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Physical and chemical characteristics of $PM_{2.5}$ and its toxicity to human bronchial cells BEAS-2B in the winter and summer*

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Abstract: With the increasing occurrence of haze during the summer, the physicochemical characteristics and toxicity differences in $PM_{2.5}$ in different seasons are of great concern. Hangzhou is located in an area that has a subtropical monsoon climate where the humidity is very high during both the summer and winter. However, there are limited studies on the seasonal differences in $PM_{2.5}$ in these weather conditions. In this test, $PM_{2.5}$ samples were collected in the winter and summer, the morphology and chemical composition of $PM_{2.5}$ were analyzed, the toxicity of $PM_{2.5}$ to human bronchial cells BEAS-2B was compared, and the correlation between $PM_{2.5}$ toxicity and the chemical composition was discussed. The results showed that during both the winter and summer, the main compounds in the $PM_{2.5}$ samples were water-soluble ions, particularly SO_4^{2-}, NO_3^-, and NH_4^+, followed by organic components, while heavy metals were present at lower levels. The higher the mass concentration of $PM_{2.5}$, the greater its impact on cell viability and ROS levels. However, when the mass concentration of $PM_{2.5}$ was similar, the water extraction from the summer samples showed a greater impact on BEAS-2B than that from the winter samples. The cytotoxicity of $PM_{2.5}$ was closely associated with heavy metals and organic pollutants but less related to water-soluble ions.

Key words: $PM_{2.5}$; Seasonal difference; Physical and chemical characteristics; Cytotoxicity
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1 Introduction

Air pollution is a major problem in many modern cities and influences billions of people worldwide (Shi et al., 2015). $PM_{2.5}$ (aerodynamic diameter ≤2.5 μm) is defined by fine particles that contain carbon and absorb various chemical compounds, such as metals, organic compounds, and salts, and biological groups, such as toxins and pollen (Spurny, 1996). These $PM_{2.5}$ compounds have a great effect on human health and can cause respiratory and cardiovascular diseases.

Most of the national heavy haze events occur during the spring and winter, but in recent years the frequency of summer haze has increased in North China (Chen and Wang, 2015). Scholars found that the source and composition of $PM_{2.5}$ in an area varied in different seasons. Some studies have shown that the inorganic ions, heavy metals, and organic compounds in $PM_{2.5}$ were generally lower in the summer than in the winter (Manzanoleón et al., 2015; Huang et al., 2016; Meng et al., 2016; Wang et al., 2016). However, the results have been inconsistent. For

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example, Perrone et al. (2010) found that $PM_{2.5}$ in Milan during the summer was richer in sulfates, Al, As, Cr, Cu, and Zn than $PM_{2.5}$ during the winter. The sources of $PM_{2.5}$ also showed seasonal differences. Zhang et al. (2013) found that the concentration of secondary inorganic aerosol was higher during the warm and rainy summers than during the winter, while secondary organic aerosols tended to be formed during the dry and cold winters. Manzanoleón et al. (2015) found that the chemical compounds of $PM_{2.5}$ during the warm and rainy season were intimately associated with the soil source, while they were closely related to combustion sources during the cold and dry season.

$PM_{2.5}$ is small enough to invade even the smallest airways and penetrate to the lungs (Shi et al., 2015), and will cause oxidative stress and inflammation in the respiratory system, and then may cause epithelial damage and abnormal cardiovascular function. The particle in vitro toxicity test with human bronchial cells is a typical method for simulating the toxicity of $PM_{2.5}$ to the respiratory system (Borgie et al., 2015a). The toxicity of $PM_{2.5}$ is not only related to its physical characteristics but is also related to various toxic chemicals adsorbed onto $PM_{2.5}$ (Ma et al., 2013). Organic compounds in $PM_{2.5}$, such as aliphatic/chlorinated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and nitro PAHs/ketones/quinones, may be an important part of $PM_{2.5}$ toxicity (Borgie et al., 2015b). The water-soluble components of $PM_{2.5}$ are also very harmful to human health (Gualtieri et al., 2009). For example, the transition metal elements Zn and Pb have a strong correlation with the A549 cells cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Liu et al., 2014).

There are some studies regarding the differences in $PM_{2.5}$ toxicity between the winter and summer (Becker et al., 2005; Perrone et al., 2010; Happo et al., 2013; Longhin et al., 2013). Some researchers have found that reactive oxygen species (ROS) levels in alveolar macrophages and the activity of A549 cells were affected more significantly by $PM_{2.5}$ during the summer than during the winter (Becker et al., 2005; Perrone et al., 2010). However, Longhin et al. (2013) found that winter $PM_{2.5}$ would cause a significant ROS level increase in cells after only one hour of exposure. Happo et al. (2013) also found that the cytotoxicity of outdoor $PM_{2.5}$ samples of the spring and summer was more cytotoxic than those collected during the winter.

