Source differences in the components and cytotoxicity of PM$_{2.5}$ from automobile exhaust, coal combustion, and biomass burning contributing to urban aerosol toxicity

Xiao-San Luo$^{1,\#,*}$, Weijie Huang$^{1,\#}$, Guofeng Shen$^{2}$, Yuting Pang$^{1}$, Mingwei Tang$^{1}$, Weijun Li$^{3}$, Zhen Zhao$^{1}$, Hanhan Li$^{1}$, Yaqian Wei$^{1}$, Longjiao Xie$^{4}$, Tariq Mehmood$^{5}$

$^{1}$International Center for Ecology, Meteorology, and Environment, School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, China
$^{2}$Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
$^{3}$Department of Atmospheric Sciences, School of Earth Sciences, Zhejiang University, Hangzhou 310027, China
$^{4}$Health Science Center, Peking University, Beijing 100871, China
$^{5}$College of Ecology and Environment, Hainan University, Haikou 570228, China

Correspondence: Xiao-San Luo (xsluo@nuist.edu.cn)

*Authors contributed equally to this work

Abstract. The combustions of fuels, including oil, coal, and biomass, are main anthropogenic sources of atmospheric fine particulate matters (PM$_{2.5}$), however, their discrepant contributions to health toxicity risks of mixed ambient aerosol pollution dominated by respective emission intensity and chemical compositions are still unclear. In order to explore the quantitative differences of these combustion emissions, ten typical types of each source PM$_{2.5}$, i.e., vehicle exhaust, coal combustion, and biomass burning, were collected by laboratory simulated combustion and dilution channel sampler. Totally thirty type combustion samples were compared with monthly urban air PM$_{2.5}$ samples, which chemical characteristics and biological effects were investigated by component analysis and in vitro toxicity assays of human lung epithelial cells (A549). Heavy metals are more plentiful in PM$_{2.5}$ from coal combustion and automobile exhaust, while carbonaceous fraction was plenteous in biomass burning. The overall cytotoxicity of PM$_{2.5}$ was automobile exhaust $>$ coal combustion $>$ biomass burning, with different toxicity pathways and triggers. The toxicity of PM$_{2.5}$ from gasoline/diesel and biomass combustion was relevant to the combination of carbonaceous and water-soluble components, but the toxicogenic capacity of coal combustion PM$_{2.5}$ was mainly related to the high content of heavy metals. All these three emission categories of anthropogenic combustion sources were more toxic than ambient PM$_{2.5}$ and should be the main independent contributors to the cytotoxicity of mixed urban air PM$_{2.5}$. Associated with the source apportionment results of positive matrix factorization (PMF) model that automobile exhaust, coal and biomass combustion contributed 27.7%, 25.2% and 13.1% of ambient air PM$_{2.5}$, respectively, the toxicological results suggest automobile exhaust and coal combustion are priority emissions with higher toxic pollutants to be reduced preferentially for precise urban PM$_{2.5}$ pollution control ensuring public health safety.
1 Introduction

As a mixture of multiple sources, ambient particulate matters (PMs) arise from anthropogenic activities are continuously deteriorating the urban air quality, particularly in developing countries. Among these, fine PMs with an aerodynamic diameter of less than 2.5 µm \((\text{PM}_{2.5})\) is recognized as a serious public health concern due to its long persistence in air, carcinogenicity and acute toxicity to humans (Al-Kindi et al., 2020). There were extensive epidemiological evidences that airborne PM, can cause serious negative effects on human health, such as cardiovascular diseases, respiratory diseases, genetic mutations, and developmental disorders (Chowdhury et al., 2022; Lelieveld et al., 2021; Smith, 2021; Clemens et al., 2017). Besides natural sources like dust and sea spray, the vast majority of aerosols come from anthropogenic activities such as energy consumptions, including the combustion of fossil fuels causing industrial emissions and automobile exhaust, and biomass burning (McDuffie et al., 2021; Wu et al., 2022). Finally, these diverse sources make the ambient air \(\text{PM}_{2.5}\) become a complex mixture with multiple chemical components varying with time and space, which consisting mainly of sulfate, nitrate, ammonium, organic carbon (OC), elemental carbon (EC), mineral and trace metals (Bari and Kindzierski, 2016). However, which specific components and which particular sources are the most critical factors dominating the aerosols' health risks, still leave puzzles unsolved.

