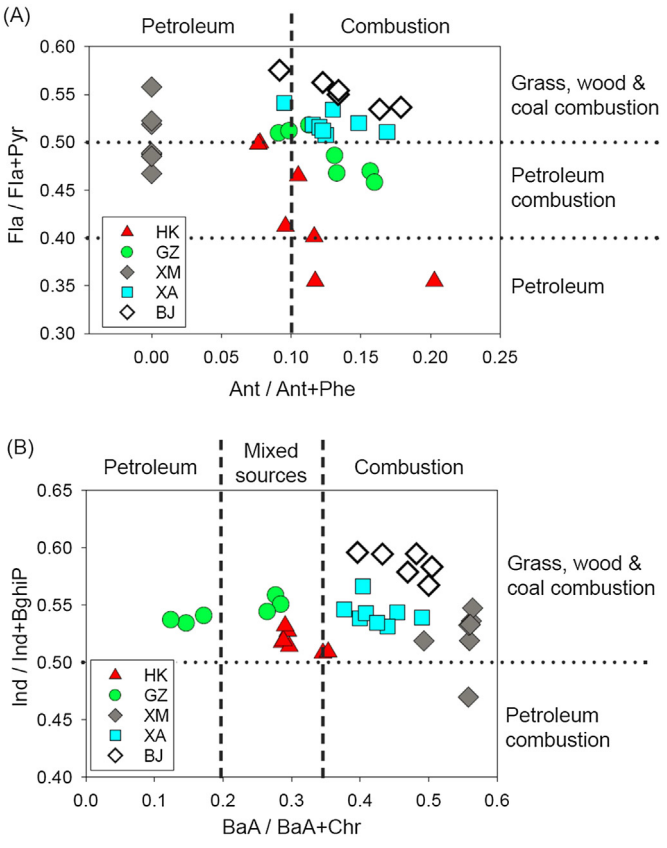

Fig. 5. Graphic illustration of the diagnostic ratios on the sources of PAHs emission.

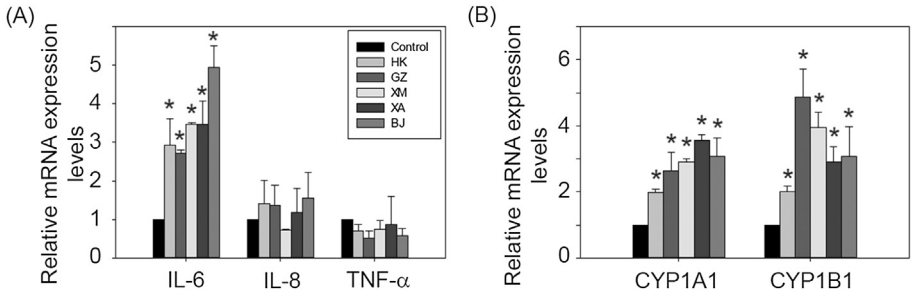

Fig. 6. Gene expression levels of pro-inflammatory cytokines and xenobiotic metabolic enzymes upon extracts treatment for 24 h in BEAS-2B cells. (A) mRNA levels of interleukin-6 (IL-6) was significantly up-regulated in all five sampling sites as compared to the blank filter control ($p < 0.01$). (B) Gene expression levels of cytochrome p450 enzymes were significantly up-regulated by the extracts exposure of all sampling sites ($p < 0.01$).