Obviously, neither the differences in the chemical composition nor the cytotoxicities of $PM_{2.5}$ during different seasons have consistent conclusions. It is important to understand this issue because it is related to the mechanism and governance of haze and the protection of public health. The current comparison studies between the winter and summer $PM_{2.5}$ have focused on the dry and cold winters and warm and rainy summers. Hangzhou (Zhejiang, China) is located in a wet area with subtropical monsoons, and the humidity is very high during both the summer and winter; however, there are limited studies regarding the seasonal differences in $PM_{2.5}$ in these weather conditions. In this paper, $PM_{2.5}$ samples from different seasons in Hangzhou were collected, the main chemical components were analyzed, the influences of $PM_{2.5}$ water extraction on the cell viability and ROS levels of BEAS-2B cells were compared, and the relationship between the cell toxicity of $PM_{2.5}$ over different seasons and its chemical factors was investigated.

2 Materials and methods

2.1 Collection and selection of $PM_{2.5}$ samples

The sampling point was located at the third floor of the Agricultural Biological and Environment Building in the Zijingang Campus of Zhejiang University, Hangzhou, Zhejiang Province, China. There are a few major roads within 1 km of the sampling point. A residential area is approximately 200 m east of the sampling point (Fig. 1). $PM_{2.5}$ samples were collected on 90-mm quartz microfiber filters (prebaked, QMA, Whatman-GE Healthcare Biosciences Corp., UK) using an air impactor monitor (Qingdao Laoying Corp., Qingdao, China) that was operated at a flow rate of 100 L/min, as recommended by China's standard method for $PM_{2.5}$ collection (Environmental Protection Department of the People's Republic of China, 2013). Samples were collected from Jan. 5 to 14 and May 20 to June 25 in 2015. The filters were changed every morning at 10:00 a.m. during the measurement period. The quartz fiber filters were baked for 5 h at 500 °C to remove impurities and then placed into desiccators for 24 h and weighted prior to deployment for $PM_{2.5}$ measurement. After weighing, the filters were stored in a refrigerator at −18 °C before the chemical analysis.

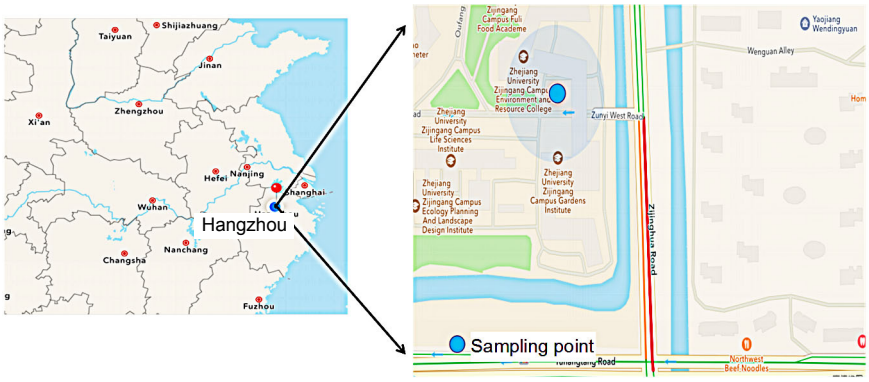

Fig. 1 Position of sampling point

Based on the filter quality differences between the before and after samplings and the sampling volume under standard conditions, the $PM_{2.5}$ mass concentration could be obtained. Then, the samples from Jan. 5 (W_H), 6 (W_M), and 14 (W_L) were selected, representing severe, light, and good winter weather conditions, respectively, and May 20 (S_H), June 25 (S_M), and June 24 (S_L) were selected, representing summer weather (Table 1).

2.2 $PM_{2.5}$ morphology and chemical composition analysis

All the selected filters were divided into four parts, which were used for the morphology, chemical composition analysis, and cell toxicity tests. The main chemical analysis methods were discussed by Liu et al. (2014).

2.2.1 $PM_{2.5}$ morphology analysis

A small piece of filter (approximately 0.5 cm× 0.5 cm) was cut from S_M, W_M, and blank sample filters, and placed in a 3-cm cylindrical stage by conductive adhesive. After gold platting, the $PM_{2.5}$ morphology was analyzed under SU-8010 type field emission scanning electron microscope (SEM) (SU-8010 Hitachi, Japan).