Past studies performed in various countries have focused on physicochemical characterization or biological effects of ambient air \(\text{PM}_{2.5}\) respectively (Weagle et al., 2018; Jia et al., 2017; Wang et al., 2020). For example, the source analysis of \(\text{PM}_{1.5}\) by photochemical modeling (Bao et al., 2018), chemical composition of regional \(\text{PM}_{2.5}\) (Chi et al., 2022), and the mechanism of \(\text{PM}_{2.5}\) toxicity was independently reported recently (Jia et al., 2020). Because differences in particle composition, sources, and toxicity appear in different urban environments (Zhao et al., 2019; Wang et al., 2022a), the source profiles of different emission inventories were applied to elucidate aerosol pollution characteristics and control strategies. For instance, it was found that straw burning during the harvest season is a major trigger of severe air pollution in many regions (Sahu et al., 2021). Aerosols from open biomass burning in the Amazon had a stronger ability to induce reactive organic species (ROS) than laboratory-generated secondary organic aerosols (Tuet et al., 2019). The particle composition of motor vehicle exhaust was related to automobile types with various fuels, engines, and loads (Lin et al., 2020). A strong catalytic reactivity of metals in PMs emitted from diesel vehicles was observed by dithiothreitol (DTT) assay (Jesus et al., 2018). Sulfate is a major component of PMs from Xi’an city, western China, mainly released from residential coal combustion activities (Dai et al., 2019). SMK-1 is an important signal pathway in the toxicogenic mechanism of \(\text{PM}_{2.5}\) from coal combustions (Sun et al., 2020). Although there were emerging studies on particle emissions from single sources, quantificationally comparative studies on multi-source pollutants as well as the composition and toxicity of various sources are still limited. Furthermore, there is a particular lack of understanding regarding the relative contribution of each specific source to ambient aerosol pollution risks considering their different components’ roles.

The main objective of current study was to compare the chemical components and corresponding toxicological effects of various representative anthropogenic combustion sources and their contributions to ambient aerosol health risks. The aim is to provide detailed guidance on the targeting and precise control of sources with prominent risks based on their pivotal toxic
components. Therefore, we collected both ambient PM$_{2.5}$ samples from urban air and abundant representative source PM$_{2.5}$ samples from automobile exhaust, coal combustion, and biomass burning. Their profiles of chemical compositions and *in vitro* cytotoxicity were investigated and intercompared, to assess the differences in source-to-receptor toxicity and to infer the core toxicogenic components and respective harmful contribution. The pivotal toxicogenic components were identified based on the source-sink bi-directional composition-effect results, which were further used to assess the health toxicity contribution of various emission sources to atmospheric PM$_{2.5}$, supported by its source apportionment through positive matrix factorization (PMF) model. This study could advance the understanding to quantify the complex source contribution to high-risk PM$_{2.5}$ emission oriented to public health, which is imperative for precise prevention and control of atmospheric PMs pollution.

2 Materials and methods

2.1 Collection of PM$_{2.5}$ samples directly from anthropogenic emissions of 30 typical combustion sources and monthly urban air

As the main anthropogenic sources of the ambient air PM$_{2.5}$ pollution, totally 30 types of PM$_{2.5}$ samples emitted directly from automobile exhaust, coal combustion, and biomass burning were respectively collected as follows for both chemical and toxicological analyses.

Based on the classification of automobile fuel types as well as load and tailpipe emission standards provided by the 2019 Annual Report on Environmental Management of Mobile Sources in China, a total of 10 types of vehicles were chosen for exhaust investigation. They were further categorized into 7 sub-groups, including small duty gasoline coaches (SDGCs), small duty diesel coaches (SDDCs), middle duty diesel coaches (MDDCs), big duty diesel coaches (BDDCs), light duty diesel vans (LDDVs), middle duty diesel vans (MDDVs), and heavy duty diesel vans (HDDVs). The detailed information of these representative local automobiles were showed in Table S1.

To cover all coal types consumed in the city, 10 representative types of coal were gathered for investigation. They were further grouped into 4 sub-groups, including 2 types of honeycomb coal (HC), 3 types of anthracite coal (AC) and 2 types of bituminous coal (BC) mainly for restaurant or household use, and 3 types of industrial coal (IC) for coal-fired power plants and steel-smelting industry. The detailed characteristic analysis of these typical coals purchased from local market were showed in Table S2.

Considering the biomass combustion in rural areas surrounding the megacity, 10 typical types of agricultural and forestry solid wastes were gathered for investigation. Because of the high annual production of three staple food crops (rice, wheat, and corn) as well as soybean, peanut and rapeseed, their straws generated after harvest are often used as fuels in rural households. In addition, woods were also common fuels. Therefore, straws of rice, wheat, corn, soybean, peanut, rape, and sesame, corncob, branches of peach and pine, were selected as biomass fuels and further divided into 2 sub-groups, including...
8 types of crop straw and 2 types of firewood. The detailed characteristic analysis of these representative biomass fuels collected from rural areas around Nanjing city were showed in Table S3.

PM$_{2.5}$ samples directly emitted from these combustion sources were collected by dilution channel sampling method (Figure S1), using a 4-channel particulate matter dilution sampler (HY-805, Hengyuan Technology Development Co., CN). Each sampling included 3 channels of quartz microfiber filter (Figure S2) and 1 channel of Teflon filter membrane with diameters of 47 mm, through a size selector for PM$_{2.5}$ with a flow rate of 160 L min$^{-1}$. Clean air was pumped for 10 min before and after each sample was collected. The blank quartz and Teflon filters were baked by a muffle furnace at 500 °C for 3 h and at 60 °C for 4 h, respectively. After being equilibrated in a constant temperature and humidity chamber for 24 h, the filters were weighed. The sampled filters were stored in a refrigerator at -20 °C before analysis.

As the actual mixture of various source particles in real environment, totally 16 ambient air PM$_{2.5}$ samples covering a year monthly were collected from December 2019 to October 2020 in the urban site of Nanjing city, Yangtze River Delta of eastern China, using a high-volume air sampler. Detailed procedures and sample information were described in previous paper (Li et al., 2022), but the purpose of using these air samples in current study was to compare them with the specific source samples for evaluating the chemical and toxicological contributions of the anthropogenic combustion sources to environmental aerosols pollution.

