

Reviewers' Comments, Author's Replies, and Revisions in the Text

Manuscript Number: egosphere-2023-598

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

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MS type: Research article

Dear editor Dr. Prof. Su and dear reviewers,

We are very grateful to you for your critical comments and helpful suggestions. Your detailed advices have enabled us to improve our work. Based on these comments and suggestions, we have made careful modifications and detailed improvements in depth to the original manuscript. The changes are shown in the red prints. The point-to-point replies, explanations, and clarifications for all of the revisions are listed below for easy reference. We have also checked all the formats and reference styles of the overall manuscript again.

We hope the revised manuscript can be published in ACP after these important modifications and significant improvements.

Responses to Reviewers' Comments:

Reviewer #1:

This manuscript has investigated the contribution of three common combustion pollutants to the ambient urban PM_{2.5} health effects. The results are interesting, which showed that particles from different combustion processes at the same concentration exert different toxic effects on the A549 cells. The English language needs to be polished to further improve the quality of this work.

Reply and revision:

We appreciate your kindly evaluations very much for our manuscript. This manuscript has been revised thoroughly according to your following advices. To improve the language, we have polished the English carefully overall again. The point-to-point replies and explanations for all revisions are listed below for easy reference.

Other comments are as follows:

lines 24-26: is there any difference between the two words “toxicity” and “toxicogenic” when the authors here used them for different types of samples? Generally, toxicogenic indicated the toxin production activity of bacteria or other organisms.

Reply and revision:

Thanks very much for your reminding. Yes, the word “toxicogenic” is inappropriate in current study, and have unified the term by using “toxicity” or “toxic”.

line 102: was the Teflon filter also baked in the muffle furnace at 500°C?

Reply and revision:

Sorry for the confusion, we describe it more clearly in the revised manuscript. Before being used for sampling, the inorganic quartz filters were incinerated by a muffle furnace at 500 °C for 3 h to remove any possible organic matters, therefore, the parallel PM_{2.5} samples collected by quartz filters could be used for analyzing carbonaceous species. The organic Teflon filters used for collecting parallel PM_{2.5} samples of inorganic analysis were not baked by so high temperature.

Although the air sample information was referred to a literature, it would be better some brief information could be provided here. For example, how about the duration of the air sample?

Reply and revision:

Thanks for your reminding. We add some detailed sampling information about the ambient air PM_{2.5} samples in the revised manuscript. As the actual mixture of various source particles in real environment, totally 16 representative ambient air PM_{2.5} samples (each time lasting 23h) covering a year monthly were collected from December 2019 to October 2020 in an urban site surrounded by traffic, residential and commercial quarters of Nanjing city, Yangtze River Delta of eastern China, using a high-volume air sampler (800 L min⁻¹) with quartz microfiber filters.

line 136: how about the PM_{2.5} concentrations for the cell stimulation experiments? If 80 mg/L, was the cellular supernatant removed before the addition of PM_{2.5} elution? Cell viability test: has the authors treated the cells with other lower or higher concentrations in addition to the one concentration here (80 mg/L)?

Reply and revision:

Yes, the selected concentration of PM_{2.5} suspension is 80 mg L⁻¹ based on our pre-experiments covering lower and higher concentrations designed for the dose-response curves. Finally, under this dose, the oxidative stress and inflammation response sensitively, while the cell viability can keep sufficient. The cellular supernatant was removed before the addition of PM_{2.5} elution, so the cells were exposed to the same PM_{2.5} dose.

Correlations between PM_{2.5} components and toxicity: has the authors measured other biological components., e.g., LPS, which is a very strong inflammation inducer and is a common component in the air?

Reply and revision:

Much thanks for your nice suggestions. Lipopolysaccharide (LPS) as a common endotoxin in the ambient air is really a strong inflammation inducer, and should be a

significant component from natural sources posing health risks. Because our current study focus on the PM_{2.5} emitted directly from combustion sources, the biological components including LPS in these anthropogenic PM_{2.5} was not measured. But it's sure an important parameter in our future bioaerosols work.

Figure 1: it is not clear the percentage of species in what?

Reply and revision:

Thanks for the reminding. We have modified the Figure 1 to indicate the proportion (%) of each component from each source accounting for the corresponding component in urban ambient air PM_{2.5} more clearly.