2.2.2 Trace heavy metal analysis

Inductively coupled plasma-mass spectrometer (ICP-MS) was used to analyze the trace heavy metals in $PM_{2.5}$. The sample filter was cut into pieces, placed in a Teflon crucible with 10.0 ml of mixed acids (5.55% (v/v) HNO_3/16.75% (v/v) HCl), and then the crucibles were heated at 100 °C for 2 h. After cooling, ultra-pure water was added to the extraction for 0.5 h. Finally, the filter pieces were removed, and the extracting solution was reduced to a constant volume of 50.0 ml. The extracting solution was filtered through a 0.45-μm Millipore filter and analyzed by NexION™ 300X ICP-MS (NexION 300, PerkinElmer, USA) to test for seven trace heavy metals (V, Mn, Ni, Cu, Cd, Ba, and Pb).

2.2.3 Water soluble ion analysis

The water solution ions were analyzed by ion chromatography. A quarter of each sample filter was cut into pieces and placed in a cleaning centrifuge tube with 20.0 ml of ultra-pure water; then, the tubes were placed in an ultrasonic water bath for 1 h. The extracting solution was filtered through a 0.22-μm Millipore filter and analyzed by ICS-3000 Multi-functional Ion Chromatography (Dionex ICS-3000, Thermo Scientific, USA) to test for eight water-soluble ions (Cl^-, SO_4^{2-}, NO_3^-, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}).

2.2.4 Organic carbon and elemental carbon analyses

The concentrations of organic carbon (OC) and elemental carbon (EC) in the samples were analyzed with a thermal/optical carbon analyzer (DRI 2001A, Atmoslytic, Inc., USA) using the IMPROVE temperature program.

2.3 Cell cytotoxicity

2.3.1 Preparation of $PM_{2.5}$ extraction solution

Based on the result of pre-test, a quarter of each sample filter was cut into pieces and placed in a clean sterile centrifuge tube with 10.0 ml of serum-free Dulbecco's modified Eagle's medium (DMEM)

Table 1 Concentrations of $PM_{2.5}$ and the chemical compounds in the samples

Sample mark	Sampling time	Mass concentration ($\mu g/m^3$)	Chemical compound concentration (ng/m^3)						
			V	Mn	Ni	Cu	Cd	Ba	Pb
W_H	2015.1.5	290	27.93	250.16	6.57	60.86	7.92	70.85	196.85
W_M	2015.1.6	96	71.41	61.71	6.57	10.96	1.87	36.10	84.53
W_L	2015.1.14	35	77.69	20.20	1.62	8.53	1.21	17.23	47.48
S_H	2015.5.20	171	112.23	115.10	16.22	32.00	1.40	84.43	50.38
S_M	2015.6.25	92	79.75	45.84	5.20	10.01	1.39	22.49	66.31
S_L	2015.6.24	47	117.62	40.33	4.11	9.91	2.20	32.47	33.86

Sample mark	Chemical compound concentration ($\mu g/m^3$)										
	Cl^-	NO_3^-	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	OC	EC	SOC
W_H	6.72	32.24	22.54	0.56	8.12	2.49	0.09	1.90	39.20	10.31	23.74
W_M	5.49	16.89	21.42	1.09	9.31	1.33	0.06	0.96	12.15	2.65	8.17
W_L	1.31	4.58	9.91	0.21	3.73	0.60	0.02	0.42	5.25	1.42	3.12
S_H	1.13	10.76	25.74	0.86	2.78	0.69	0.32	7.16	20.39	4.79	13.20
S_M	0.16	11.86	24.19	0.23	6.07	0.27	0.07	0.74	12.24	2.47	8.53
S_L	0.77	4.39	10.88	0.11	0.93	0.10	0.06	0.51	10.20	2.31	6.73

OC: organic carbon; EC: elemental carbon; SOC: secondary organic carbon. $SOC=OC-OC\times(OC/EC)_{min}$, where $(OC/EC)_{min}$ is the minimum value of the OC/EC during the sampling period, and 1.5 is used in this test

(Gibco, USA); then the tube was placed in an ultrasonic water bath for 1 h and the temperature was kept at 30 °C. The step was repeated three times. After filtering through a 0.22-μm sterile filter, the $PM_{2.5}$ extractions were kept in −20 °C storage before the cytotoxicity tests.

2.3.2 Cytotoxicity test

Human bronchial cells BEAS-2B (ATCC No. ATCC® CRL-9609™) were cultured at 37 °C in a 5% CO_2 box (Eppendorf China Ltd., Shanghai, China) before the cell viability and ROS tests. When the culture dish was covered in mono-layer cells, the cells were digested and matched into a single cell suspension by a DMEM medium containing 10% (v/v) fetal bovine serum and 1% (v/v) Penicillin-Streptomycin solution, and then vaccinated to Corning 96-well plates in 10000 densities and cultured for 24 h before the $PM_{2.5}$ cytotoxicity experiments.