2.2 Chemical composition analysis
All collected source and ambient PM$_{2.5}$ samples were conducted various component analysis. For the concentrations of heavy metals in particulates, samples were acid digested and determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima8000, PerkinElmer), while some elements at lower concentrations were measured by ICP mass spectrometry (ICP-MS; NexIONTM300X, PerkinElmer). Blank filter, reagent blank, and reference material (NIST SRM 1648a, urban dust) were adopted for analytical quality control. Carbonaceous species (OC and EC) in PM$_{2.5}$ were determined using a DRI-2001A OC/EC (Atmoslytic Inc., Calabasas, CA, USA). For the concentrations of water-soluble ions (WSIs), the main cations (Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, NH$_4^+$) and anions (NO$_3^-$, SO$_4^{2-}$, Cl$^-$, F$^-$) in PM$_{2.5}$ were measured by ion chromatography (IC, Thermo Fisher Scientific, USA), using the Metrosep C6-150/4.0 column for cations and the Metrosep A Supp 5 150/4.0 column for anions, respectively.

2.3 Preparing PM$_{2.5}$ suspension for cell exposure
Totally 30 source and 16 ambient PM$_{2.5}$ samples were also performed cytotoxicity tests. In order to elute the particles completely from the quartz membranes, the PM$_{2.5}$-loaded sample filter was cut into small pieces, immersed in ultrapure water and extracted six times (30 min for each) in an ultrasonic bath at 0 °C. The extract was then suction filtered through a 2.6 μm pore-size nylon membrane to remove possible quartz fragments, and the bulk filtrate was freeze-dried back to pure PM$_{2.5}$ powder. Ultimately, based on particle mass, the gathered PM$_{2.5}$ was dispersed by sterile phosphate-buffered saline (PBS) to a
concentration of 400 mg L\(^{-1}\), and then diluted to PM\(_{2.5}\) suspension of 80 mg L\(^{-1}\) with serum-free Dulbecco's modified eagle medium (DMEM) medium for following \textit{in vitro} cell exposure (Li et al., 2022).

### 2.4 Cell culture and \textit{in vitro} PM\(_{2.5}\) exposure

The A549 human lung epithelial cells were cultured in RMPI-1640 medium (Gibco, USA) supplemented with 10\% fetal bovine serum (FBS, Hyclone, USA) and 1\% antibiotic penicillin-streptomycin (100 U mL\(^{-1}\)) at 37 °C in a 5\% CO\(_2\) incubator. Cell viability was assayed by methylthiazoletetrazolium (MTT) (Chen et al., 2019). After trypsin action, the density of cells in the logarithmic growth phase was adjusted to 1 \times 10^5 mL\(^{-1}\). Cell suspensions were inoculated into 96-well plates (Costar, USA) at 100 μL per well. The blank control well (without medium and PM\(_{2.5}\) suspension) and reagent control well (with medium but without PM\(_{2.5}\) suspension) were set together. After incubation for 24 h, various types of PM\(_{2.5}\) suspension (80 mg L\(^{-1}\)) were added to 96-well plates and incubated for 24 h. Fresh medium and MTT reagent (Solarbio, Beijing, CN) were added to each well and the supernatant was discarded, then 100 μL of formazan lysate was added to each well. The optical density (OD) values were measured at 490 nm using a microplate reader (Thermo MULTISKAN FC, USA). Cell viability (%) = (OD\(_{\text{treatment}}\) – OD\(_{\text{blank control}}\)) / (OD\(_{\text{reagent control}}\) – OD\(_{\text{blank control}}\)). The oxidative stress (ROS) and inflammatory cytokines (TNF-α and IL-6) in the supernatant were analyzed by enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Enzyme Biotechnology Co., Ltd., CN), and OD values were measured at 450 nm (Huang et al., 2020; Pang et al., 2020).

### 2.5 Data analysis

The statistical analysis was performed by IBM SPSS statistics 24 and plotted by Origin 2020b software. Pearson correlation coefficients were produced by the correlation analysis. The variance was statistically significant when the statistical test level was P < 0.05, and extremely significant when P < 0.01.

The source apportionment of PM\(_{2.5}\) mass in ambient air was conducted by the receptor model PMF (EPA PMF version 5.0). Major constituents (OC, EC, Cu, Cr, Co, Ni, As, Pb, Mn, V, Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), NH\(_4\)+, Cl\(^-\), F\(^-\), NO\(_3\)^- , and SO\(_4\)^{2-}\) were selected as input data, and a four-factor solution was chosen as the optimal solution based on an assessment of the interpretability of the source profiles and the seasonal variability of the source contributions.

### 3 Results

#### 3.1 Contributions of combustion sources to urban ambient air PM\(_{2.5}\)

As shown in Figure S3, although have been significantly improved with the national air quality in recent years, the daily PM\(_{2.5}\) concentrations of typical Nanjing city still exceeded the healthy guidelines obviously, with higher urban air PM\(_{2.5}\) pollution level in the cold season\(^{23}\). Four major sources of the ambient PM\(_{2.5}\) were produced by the PMF model, i.e., secondary aerosols, automobile exhaust, coal combustion, and biomass burning, which account for 34\%, 27.7\%, 25.2\%, and 13.1\% of total PM\(_{2.5}\) mass concentration, respectively. Their source profiles and proportions were showed in Figure 1. Therefore, although the
contribution of secondary aerosols cannot be ignored, the main anthropogenic sources of urban air PM$_{2.5}$ were primary emissions from the various fuel combustions.