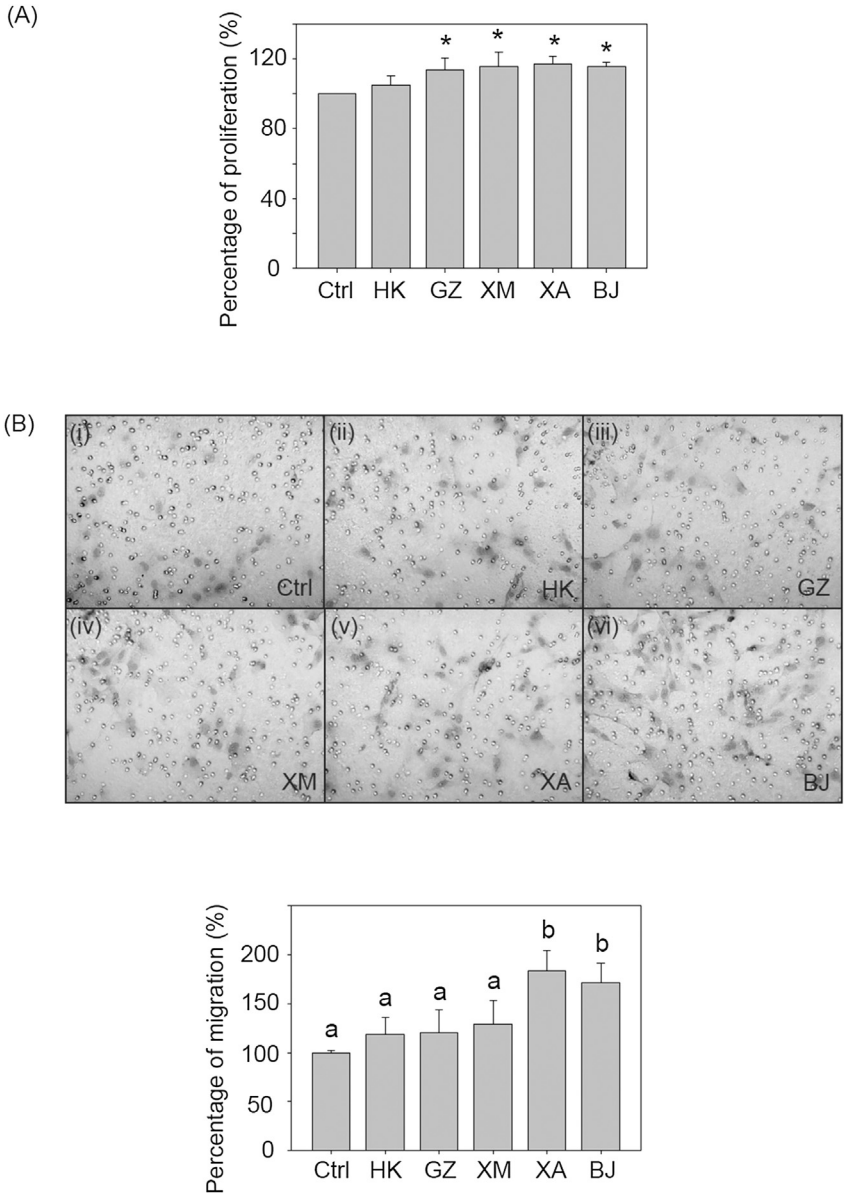

Fig. 7. Exposure to PM extracts increased the migration of BEAS-2B cells in cell migration assay. (A) The proliferation assay demonstrated the increase in proliferation in the cells treating with GZ, XM, XA and BJ extracts in MTT assay ($p < 0.05$). (B) Micrographs showing the representative pictures of cell migration assay (200×). The percentage of migration of cells treated with XA and BJ were significantly higher than that of other cities and the blank filter ($p < 0.05$).

inflammatory response may be triggered by the exposure of Beas-2b cells to PM extracts from the five cities.

Xenobiotic metabolic enzymes were also up-regulated upon exposure to $PM_{2.5}$ extracts (Fig. 6B). CYP1A1, a phase I enzyme of xenobiotic metabolic pathway, was known to be induced by PAHs (Cachon et al., 2014; Dergham et al., 2012; Val et al., 2011).

3.5. $PM_{2.5}$ from XA and BJ induced migration of Beas-2b cells

Cell proliferation and migration are important biological process found in tumor metastasis and progression. Treatments of Beas-2b cells with $PM_{2.5}$ extracts from BJ, XA, XM or GZ induced cell proliferation (Fig. 7A). For the transwell cell migration assay, $PM_{2.5}$ extracts from XA and BJ caused significant increases in cell migration as compared to the filter control, and extracts from HK, GZ and XM (Fig. 7B). Although most of the $PM_{2.5}$ extracts induced cell proliferation and the expression levels of IL-6, the extracts from the Northern cities (BJ and XA) might contain more carcinogenic substances that promoted tumorigenicity. This observation is consistent with the highest calculated values of the excess lifetime cancer risks in BJ and XA (Fig 4). However it may warrant a further investigation if there are other unidentified carcinogenic substances in the air of the Northern cities.

4. Conclusion

Ten priority PAHs were identified in the PM samples in all five cities; while all 16 PAHs can be detected in samples of XA and BJ. Chemical analysis results showed the higher levels of PAH contaminations were found in XA and BJ, indicating the pollution in these two cities were much severe than HK, GZ and XM. We used TEF to evaluate the possibility of cancer risks in equivalent to human carcinogen BaP. The analysis suggested that a long term exposure to such environment may cause adverse health effects, such as a higher risk of getting lung cancers. New policy measures on $PM_{2.5}$ emission should be adopted to protect the deteriorating air quality and public health against the air pollution, especially in northern cities in China.

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