A CCK-8 cell activity detection kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to test the cell activity, and an ROS detection kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to test intracellular ROS. In the experiment, the non-cell group was used as a blank control, and none of the $PM_{2.5}$ exposure groups was used as a control group. Each sample was provided with three sets of parallel samples. The experimental operation was carried out strictly in accordance with the specification. After 24 h of exposure, the morphology of the cells was observed under a Nikon Eclipse TS100 microscope (Eclipse TS100 Nikon, Japan).

2.4 Data analyses

Data were analyzed using Origin 8.0 and Microsoft Excel 2010. The correlation analysis was finished by Pearson's correlation analysis program in IBM Statistics SPSS 19.0.

3 Results and discussion

3.1 Chemical analysis of $PM_{2.5}$ samples

The $PM_{2.5}$ mass concentration and chemical components of the samples are shown in Table 1, and the percentages of the main compounds are shown in Table 2. The $PM_{2.5}$ mass concentrations of the six samples were much higher than the daily average concentration limit of the World Health Organization (WHO) (25 $\mu g/m^3$). The most abundant components in the $PM_{2.5}$ of each sample were water-soluble ions (especially SO_4^{2-}, NO_3^-, and NH_4^+), which accounted for 25.74%–59.37%. The concentrations of SO_4^{2-} and NO_3^- are directly related to the contents of SO_2 and NO_x, which are mainly from fixed sources, such as coal combustion, and mobile sources, such as vehicle emissions, indicating that the use of fossil fuels, such as coal and oil, has a significant impact on the ionic

composition of $PM_{2.5}$. The mass ratio of NO_3^- and SO_4^{2-} ($[NO_3^-]/[SO_4^{2-}]$) is typically used to compare the contribution of fixed sources (such as coal combustion) and mobile sources (such as vehicle emission) to sulfur and nitrogen in the atmosphere. The results showed that except for W_H, the $[NO_3^-]/[SO_4^{2-}]$ mass ratios for the remainder of the samples were less than 1, while the $[NO_3^-]/[SO_4^{2-}]$ mass ratios of W_M and W_L were closer to 1, which indicated that although the fixed sources have a great contribution in the winter, the impact of mobile sources should not be ignored. It should be noted that Cl^- and K^+ were higher in the winter than in the summer, since Cl^- and K^+ come from biomass burning, indicating that $PM_{2.5}$ is affected more by biomass burning in the winter than in the summer.

Table 2 Percentages of water-soluble ions, total carbon, total metals, and SOC in the samples

Sample mark	Water-soluble ions (%)	Total carbon (%)	Total metals (%)	SOC (%)
W_H	25.74	17.07	0.21	8.18
W_M	58.91	15.42	0.28	8.51
W_L	59.37	19.06	0.50	8.90
S_H	28.91	14.73	0.24	7.71
S_M	47.38	15.99	0.25	9.22
S_L	37.77	26.62	0.51	14.26

SOC: secondary organic carbon

The contents of OC and EC were 14.73%–26.63%, which were slightly inferior to the water-soluble ions. The percentage of EC in the summer samples was similar to that in the winter, while the percentages of OC and SOC in the summer samples were higher than those in the winter samples. Because EC is mainly from primary sources, such as coal emission, and OC is derived from primary sources and secondary reactions in the atmosphere, which indicated that the contribution of primary sources to $PM_{2.5}$ was relatively stable in the winter and summer, while $PM_{2.5}$ in the summer is influenced more by secondary sources than that in the winter. This differed from the prior results (Zhang et al., 2013), maybe because the temperature has a greater effect on the formation of SOC when the humidity was high both in the summer and winter, and thus the summer climate in Hangzhou was more conducive to the formation of SOC.

The heavy metals only accounted for less than 1% of all selected samples. V, Mn, Pb, and Cu were relatively rich among the seven metals. Mn, Pb, and Cu increased with increases in the $PM_{2.5}$ mass concentration and were higher in the winter than in the summer, while V decreased with increases in the $PM_{2.5}$ mass concentration and was higher in the summer. Coal, fossil fuels, and industrial emissions were the main sources of V, Mn, and Pb. There are no industrial parks around the sampling point, which indicated a contribution of long distance transmission to $PM_{2.5}$. In addition, the resuspension of soil could also increase the Mn content in the $PM_{2.5}$. Notably, although the concentration of Pb did not exceed the WHO limit value (500 ng/m^3), it was higher than that in other cities in China, such as Guangdong, Nanjing, and Macao, and was slightly higher in the winter (Zheng et al., 2014).

3.2 $PM_{2.5}$ morphology

The morphological characteristics of $PM_{2.5}$ affect its adsorption of toxic and harmful substances and $PM_{2.5}$ toxicity. SEM images are widely used in the study of atmospheric particle morphology, and can directly show the particle size, morphology, aggregation characteristics, composition, and even sources of fine particles (McMurry, 2000). In this report, SEM was used to analyze the morphological characteristics of $PM_{2.5}$ on the filter. There was no significant difference between the winter and summer particle samples (Fig. 2). In contrast to the blank quartz filter, the sample filter was not as smooth and the adhered fine particles made the fiber coarser. $PM_{2.5}$ was not only comprised mainly of irregularly shaped agglomerate particles but also contained spherical, elongated, and flocculent particles.