3.2 Chemical compositions of PM$_{2.5}$ from 30 different emission sources and ambient air

Representative chemical components including carbonaceous fractions, heavy metals and WSIs of all PM$_{2.5}$ samples from both ambient urban air and 30 typical anthropogenic combustion sources (covering different categories of automobile exhaust, coal combustion, and biomass burning) were analyzed and compared with each other.

According to the comparison of PM$_{2.5}$ bound carbonaceous fractions (Figure 2), automobile and biomass sourced aerosols contained significantly higher total carbon (TC) content than coal combustion and ambient air, while the OC/EC ratio trend was ambient air > coal combustion > biomass burning > automobile exhaust sources. It indicated that the carbon content of ambient PM$_{2.5}$ mixture was lower and dominated by OC than that of anthropogenic combustion sources. Figure S4-S7 showed the detailed carbon fraction characteristics (contents and ratio) of PM$_{2.5}$ from each specific source. Carbonaceous fractions in automobile exhaust PM$_{2.5}$ were high but the difference between OC and EC content was small. Depending on the diverse automobile fuels, loads and tailpipe emission standards, the concentrations of carbon fractions in exhaust PM$_{2.5}$ varied widely with vehicle categories. The carbonaceous portion of PM$_{2.5}$ gradually declines as emission regulations rise, and EC likewise declines dramatically (Figure S4). However, such differences among coal types were less, except the bituminous coal with extreme high OC (Figure S5). The biomass combustions differed from plant species that tree branches generally contained higher carbon contents than crop straws (Figure S6).

Based on the grouped (Figure 3) and detailed (Figure S8-S11) distributions of the measured heavy metals in various PM$_{2.5}$, the V concentrations of combustion sources were generally higher while Co and Mn were lower than ambient urban air. Coal combustion emissions carried highest levels of Pb and were enriched in Cu and As (Figure S9), while biomass combustions were rich in Cr and Ni (Figure S10). However, automobile exhausts were enriched in most heavy metals, especially Cu, and Cr, Ni, V, Mn (Figure S8). Heavy metals from different types of automobile exhausts with the same emission standard varies greatly. Anthracite and industrial coal combustions contain similar heavy metals much more than bituminous coal. Generally, Pb, V, Mn, As, and Cu in branches were higher than straws, while Cr, Ni, and Co were dominant and higher in straw burning emissions. A special discovery was that corn cob burning PM$_{2.5}$ carried more heavy metals than corn straw and was the biomass with the highest emission levels of heavy metals. Correspondingly, ambient air PM$_{2.5}$ were also rich in most metals, especially Mn, Pb, and Ni, Cu, Cr. Therefore, coal combustion sources might contribute most Pb to urban ambient air, and contribute significant Cu and As with automobile exhaust emissions, while biomass burning and automobile sources contribute the Cr and Ni. Besides natural dust, automobile exhaust should be the main anthropogenic source of airborne Mn. Considering the PMF source apportionments of ambient aerosols, automobile exhaust should be the main source of Cr in urban air PM$_{2.5}$, and also the source for Cu together with coal combustion.
According to the comparisons of water-soluble cation and anion concentrations in various PM$_{2.5}$ (Figure 4), coal combustions contained highest SO$_4^{2-}$ and NH$_4^+$, automobile exhausts had highest contents of NO$_3^-$ and Na$^+$, Ca$^{2+}$, while biomass burning sources contained highest K$^+$ and Cl$^-$, but Mg$^{2+}$ was the lowest for all sources. However, the urban ambient air PM$_{2.5}$ contained highest NO$_3^-$ and were also dominated by SO$_4^{2-}$ and NH$_4^+$, for which NO$_3^-$ should be mainly contributed by secondary aerosols and automobile primary source, SO$_4^{2-}$ and NH$_4^+$ should be significantly from coal combustions. Besides NO$_3^-$, Na$^+$ and Ca$^{2+}$, automobile source PM$_{2.5}$ also had the highest F$^-$ and Mg$^{2+}$ concentrations than other sources. The detailed concentration distributions of WSIs in PM$_{2.5}$ from each specific source were provided in the supplementary information (Fig S12-S14). The WSIs levels vary widely with specific source categories. LDDVS-2 had the lowest amount of WSIs compared to the other automobile exhausts (Figure S12). Similar to the metal composition, bituminous coal also had the lowest WSIs among all coals (Figure S13). Compared to branches, crop straws had much greater levels of K$^+$, Cl$^-$, SO$_4^{2-}$ and less levels of F$^-$, NO$_3^-$ (Figure S14).

