Cover Sheet

Manuscript Title	Winter Polycyclic Aromatic Hydrocarbon-Bound Particulate Matter from Peri-urban North China Promotes Lung Cancer Cell Metastasis
Complete List of Authors	Huifeng Yue[1], Yang Yun[1], Rui Gao, Guangke Li, Nan Sang*
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FIGURE LEGENDS

Figure S1. Carton graph for sampling filter treatment and chamber set of migration and invasion assays.

Figure S2. Ring distribution of PAHs in $PM_{2.5}$ and PM_{10} collected from different seasons. The x-axis represents different seasons, and y-axis represents the mass percentages of PAHs with different rings.

Figure S3. Concentration- and time-dependent effects of PM on A549 cell migration determined by a wound-healing assay. A549 cells were treated with winter $PM_{2.5}$ and PM_{10} (0.1, 0.3, 1, 3 and 10 μg/mL) for 6, 12 and 24 h, and the invasive ability was evaluated by a wound-healing assay (200×). For $PM_{2.5}$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ *vs* the control group; For PM_{10}, (##) $p < 0.01$, (###) $p < 0.001$ *vs* the control group.

TABLES

Table S1. Primer sequences used for real-time RT-PCR

Gene	Accession No.	Sequence
Fib	NM_002026.2	
	forward	5′- AGGAGCACCACCCCAGACATTACT-3′
	reverse	5′-CCAGGCCGGGACTCAGGTTAT-3′
MMP-2	NM_001127891.2	
	forward	5′-CACGCTGGGCCCTGTCACTC-3′
	reverse	5′-GGGCCTCGTATACCGCATCAATCT-3′
TIMP-2	NM_003255.4	
	forward	5′-CCGCTCAAATACCTTCACA-3′
	reverse	5′-TACGGCAGCAAGTCCAAT-3′
E-cad	NM_004360.3	
	forward	5′-AATGCCGCCATCGCTTAC-3′
	reverse	5′-AGGCACCTGACCCTTGTA-3′
β-actin	NM_001101.3	
	forward	5′-CTGGAACGGTGAAGGTGACA-3′
	reverse	5′-AAGGGACTTCCTGTAACAATGCA-3′

Table S2. The concentrations of 18 PAHs bound to $PM_{2.5}$ and PM_{10} collected from different seasons

PAH	$PM_{2.5}$ (ng/m^3)				PM_{10} (ng/m^3)			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
NA	0.09	0.08	0.07	0.15	0.06	0.08	0.06	0.12
ACL	0.05	0.09	0.07	0.12	0.04	0.05	0.05	0.22
AC	0.36	0.23	0.04	0.31	0.40	0.23	0.35	0.28
FL	0.32	0.16	0.24	1.15	0.36	0.17	0.30	1.22
BghiP	9.96	4.63	5.42	23.01	11.09	5.76	6.38	27.46
IP	8.65	4.37	5.33	33.19	9.82	5.08	6.13	39.42
DBahA	3.10	1.51	1.82	6.87	3.55	1.72	1.84	8.63
BbFA	14.26	6.88	8.83	37.54	16.24	8.12	10.67	48.47
COR	4.60	2.50	2.97	7.00	5.88	3.28	3.40	10.64
PHE	1.33	1.06	1.63	6.11	1.65	1.21	2.05	9.15
AN	0.19	0.15	0.20	1.17	0.22	0.14	0.19	1.76
FA	4.08	2.61	3.71	26.14	4.59	3.05	4.71	36.12
BaA	5.51	1.82	2.75	26.43	6.33	2.06	3.02	30.87
CHR	8.32	3.56	5.40	27.15	9.66	4.21	6.17	35.78
PY	2.62	1.62	2.30	18.12	2.80	1.79	2.68	24.98
BaP	5.52	2.09	2.66	22.44	5.85	2.17	3.48	26.58
BeP	7.69	3.36	4.14	21.48	8.88	3.75	5.38	27.62
BkFA	3.24	1.49	1.68	11.31	3.47	1.47	2.20	11.46
PAHs	79.89	38.21	49.26	269.69	90.89	44.34	59.06	340.78

Table S3. Diagnostic ratios of PAHs bound to $PM_{2.5}$ and PM_{10} collected from different seasons

	IP/(IP+BghiP)		Bap/BghiP		FL/(FL+PY)		AN/(AN+PHE)		BaA/(BaA+CHR)	
	PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$
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	
Spring	0.47	0.46	0.53	0.55	0.11	0.11	0.12	0.13	0.40	0.40
Summer	0.47	0.49	0.38	0.45	0.09	0.09	0.11	0.12	0.33	0.34
Autumn	0.49	0.50	0.55	0.49	0.10	0.09	0.08	0.11	0.33	0.34
Winter	0.59	0.59	0.97	0.98	0.05	0.06	0.16	0.16	0.46	0.49