Irregularly shaped agglomerate particles might be large particles that absorbed various substances and mainly came from soil or construction dust; spherical particles and disportionate spherical particles could be coal fly ash, which was mainly from coal burning or nitrate and sulfate particles formed through atmospheric reactions (Du et al., 2015). Spongy spherical particles were probably spongy carbon particles (Masiol et al., 2013). Elongated particles had a regular shape and a smooth surface, which were supposedly mineral particles, such as sulfate and nitrate particles, and mainly came from secondary

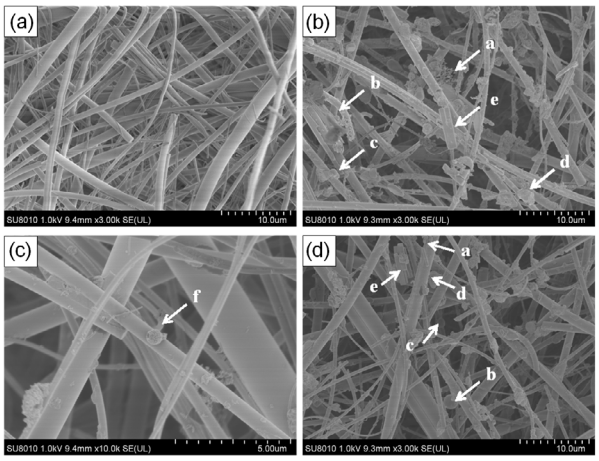

Fig. 2 SEM images of $PM_{2.5}$

(a) SEM results of blank filter (magnified by 3000 times); (b) SEM results of S_M sample (magnified by 3000 times), (c) SEM results of S_M sample (magnified by 10000 times); (d) SEM results of W_M sample (magnified by 3000 times). a: flocculent particles; b: spherical particles; c: deformation of spherical particles; d: irregularly shaped agglomerate particles; e: elongated particles; f: spongy spherical particles

particles formed in atmospheric chemical reactions. Flocculent particles were fluffy and might be soot and soot aggregates, which were mainly from coal burning and motor vehicle exhaust (Du et al., 2015).

It could be speculated that the spherical particles and soot aggregates in $PM_{2.5}$ can enable the fine particles to easily adsorb toxic and harmful substances, such as heavy metals, volatile organic contaminants, and semivolatile organic pollutants. The study of nanoscale characteristics of $PM_{2.5}$ confirmed that soot aggregates (flocculent particles) had high adhesion to adsorb other types of particles, resulting in an enhanced complexity and toxicity, and spherical carbon particles will be associated with soot aggregates when transferred, which increased the toxicity of $PM_{2.5}$ (Shi et al., 2015).

3.3 Impacts of $PM_{2.5}$ on cell vitality and ROS level

Cell viability refers to the percentage of living cells in total cells among a group of cells. It is a simple and intuitive index to test whether the cell is damaged in the experiment. The balance of oxidation and antioxidant system in cells is a key factor in determining the survival state of cells. Intracellular ROS, which are oxygen free radical and its derivatives, will rapidly increase when the body suffers from harmful stimuli. ROS will attack DNA, RNA, protein, fat, and other macromolecules in cells and ultimately lead to cell damage and even cell death.

The states of cells under the microscope (magnified by 200 times) after being exposed to $PM_{2.5}$ extractions of different seasons for 24 h were showed in Fig. 3. The changes in the cell states were similar when exposed to $PM_{2.5}$ extractions from different seasons. The cells of the control group grew well, and cell transparency was good, but after being exposed to $PM_{2.5}$ extractions for 24 h, the cells became irregular and detached from the bottom of the culture dish. Moreover, the gap between the cells increased. The number of living cells in the field of vision decreased sharply with increases in the $PM_{2.5}$ sample mass concentration.