To summarize, the overall concentrations of measured TC, cumulated heavy metals and WSIs in PM$_{2.5}$ from each source type were showed in Figure 5. Among all source emission and environmental receptor samples, the cumulated heavy metals from coal combustion was highest and automobile exhaust was higher than ambient air PM$_{2.5}$, the overall carbon contents from automobile exhaust and biomass burning were both higher than ambient PM$_{2.5}$, while only the cumulated soluble ions in PM$_{2.5}$ from primary source of coal combustion was equivalent to the ambient aerosols. In a word, chemical compositions of PM$_{2.5}$ distributed much diversely and varied significantly with the specific source types of anthropogenic combustion emissions.

### 3.3 Cell viability, oxidative stress and inflammation levels exposed to various PM$_{2.5}$

After 24 h exposure to the same dose of different PM$_{2.5}$ obtained from specific emission sources, the A549 lung cells also showed varied toxicological responses (Figure 6). The survival rate of cells exposed to automobile exhaust PM$_{2.5}$ was much lower than ambient air PM$_{2.5}$ (Figure 6a). Automobile exhaust PM$_{2.5}$ induced the highest ROS production in cells higher than biomass burning and both sources were also much higher than ambient PM$_{2.5}$ (Figure 6b). Coal combustion induced the highest cellular IL-6 production followed by automobile exhaust that was also higher than ambient air PM$_{2.5}$, while the PM$_{2.5}$ from automobile exhaust and biomass burning induced similarly higher cellular production of TNF-$\alpha$ than ambient PM$_{2.5}$ (Figure 6c, d). These results suggested that, anthropogenic combustion emission PM$_{2.5}$ had stronger ability to induce oxidative stress and inflammatory injury in cells than ambient air PM$_{2.5}$, thus resulted in the higher probability of apoptosis induction. Generally, the PM$_{2.5}$ from primary source of automobile exhaust posed the strongest overall toxicity. Therefore, to protect public health by controlling PM$_{2.5}$ pollution, the anthropogenic combustions were key target sources, especially the most toxic automobile PM$_{2.5}$ should be reduced preferentially.

### 3.4 Correlations between various PM$_{2.5}$ components and toxicity endpoints

Pearson correlation coefficients between chemical compositions and cellular toxicological response indicators were applied to screen the key components of all PM$_{2.5}$ involved in cell injury (Figure 7). It was found that, the degrees of correlations varied...
with the toxicogenic mechanisms of different airborne chemicals. Based on the overall PM$_{2.5}$ samples from various sources, the proinflammatory cytokine IL-6 showed significantly strong positive correlations with SO$_4^{2-}$ and some heavy metals (Pb, As, Cu), while TNF-α and oxidative stress (ROS) had similar significant positive correlations with aerosol components of carbon fractions (OC, EC), WSIs (Ca$^{2+}$, Mg$^{2+}$), and heavy metals (Ni, Cr). The TNF-α also showed positively correlation with water soluble Cl$^{-}$ and K$^+$, and ROS correlated with F$^-$ and Na$^+$. 

4 Discussion

4.1 New chemical markers for source apportionments of ambient air PM$_{2.5}$
Combustion emissions are key anthropogenic sources contributing to urban air PM$_{2.5}$, through both primary and secondary aerosols, which were 66% and 34% calculated by PMF model, respectively (Figure 1). The high concentrations of chemical markers are usually used in source analysis, such as ammonium sulfate and nitrate for secondary aerosols which are originated mainly from the gaseous precursors (e.g., NH$_3$, SO$_2$ and NO$_X$) (Mahilang et al., 2021), the EC, Cu, Mn, and Ni for vehicle exhaust (Srivastava et al., 2021), the As, Pb, OC, EC, SO$_4^{2-}$ and relatively low NO$_3^{-}$/SO$_4^{2-}$ ratios for coal combustion (Dai et al., 2020), soluble K$^+$ and Cl$^{-}$ for biomass burning (Jain et al., 2020). The detailed chemical species of these specific source emission PM$_{2.5}$ samples also supported the results. Moreover, low OC/EC ratio of high TC content, high NO$_3^{-}$, F$^-$, Na$^+$, Ca$^{2+}$ and Mg$^{2+}$, V and Mn of automobile exhaust, Pb and As, SO$_4^{2-}$ and NH$_4^+$ of coal combustion, soluble K$^+$ and Cl$^{-}$, and high OC/EC ratio of high TC for biomass burning found in current study (Figure 2-5), could also be corresponding potential aerosol source markers. The principal aim of this paper was to assess and contrast the chemical composition and potential harmfulness of particulate matter arising from diverse anthropogenic sources. As such, natural sources, like road dust, were not included in the source examination.