Table S4. The BaP_{eq} concentrations of PAHs bound to $PM_{2.5}$ and PM_{10} collected from different seasons

PAH species	$PM_{2.5}$ BaP_{eq}(ng/m^3)				PM_{10} BaP_{eq}(ng/m^3)			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
NA	0	0	0	0	0	0	0	0
ACL	0	0	0	0	0	0	0	0
AC	0	0	0	0	0	0	0	0
FL	0	0	0	0	0	0	0	0
BghiP	0.1	0.05	0.05	0.23	0.11	0.06	0.06	0.27
IP	0.87	0.44	0.53	3.32	0.98	0.51	0.61	3.94
DBahA	3.41	1.67	2.01	7.55	3.9	1.89	2.02	9.49
BbFA	1.43	0.69	0.88	3.75	1.62	0.81	1.07	4.85
COR	0	0	0	0.01	0.01	0	0	0.01
PHE	0	0	0	0.01	0	0	0	0.01
AN	0	0	0	0.01	0	0	0	0.02
FA	0.2	0.13	0.19	1.31	0.23	0.15	0.24	1.81
BaA	0.55	0.18	0.28	2.64	0.63	0.21	0.3	3.09
CHR	0.25	0.11	0.16	0.81	0.29	0.13	0.19	1.07
PY	0	0	0	0.02	0	0	0	0.02
BaP	5.52	2.09	2.66	22.44	5.85	2.17	3.48	26.58
BeP	0.08	0.03	0.04	0.21	0.09	0.04	0.05	0.28
BkFA	0.32	0.15	0.17	1.13	0.35	0.15	0.22	1.15
ΣPAHs	12.73	5.54	6.97	43.44	14.06	6.12	8.24	52.59

Table S5. Comparison of BaP_{eq} values of PAH-bound $PM_{2.5}$ and PM_{10} in cities China and abroad

PM	cities				
$PM_{2.5}$	Liaoning	E'erduosi	Xian	Beijing	Nanjing
	40.05	11.94	9.61	9.04	7.1
	Fuzhou	Guangzhou	Hong Kong	Xiamen	
	2.43	1.68	0.79	1.00	
PM_{10}	E'erduosi	Mexico city	Kuala lumpur		
	13.65	1.25	0.33		
PM	Florence				
	0.92				

Table S6. Conditions for $PM_{2.5}$ and PM_{10} collection during 2012-2013

	Max T °C	Min T °C	Min T°C	Pressure hPa	Hunidity %	Precipitation mm	Visibility km	Wind km/h
Spring	23.76	14.27	5.62	1010.11	28.24	0.11	15.89	15.03
Summer	29.75	21.68	14.68	1014.33	50.07	2.18	15.62	12.01
Autumn	12.75	5.90	-0.52	1017.51	57.95	0.72	15.35	13.71
Winter	9.98	2.62	-4.62	1017.18	47.9	0.21	15.48	14.2

Table S7. Correlations between PAH-bound winter PM with ROS generation and invasion of A549 cells

PAH species	$PM_{2.5}$		PM_{10}	
	Invasion	ROS	Invasion	ROS
NA	0.921	0.757	0.952*	0.959*
ACL	0.492	0.794	0.987*	0.978*
AC	0.470	-0.052	-0.493	-0.229
FL	0.917	0.921	0.925	0.941
BghiP	0.960*	0.816	0.914	0.896
IP	0.953*	0.853	0.920	0.910
DBahA	0.965*	0.812	0.907	0.879
BbFA	0.953*	0.862	0.927	0.919
COR	0.955*	0.628	0.873	0.864
PHE	0.870	0.963*	0.954*	0.972*
AN	0.854	0.950*	0.968*	0.942
FA	0.860	0.947	0.967*	0.952*
BaA	0.902	0.918	0.942	0.908
CHR	0.936	0.900	0.935	0.920
PY	0.857	0.946	0.968*	0.946
BaP	0.912	0.904	0.942	0.919
BeP	0.946	0.861	0.921	0.909
BkFA	0.920	0.890	0.926	0.915

[a]Note. * $p < 0.05$

Table S8. The concentrations of different ions in $PM_{2.5}$ and PM_{10} collected from different seasons