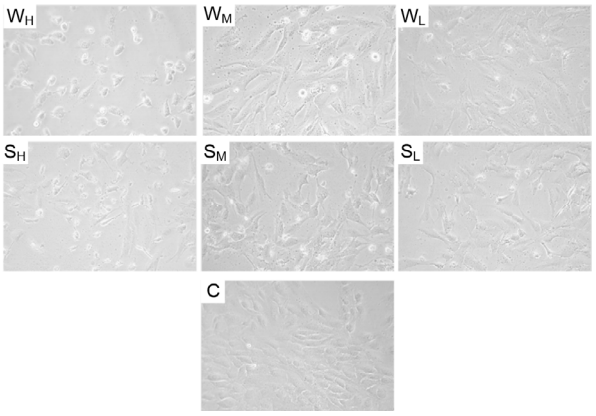

Fig. 3 States of cells under the microscope after exposure to the water extraction solution of $PM_{2.5}$ (magnified by 200 times)

Fig. 4 shows the results of changes of the cell viability after exposure to $PM_{2.5}$ extractions from different seasons for 24 and 48 h. The results showed that all $PM_{2.5}$ extractions had an effect on the cell viability, and the higher the mass concentration of $PM_{2.5}$, the lower the cell viability. In addition, the longer the exposure time, the greater the effect on the cell viability. The results also showed that the $PM_{2.5}$ extraction of W_H had a significant effect on the cell viability. The cell viability decreased to only 9% after 24 h exposure, and the cells almost died after 48 h, while the viability decreased to approximately 10% after 48 h in the other experimental groups. For the exposure groups of $PM_{2.5}$ samples of the winter, the cell viability showed a dose-response relationship with the $PM_{2.5}$ concentration in the early exposure period, which implied that the higher the $PM_{2.5}$

sample mass concentration, the lower the cell viability. However, the cell viability of the group exposed to $PM_{2.5}$ extraction of S_L was lower than that of S_M. This may due to the equal content of heavy metals in S_L and S_M, where some elements were even higher in S_L than in S_M. For example, the concentration of the toxic element V was 117.62 ng/m^3 in S_L, while it was only 79.75 ng/m^3 in S_M. Meanwhile, OC (10.20 μg/m^3) was higher in S_L, which may lead to an increase in the release of organic compounds in the water and have a notable effect on cell viability.

In addition to the ROS components of the cells, $PM_{2.5}$ can also stimulate cells to produce a large number of ROS. As shown in Fig. 5, the ROS content increased and was approximately two times greater compared to the control group after 3 h of $PM_{2.5}$ stimulation. As time elapsed, ROS secretion in the 6 h experimental groups decreased. This may be the result of the balance between intracellular oxidation and antioxidant system, e.g. the intracellular superoxide dismutase (SOD) might play a role in scavenging free radicals so that the level of ROS decreased. This was similar to the results reported previously which used A549 cells (Deng et al., 2013).

In addition, the $PM_{2.5}$ extraction samples of the summer induced higher increments of intracellular ROS than the winter samples after 6 h of exposure (Fig. 5). In contrast to the cell viability data, the effect of $PM_{2.5}$ extractions of the summer on cell viability was also greater in the early 24 h exposure time, which is similar to the results reported before (Becker et al., 2005; Perrone et al., 2010). It could be conjectured that a considerable mass concentration of $PM_{2.5}$ in the warm months may have a greater effect on the cell viability of BEAS-2B cells and the increase in intracellular ROS than that in the cold months.

3.4 Correlation between the chemical composition and $PM_{2.5}$ cytotoxicity

Previous studies have shown that the cytotoxicity of $PM_{2.5}$ may be related to the metal element and organic pollutant content (de Kok et al., 2006; Borgie et al., 2015b). According to the study of Xiang et al. (2016), the ingredients in water extraction of $PM_{2.5}$ should be water-soluble ions and heavy metals, which are consistent with the chemical compounds of $PM_{2.5}$ in Section 3.1. From the perspective of human health, when the $PM_{2.5}$ invades the lungs, the encounter is mainly inorganic salt water extraction and lung fluid. Thus, the correlation between the chemical components and the cytotoxicity of $PM_{2.5}$ was analyzed, and the results showed that the metal element and OC, EC, and SOC content had different correlations with the cytotoxicity results.

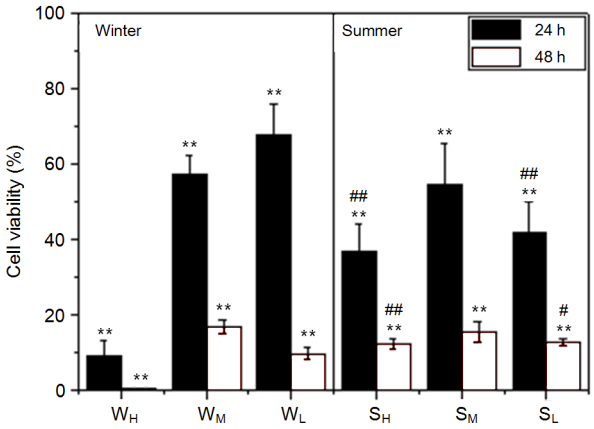

Fig. 4 Cell viability after exposure to the water extraction solution of $PM_{2.5}$