4.2 Common PM$_{2.5}$ components related to specific combustion sources
The OC/EC ratio of ambient aerosols greater than 2.0 is usually associated with secondary organic carbon (SOC) (Wang et al., 2022b), while the EC characteristics of primary PM$_{2.5}$ from various combustion emissions in current study also indicate their sources. The automobile exhaust source had high TC content and low OC/EC value with considerable EC content (Figure 2), varying with specific vehicle types (Figure S4). The contents of the carbon fractions from diesel vehicles were higher than gasoline exhausts, and the OC/EC ratios of diesel exhausts were much lower than gasoline vehicles, owing to both considerable contents of EC and OC from diesel vehicle emission PM$_{2.5}$. Some diesel vehicles showed higher EC emissions with age, so exhaust cleaning devices for them are suggested. In addition, the amounts of OC and EC in exhausts gradually decreased with the strengthened emission standards (Wong et al., 2020). In PM$_{2.5}$ samples obtained from coal combustion (Figure S5), the TC contents of bituminous coals were significantly higher than that of honeycomb coals, anthracite coals, and industrial coals, because bituminous coals contain higher volatile fraction, which is composed of organic matter. Therefore, besides the way of
combustion and the use of combustion stoves, the coal quality related to different coal types and origins determine the carbonaceous fractions of the PMs emitted from coal combustion (Zhang et al., 2022a). In the PM$_{2.5}$ samples from biomass combustion (Figure S6), OC contents were generally higher than EC contents, except that pine branches contained higher EC and rapeseed straw had considerable contents of EC and OC. Dominated by OC (Figure S7), the concentrations of carbonaceous fractions in ambient air samples varied seasonally (Flores et al., 2020; Xu et al., 2019). Combining the TC contents and OC/EC ratios, carbonaceous components in ambient PM$_{2.5}$ mainly originate from SOC (Wang et al., 2018) and anthropogenic combustion primary emissions for OC (Kang et al., 2018), and automobile exhaust for EC (Barraza et al., 2017). Thus, to control ambient carbon aerosol pollution, besides reducing the SOC precursor emissions, controlling primary aerosols especially EC from diesel vehicles were key measures.

Airborne heavy metals are usually recognized as contributing the oxidation stress of PM$_{2.5}$. Different types of automobiles emitted diverse metal contents (Figure S8). Because Mn is a common antidetonator that delays and prevents the oxidation of hydrocarbons and increases the octane number, which not only increases the thermal efficiency of the engine but also improves the emission performance of the vehicle (Cheung et al., 2010), the Mn content was greater in gasoline vehicle exhausts than in diesel vehicles. Although there are multi-sources of traffic Pb emissions such as fuel combustion and brake wear (Wang et al., 2019; Panko et al., 2019), the automobile exhaust Pb content of gasoline vehicles were greater than diesel vehicles owing to oil combustion. Moreover, for the same vehicle type (LDDVs-1 and 2; HDDVs-1 and 2; SDGCs-1 and 2), the stricter the emission standard, the lower the exhaust metal contents. The metal contents in the PM$_{2.5}$ of trucks was higher than that of passenger cars (Wu et al., 2016). In the combustion PM$_{2.5}$ of 10 coal types (Figure S9), Pb contents were the highest than other heavy metals, similar to available findings (Zhang et al., 2020). The PM$_{2.5}$ metals from bituminous coal were significantly lower than other coal types, because indicated by the coal quality analysis, bituminous coal has a low ash content which is mainly derived from non-combustible minerals in coal. These findings suggested that coal maturity might be an important factor influencing the metal composition of particulates emitted from coal combustion (Shen et al., 2021; Zhang et al., 2021). Heavy metal contents in burned PM$_{2.5}$ varied much widely with biomass types (Figure S10), although dominated by Cr and Ni. Because of the high enrichment coefficients of some metals for crop straws (Zhang et al., 2016; Sun et al., 2019), they also released more Cr, Ni, and Co during burning than fuelwoods. Total metal emissions were highest in corn cob but lowest in peanut straw burning. The heavy metals enriched in urban ambient air PM$_{2.5}$ demonstrated a seasonal pattern (Chen et al., 2018; Hsu et al., 2016) (Figure S11). Contents of V, Co, and As were relatively low and are less affected by seasonal changes. Accordingly, supported by the metal profiles of anthropogenic combustion sources and ambient aerosols, to control the environmental airborne heavy metal pollution, the Pb, Cu and As from honeycomb, anthracite and industrial coal combustion, Cu from vehicle exhausts and especially V from light duty diesel van with the CN.III emission standard and Mn from gasoline vehicles, Cr and Ni from biomass especially crop straws burning, should be key targets.

Epidemiological studies have also shown the mortality closely related to the WSIs such as sulfate and nitrate in aerosols (Ostro et al., 2009; Liang et al., 2022). Among the WSIs contents of various automobile exhausts (Figure S12), NO$_3^-$ and Ca$^{2+}$
were the most abundant anion and cation, respectively. The high NO$_3^-$ in the automobile PM$_{2.5}$ may be due to NO$_x$ production during high-temperature combustion (Zhang et al., 2022b), while the high Ca$^{2+}$ content should be related to additives in automobile fuels and calcium-based lubricants (Hao et al., 2019; Yang et al., 2019). Moreover, the exhaust WSIs decreased with the strengthened automobile emission standards. Coal combustion PM$_{2.5}$ contained relatively higher SO$_4^{2-}$ and NH$_4^+$ concentrations followed by Cl$^-$ than other WSIs species (Figure S13). Among various coal types, industrial coals emitted highest SO$_4^{2-}$ followed by honeycomb and industrial coal with also high NH$_4^+$, but bituminous coals emitted low WSIs which were mainly NO$_3^-$, F and Na$, Ca^{2+}$. The WSIs emission factors of honeycomb coal were generally higher than those of lump coal (Yan et al., 2020). For biomass combustion emissions (Figure S14), Cl$^-$ and K$^+$ were dominant WSIs in PM$_{2.5}$ from straw-based biomass fuels (Tao et al., 2016; Sillapapiromsuk et al., 2013), but fuelwood-based combustion emitted high NO$_3^-$. Plant species absolutely determine the emissions (Liao et al., 2021). Finally, there were also high levels of NO$_3^-$, SO$_4^{2-}$, and NH$_4^+$ in ambient air PM$_{2.5}$ (Zhang et al., 2019) (Figure S15). Consequently, implied by the WSIs species distributed in anthropogenic combustion sources and environmental PM$_{2.5}$, to control the aerosols ions pollution, the NO$_3^-$ from vehicle exhausts and fuelwood burning, SO$_4^{2-}$ and NH$_4^+$ from honeycomb, anthracite and industrial coal combustion, Cl$^-$ and K$^+$ from biomass especially crop straw burning, should be principal targets, by stricter automobile emission standards or using clean coals.