	$PM_{2.5}$ (μg/mg)				PM_{10} (μg/mg)			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Fluorinion	1.228	0.750	0.838	1.359	0.899	0.749	1.141	1.573
Chloridion	4.328	0.872	2.243	15.659	2.827	0.680	1.575	8.387
Nitrate ion	8.195	6.787	9.770	4.002	4.441	6.239	7.518	2.286
Sulfate ion	11.089	16.607	24.805	12.825	5.630	11.267	13.064	6.667
Sodion	192.991	121.918	217.448	154.958	90.220	77.674	103.611	75.699
Ammonium ion	231.086	228.377	397.174	307.017	100.190	124.247	153.844	133.392
Potassium ion	82.000	62.228	110.981	92.296	38.596	38.039	55.059	47.549
Magnesium ion	8.732	8.691	12.350	10.912	4.947	7.062	8.207	5.583
Calcium ion	136.935	210.220	171.196	106.171	101.777	204.107	236.276	109.803

Table S9. Correlations between 9 ions in $PM_{2.5}$ and PM_{10} with A549 cell invasion

ion species	$PM_{2.5}$	PM_{10}
Fluorinion	0.9645125	0.8486777
Chloridion	0.9104322	0.8858945
Nitrate ion	-0.5579098	-0.7229452
Sulfate ion	-0.5276417	-0.2884718
Sodion	0.1952041	-0.6196579
Ammonium ion	0.1166037	0.2893317
Potassium ion	0.3786937	0.2488081
Magnesium ion	0.2280257	-0.2281295
Calcium ion	-0.995165	-0.354808

TEXT

PM Sample Collection and Filter Treatment. Samplings were collected during the period 2012-2013: spring, March 1, 2012 to April 30, 2012; summer, May 1, 2012 to July 31, 2012; autumn, August 1, 2012 to October 31, 2012; and winter, November 1, 2012 to February 28, 2013 (considering the annual heating period in Northern China). The samples were collected onto glass filters (GF/A-1820-090, Whatman, UK) using two PM middle volume air samplers (TH-150CIII and TH-150C, Wuhan, China), and the samplers were placed on rooftop of a building approximately 15 m tall. The sampling point was located at Shanxi University (112°21-34'E longitude, 37°47-48'N latitude) in Taiyuan, and the location was surrounded by three villages inside the city, highway, schools, and a railway station within two kilometers. We collected PM samples every day except days of precipitation, equipment failures, power outages and student vacations. In total, we collected 41, 63, 61, and 78 samples in spring, summer, autumn, and winter, respectively, during our one-year field study. The sampling conditions were the following: the flow rate was 100 L/min; the sampling time started at 9:00 a.m. and lasted 24 h; the average temperature was -5 °C to 30 °C and the barometric pressure was 93.3 kPa.

Prior to sample collection, the filters were calcined at 450 °C for 12 h, equilibrated in a desiccator at room temperature within 24 h, and then weighed by a microbalance (ME36S, Sartorius, Germany) with balance sensitivity ± 1 μg. After sampling, the filters were wrapped in aluminum foil, weighed and stored at 4 °C for further analysis in our laboratory. We pooled PM samples from volume filters collected every Monday/Tuesday/Wednesday/Thursday/Friday from a particular season as one group, and obtained 8-12 groups of data for a particular season. Following this, we cut 1/8 volume filters for PAH measurement and another 1/8 volume filters for *in vitro* experiments. To prepare extractions for PAH identification, the filter samples were ultrasonically extracted three times (20 min each time) with 150 mL of dichloromethane. Following this process, the extracts were concentrated to 1 ml under pure nitrogen gas flow using rotary evaporation and then transferred into a separation column for eliminating impurities with a mixed solution of n-hexane and dichloromethane (1:1). Subsequently, the purified samples were concentrated to 1.0 mL under nitrogen. For *in vitro* exposure, the PM extracts were prepared by socking the filters with ultrapure water, drying under nitrogen, and reconstituting to 10 mg/mL. The PM extracts were stored at -80 °C until use in bioassays.