Sample groups are compared to control groups, where ** indicates a significant difference. Summer severe, mild, and good weather groups are compared to the corresponding winter samples, where # indicates a significant difference at 0.05, and ## indicates a significant difference at 0.01

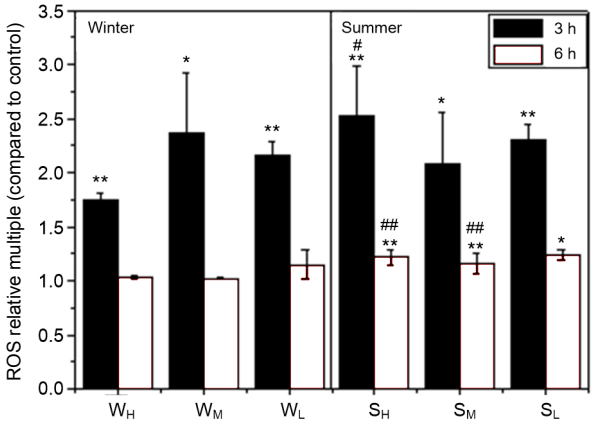

Fig. 5 Relative multiple of ROS in cells compared to the control group after exposure to the water extraction solution of $PM_{2.5}$

Sample groups are compared to control groups, where * indicates a significant difference at 0.05, and ** indicates a significant difference at 0.01. Summer severe, mild, and good weather groups are compared to the corresponding winter samples, where # indicates a significant difference at 0.05, and ## indicates a significant difference at 0.01

3.4.1 Correlation between heavy metals and $PM_{2.5}$ cytotoxicity

Many studies have confirmed that heavy metals in $PM_{2.5}$ were harmful to human health (de Kok et al., 2006). For example, transition metal elements can induce cells to produce ROS, which leads to excessive free radicals that can cause lipid peroxidation and the breakdown of DNA strands, possibly causing cell death.

Based on Table 3, it was notable that V had relevance to cell viability and intracellular ROS formation, while the correlations between other elements and the cell viability and the intracellular ROS content were not significant. V had a high correlation with the change in the intracellular ROS content, indicating that it had a substantial influence on the oxidative stress of cells. This agreed with Okeson's conclusion that the cytotoxicity of $PM_{2.5}$ was significantly correlated with the content of V (Okeson et al., 2003). Pb had a strong correlation with the cell viability and intracellular ROS content, which meant that although the content of Pb in $PM_{2.5}$ did not exceed the concentration limit of the WHO, its toxicity could not be ignored. Meanwhile, Cu, Cd, and Mn also showed high negative correlations with the cell viability. The results agreed with previous studies (de Kok et al., 2006; Gualtieri et al., 2009), which demonstrated that transition metals, such as Cu and Mn, were toxic to cells and could damage the membrane lipid, protein, and DNA, possibly causing cell death. Because the concentrations of V, Mn, and Pb differed between the summer and winter, this may be one of the reasons for the differences in the toxicity of $PM_{2.5}$ between the summer and winter.

3.4.2 Correlations between OC, EC and $PM_{2.5}$ cytotoxicity

Organic compounds in $PM_{2.5}$ have been linked to its toxicity (Borgie et al., 2015b; Liu et al., 2016). The correlation coefficients (Table 3) showed that OC, EC, and SOC were negatively correlated with the cell viability, and the correlations were especially high during the early exposure (24 h). This indicated that there were some organic matters in the extraction of $PM_{2.5}$, which affected the growth of the cells. Therefore, cell viability may be affected by the synergism or overlapping of water-soluble heavy metals and organic compounds. Moreover, this phenomenon was most obvious during the early exposure period. This result was similar to that reported by Huang et al. (2015), who used a water extraction of dust and $PM_{2.5}$ to interact with human cells and obtained a similar conclusion. However, OC, EC, and SOC had a smaller effect on ROS, which may be because the cells had no phagocytic function. Because of the phagocytic function of macrophages, when exposed to $PM_{2.5}$ extractions, the non-water-soluble components, which are presented as a foreign matter, can be easily identified by macrophages. In the process of its phagocytosis, the oxygen resulted in a large number of ROS, which led to the death of the macrophages (Imrich et al., 2000). However, cells do not have phagocytosis, such as A549 cells, non-water-soluble composition has little influence on cells, and water-soluble transition metal ions and other substances can more easily induce the production of free radicals and aggravate oxidative stress damage (Cao et al., 2008). Therefore, it could be conjectured that because of the BEAS-2B cells not having phagocytosis, OC, EC, and SOC have a low impact on ROS production. The different contents of OC and EC in the summer and winter would influence $PM_{2.5}$ cytotoxicity during the two seasons.