4.3 PM$_{2.5}$ toxicity related to specific sources by pivotal chemical components

The complexity of the sources and compositions of atmospheric PM$_{2.5}$ leads to different toxicological effects (Newman et al., 2020; Kelly, 2021). Our results demonstrated that the toxicities of anthropogenic combustion emission PM$_{2.5}$ were much greater than the urban ambient air PM$_{2.5}$ (Figure 6), owing to the higher concentrations of specific toxic components in PM$_{2.5}$ from these sources. The supplementary information had included exhaustive cytotoxicity indicators from each individual source (Figure S16-S19). While the survival rate of cell exposed to CN.III emission standard PM$_{2.5}$ was the lowest and the capacity to induce cells to produce ROS was the highest for CN.IV, automobile exhaust had a similar potential to cause cells to produce inflammatory cytokines (Figure S16). The capability to induce IL-6 production in cells was highest in industrial coal, whereas bituminous coal had the highest survival rate of cells and TNF-α induction capacity (Figure S17). From the Figure S18 above we can see that the cytotoxicity of straw and branches was analogous, but it should be noted that the survival rate of cell of various straw varies significantly. Carbon fractions bound in PM$_{2.5}$ could be transformed into reactive metabolites and then induce ROS production in cells (Stevanovic et al., 2019). The PM$_{2.5}$ bound heavy metals could also induce ROS production through the Fenton reaction and disrupt the function of enzymes in cells (Verma et al., 2010; Sørensen et al., 2005). Water-soluble components of PM$_{2.5}$, especially water-soluble transition metal ions could accelerate the induction of free radicals, which can cause inflammatory damage (Zou et al., 2016). Oxidative stress can lead to inflammatory infiltration of neutrophils and stimulate immune cells to produce inflammatory cytokines, among which TNF-α and IL-6 play important roles in the inflammation development (Xu et al., 2020). Ultimately, excessive production of ROS leads to dysfunctional endoplasmic
reticulum responses and dysfunctional lipid metabolism in ROS bursts can result in cell membrane damage and even cell death (Piao et al., 2018; Zhao et al., 2004).

These mechanisms were supported by the relationships between the measured components with cytotoxicity indicators of PM$_{2.5}$ from various specific sources (Figure 7). By relating to the chemical components overall, IL-6 was significantly positively correlated with SO$_4^{2-}$, Pb, As, and Cu, which were main contributors of coal combustion sources. Both TNF-$\alpha$ and ROS were significantly positively correlated with carbonaceous fractions, Ni, and Cr, which were main contributors of automobile exhausts and biomass burning; and were correlated with water soluble Ca$^{2+}$ and Mg$^{2+}$, which were main contributors of automobile exhausts. Moreover, the TNF-$\alpha$ was also positively correlated with water soluble Cl$^{-}$ and K$^+$, which were main contributors of biomass burning, and ROS correlated with F$^{-}$ and Na$^+$, which were main contributors of automobile exhausts. According to the source-based aerosols cytotoxicity comparisons (Figure 6), automobile exhaust PM$_{2.5}$ induced the highest lethality and cellular ROS and TNF-$\alpha$ production, coal combustion PM$_{2.5}$ induced the highest cellular IL-6 production, biomass burning PM$_{2.5}$ induce considerable cellular TNF-$\alpha$ and ROS production. It has been reported that PM$_{2.5}$ from coal combustion decreased cell viability, increased overall DNA methylation, and led to cellular DNA oxidative damage (Møller et al., 2014). Some studies proposed that the toxicity of PAHs in PM$_{2.5}$s from biomass burning was likely underestimated (Sarigiannis et al., 2015; Yin and Xu, 2018). The OC and EC were reported significantly associated with biological responses of PM$_{2.5}$s from vehicle emissions collected in tunnels (Niu et al., 2020).

Therefore, the accumulations of some organic matters with high carbonaceous content (OC, EC) in PM$_{2.5}$ typically from automobile exhausts and biomass burning, heavy metals (Pb, As, Cu, Ni, Cr) and water-soluble anions (SO$_4^{2-}$, Cl$^-$, F$^-$) and cations (Ca$^{2+}$, Mg$^{2+}$, K$^+$) contributed by various anthropogenic combustions, might induce ROS production in cells, cause cellular damage through oxidative stress and inflammatory responses, impair cell viability and finally harm human health. To improve the urban environmental air quality for best public health benefits by controlling aerosols pollution, considering the toxicity intensity of each chemical component and their contributions from various sources to ambient aerosols, preferential targets of specific primary PM$_{2.5}$ sources and bound pollutants to be controlled are suggested as following sequence: Reducing all anthropogenic combustions, especially decreasing the automobile exhaust with high contents of EC, Ca$^{2+}$, Mg$^{2+}$, F$^-$, and Na$^+$ from diesel exhausts by strengthening the emission standards, then controlling the coal combustion with high SO$_4^{2-}$, Pb, As, and Cu from by replacement with low-ash clean coals, and depressing the biomass burning with high OC, Ni, Cr, Cl$^-$, and K$^+$ from crop straw emissions.