Determination of PAHs in PM. Reserve liquid (1000 mg/L), including phenanthrene-d10,

acenaphthene-d10, perylene-d12 and chrysene-d12, was diluted by n-hexane to 20 mg/L and used as an internal standard solution. Deuterated PAH internal standards were added to the extracts for GC-MSD analysis (6890 N, HP; 5973, HP) with a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J &W Scientific Co. Ltd., Folsom, CA, USA), as described in the USEPA Standard method 8270C. In this study, 18 types of PAHs were analyzed, including the native PAH standards [naphthalene (NA), acenaphthene (AC), acenaphthylene (ACL), anthracene (AN), fluoranthene (FA), pyrene (PY), fluorene (FL), benzo(a)anthracene (BaA), chrysene (CHR), phenanthrene (PHE), dibenzo(a,h)anthracene (DBahA), benzo(b)fluoranthene (BbFA), benzo(k)fluoranthene (BkFA), benzp(e)pyrene (BeP), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (IP), benzo(g,hi)perylene (BghiP), and coronene (COR)]. QA/QC analysis. The limit of detection (LOD) was measured with a signal-to-noise ratio of 3, ranging from 0.05 to 0.15 ng/g. The recoveries of individual PAHs ranged from 74 to 108%. A spiked blank, sample duplicate, method blank (solvent) and standard reference material were analyzed. The difference in the coefficients of PAH concentrations for duplicated samples was less than 10%. The PAH concentrations in the method blank were less than the LOD.

Considering that the glass filter is readily adsorbing ambient air concentrations of HNO_3, NO_2 and SO_2, and the process may affect the results in PAHs cycling, sources and *in vitro* bioassays, we conducted the following analyses. (a) High concentrations of secondary aerosol precursors, including pPAHs,[1] SO_2 and NO_x,[2] have often been found in ambient air in northern China; the presence of these compounds could favor the formation secondary fine aerosol and lead to elevated concentrations of nPAHs and oPAHs.[3] The annual average concentrations of NO_2 and SO_2 in the sampling site of Taiyuan were 20 and 68 ng/m^3,[4] whereas that of nPAHs and oPAHs were 1.24 ng/m^3 and 33.89 ng/m^3, respectively.[3] Compared with other cities, the concentrations of atmospheric PAHs (209 ng/m^3 in urban, 199 ng/m^3 in rural) in Taiyuan were substantially higher than that of nPAHs and oPAHs.[5] (b) Ambient air HNO_3, NO_2, and SO_2 may also transform to ions such as nitrate ion and sulfate ion. To investigate their possible effect, we detected the levels of ions in PM, and did not find significant seasonal changes. Especially, no significant correlation occurred between ion concentration and the effect on A549 cell migration and invasion (see Table S8 and Table S9).

Data Analyses. To identify the differences in the PAH composition profiles in $PM_{2.5}$ and PM_{10} in four seasons, the parameter was determined as follows:

$$CD_{jk} = \sqrt{\frac{1}{p}\sum_{i=1}^{p}\left(\frac{x_{ij}-x_{ik}}{x_{ij}+x_{ik}}\right)^2}$$

where j and k stand for the two profiles for sampling sites or fractions, p is the number of investigated components, and x_{ij} and x_{ik} represent the average mass concentrations of chemical component i for j and k.[6,7] If the CD_{jk} value approaches zero, sources j and k are similar, and if the CD_{jk} value approaches one, the sources are significantly different.[6-8]

Benzo(*a*)pyrene-equivalent (BaP_{eq}) can be used to estimate the carcinogenic risks for humans. BaP_{eq}=TEF×C (PAH), where TEF was the toxic equivalent factor of each PAH and where C (PAH) was the corresponding concentration of PAHs.[9]

The diagnostic ratio has been reported to be a convenient method for identifying possible emission sources.[10] In the present study, five diagnostic ratios were calculated: (Bap/BghiP) = Concentration (Bap)/Concentration (BghiP); IP/(IP+BghiP) = Concentration (IP)/[Concentration (IP) + Concentration (BghiP)]; AN/(AN+PHE) = Concentration (AN)/[Concentration (AN) + Concentration (PHE)]; FL/(FL+PY) = Concentration (FL)/[Concentration (FL) + Concentration (PY)]; BaA/(BaA+CHR) = Concentration (BaA)/[Concentration (BaA) + Concentration (CHR)].

Real-time Quantitative Reverse Transcription-PCR. Briefly, total RNA was isolated, quantified and synthesized to complementary DNA (cDNA) using TRIzol Reagent (Invitrogen, USA) and a reverse transcription kit (TaKaRa, China) according to the manufacturer's instructions. The cDNA product was stored at -20 °C until use. The sequences of each gene-specific primer are provided in Table S1. Each 20-μL PCR reaction contained 2 μL of cDNA (6-fold dilution of original cDNA product), 10 μL of SYBR Premix Ex Taq II (TaKaRa, China), 7 μL of RNase Free H_2O, and 0.5 μL of each primer. The real-time PCR was run for 40 cycles at 95 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s on a qTOWER 2.2 Real-Time PCR (Analytik Jena AG, Jena, Germany). Each cDNA sample was run in triplicate, and the relative quantification of the gene expression was determined with β-actin as an internal control.

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