Table 3 Correlation coefficients of different chemical compounds in $PM_{2.5}$ and the cell viability and intracellular ROS level

Chemical component	Cell viability		Intracellular ROS level	
	24 h	48 h	3 h	6 h
V	0.5440*	0.8456*	0.8456*	0.8714*
Mn	−0.9370	−0.2874	−0.2874	−0.4838
Ni	−0.4064	0.0296	0.4770	0.1994*
Cu	−0.9237	−0.8479	−0.5440	−0.3936
Cd	−0.8284	−0.8432	−0.7824	−0.5694
Ba	−0.7257	−0.4213	0.0586	−0.0560
Pb	−0.8169	−0.8177	−0.7887	−0.7605
OC	−0.9639	−0.7943	−0.5750	−0.4507
EC	−0.9436	−0.8360	−0.6092	−0.4644
SOC	−0.9729	−0.7621	−0.5490	−0.4392
Na^+	−0.1803	0.1112	0.3471	−0.5110
NH_4^+	−0.3339	−0.2694	−0.4487	−0.9418
K^+	−0.6900	−0.7343	−0.6019	−0.8352
Mg^{2+}	−0.3634	−0.0062	0.4817	0.3741
Ca^{2+}	−0.3632	−0.0739	0.4537	0.2974
Cl^-	−0.4896	−0.4885	−0.4589	−0.9101
NO_3^-	−0.8384	−0.7056	−0.6617	−0.7764
SO_4^{2-}	−0.6335	−0.1494	−0.0206	−0.2741

* Significant correlation at the 0.05 probability level

3.4.3 Correlation between water-soluble ions and $PM_{2.5}$ cytotoxicity

Previous studies have found that the high levels of SO_4^{2-} and NO_3^- in $PM_{2.5}$ could change the pH of the exposed liquid, thereby affecting the growth of cells (Huang et al., 2015). From the correlation results (Table 3), water-soluble ions showed negative correlations with cell viability and intracellular ROS level. In addition to the higher correlations between K^+, NO_3^- and other indices, other components showed a low correlation with the cytotoxicity results.

The toxic effect of K^+ on cells could be related to the change in the membrane potential of cells, while NO_3^- may change the pH of the exposure solution and indirectly affect the growth of cells.

4 Conclusions

During both the summer and winter, the main chemical components of $PM_{2.5}$ in Hangzhou were water-soluble ions, particularly SO_4^{2-}, NO_3^-, and NH_4^+, followed by organic compounds and then heavy metals. The toxicity tests of water extractions of $PM_{2.5}$ showed that $PM_{2.5}$ had greater influences on cell viability and ROS levels when the mass concentration increased. However, when the mass concentration of $PM_{2.5}$ was considerable in the summer and winter, the extraction of $PM_{2.5}$ collected in the summer showed greater effects on cell viability and ROS levels. The difference in the contents of trace heavy metals, such as V and Pb, and organic compounds, such as OC and EC, during the winter and summer was one of the reasons for the seasonal toxicity difference of $PM_{2.5}$. Organic compounds in the extracts of $PM_{2.5}$ together with trace heavy metals would produce synergistic or overlapping toxic effects on cells, causing the cell viability decreasing. However, because of the low content of organic compounds released into the aqueous extraction and BEAS-2B cells did not have the function of phagocytosis, water-soluble organic compounds had less effect on ROS level. Although water-soluble ions were the main components in $PM_{2.5}$, most of them were not related to the cytotoxicity of $PM_{2.5}$.

Compliance with ethics guidelines

Hui-hui ZHANG, Zheng LI, Yu LIU, Ping XINAG, Xin-yi CUI, Hui YE, Bao-lan HU, and Li-ping LOU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：冬夏季 $PM_{2.5}$ 的理化特征及其对人支气管细胞 BEAS-2B 的毒性

创新点：对东南沿海地区典型亚热带季风气候影响下的不同季节 $PM_{2.5}$ 颗粒物的水提液进行了毒性探究。

方　法：对比了冬夏 $PM_{2.5}$ 样品的化学成分，及其水提液对人肺支气管细胞 BEAS-2B 的毒性，并探讨了其毒性和化学组成间的相关性。

结　论：冬夏季节 $PM_{2.5}$ 样品均以水溶性离子，尤其是 SO_4^{2-}、NO_3^-和 NH_4^+为主，其次是有机组分，重金属含量较少。$PM_{2.5}$ 浓度越高，暴露时对细胞活力的影响越大，对细胞内活性氧（ROS）增加的影响也越大。但是当 $PM_{2.5}$ 浓度相当时，夏季采集的 $PM_{2.5}$ 的暴露液对细胞活力和细胞内 ROS 水平的影响更大。$PM_{2.5}$ 的细胞毒性与重金属和有机污染物关系密切，而与质量比重最高的水溶性离子关系不大。

关键词：$PM_{2.5}$；季节差异；理化特征；细胞毒性