5 Conclusions

In current study, we found that 2/3 mass of urban ambient air PM$_{2.5}$ in a typical megacity of eastern China originated from primary anthropogenic combustion sources. Because of the significant differences in the chemical compositions, the PM$_{2.5}$ from both ambient air and combustion sources showed much diverse in vitro toxicity to the human lung epithelial cells, either
for the environmental aerosol samples collected from different seasons, or for the direct \( \text{PM}_{2.5} \) emissions from various source types and even from the specific sub-groups of each fuel source category. According to the comparative study and correlation analysis, the carbonaceous fractions (EC, OC), water-soluble ions (SO\(_4^{2-}\), Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), K\(^{+}\), F\(^{-}\), Cl\(^{-}\)), and heavy metals (Pb, As, Cu, Ni, Cr) might play important roles in inducing cellular ROS production, causing oxidative stress and inflammation, resulting in cell injury and apoptosis, thus damage human health. These toxic pollutants accumulated in specific sources varied by the fuel properties. Combined with chemical composition and cytotoxicity sequence, the preferential controlling targets of specific combustion sources should be automobile exhaust (diesel vehicles with emission standards inferior to CN.IV), coal combustion (high ash and high sulfur coals), and biomass burning (crop straws). Although showing the synthetic effects of mixed compositions and complex sources, besides preventing the secondary aerosols from combustions, targeted reductions of these primary sources of toxic \( \text{PM}_{2.5} \) direct emissions, would produce the greatest benefits for public health with improved ambient air quality. Overall, this paper provides a precise, oriented, effective, efficient, and economical composition-source-based strategies for urban aerosols pollution control. However, as a prospect, the detailed mechanisms for toxicity of PMs with complicated components from various sources and their quantitative contributions to the health effects of ambient air \( \text{PM}_{2.5} \) mixture still need in-depth study.

**Supplementary materials**

There are 19 figures (Figure S1-S19) and 3 tables (Table S1-S3) in the Supporting Information.

**Data availability**

All raw data can be provided by the corresponding authors upon request.

**Author contributions**

XSL conceived and supervised the study; WH, YP, MT, HL, and ZZ collected the samples; WH, YP, MT, WL, HL, ZZ, GS, and LX analyzed the chemical compositions; WH, YP, and MT performed the toxicity tests; WH, YP, MT, and XSL analyzed the data; WH and XSL wrote the manuscript draft; XSL, WH, GS, and TM reviewed and edited the manuscript.

**Competing interests**

The authors declare that they have no conflict of interest.

**Financial support**

This work was supported by the National Natural Science Foundation of China (NSFC 41977349, 41471418).
References


Zhang, X., Zhao, X., Ji, G., Ying, R., Shan, Y., and Lin, Y.: Seasonal variations and source apportionment of water-soluble...


Captions of figures

**Figure 1.** PMF factor profiles and source percentages of secondary aerosol, automobile exhaust, coal combustion, and biomass burning contributing to urban ambient air PM$_{2.5}$.

**Figure 2.** Carbon contents (mg kg$^{-1}$) and ratio in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).

**Figure 3.** Heavy metal contents (mg kg$^{-1}$) in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).

**Figure 4.** Water-soluble ion (WSI) contents (mg kg$^{-1}$) in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).

**Figure 5.** Cumulated typical measured components (mg kg$^{-1}$) in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).

**Figure 6.** Cell viability, oxidative stress and inflammation levels of human alveolar epithelial cell lines (A549) exposed to PM$_{2.5}$ suspension (80 mg L$^{-1}$) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).

**Figure 7.** Overall correlations between typical cellular toxicological responses and chemical compositions of PM$_{2.5}$ from various specific sources (*p < 0.05, #p<0.01; n=46).
Figure 1. PMF factor profiles and source percentages of secondary aerosol, automobile exhaust, coal combustion, and biomass burning contributing to urban ambient air PM$_{2.5}$.
Figure 2. Carbon contents (mg kg$^{-1}$) and ratio in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).
Figure 3. Heavy metal contents (mg kg$^{-1}$) in PM$_{2.5}$ from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).
Figure 4. Water-soluble ion (WSI) contents (mg kg\(^{-1}\)) in PM\(_{2.5}\) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).
Figure 5. Cumulated typical measured components (mg kg\(^{-1}\)) in PM\(_{2.5}\) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).
Figure 6. Cell viability, oxidative stress and inflammation levels of human alveolar epithelial cell lines (A549) exposed to PM$_{2.5}$ suspension (80 mg L$^{-1}$) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air).
Figure 7. Overall correlations between typical cellular toxicological responses and chemical compositions of PM$_{2.5}$ from various specific sources (*p < 0.05, #p<0.01; n=46).