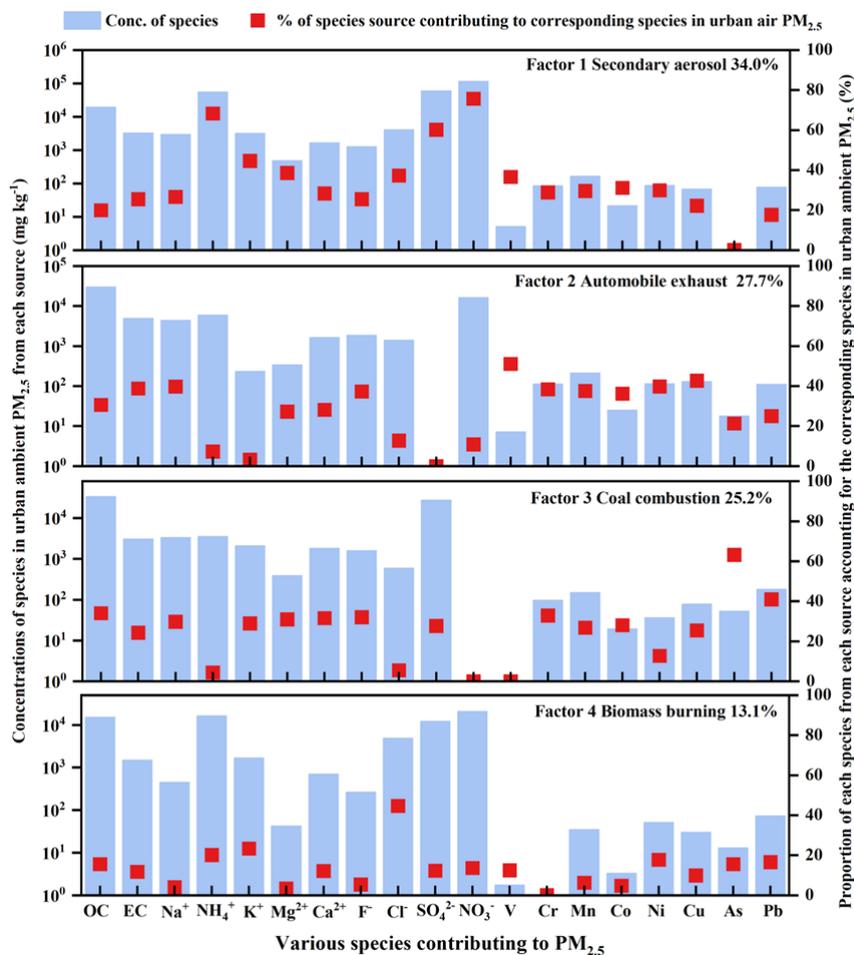


Figure 1. The PMF factor profiles of various components and source percentages of secondary aerosol, automobile exhaust, coal combustion, and biomass burning contributing to the urban ambient air PM_{2.5}.

Figure 5: were there any statistically significant difference for each component in different types of samples?

Figure 6: similar to the last question, statistical test?

Reply and revision:

Thanks very much for the reminding. We performed a significance analysis based on the Kruskal-Wallis test to modify Figure 5 and 6 in the revised manuscript.

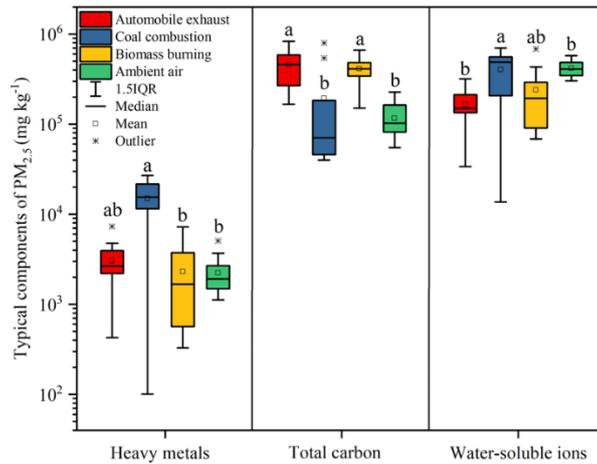


Figure 5. Cumulated typical measured components (mg kg⁻¹) in PM_{2.5} from various specific sources (n=10 for each combustion source and n=16 for urban ambient air). The letters a and b are significant groups classified by Kruskal–Wallis test, p < 0.05.

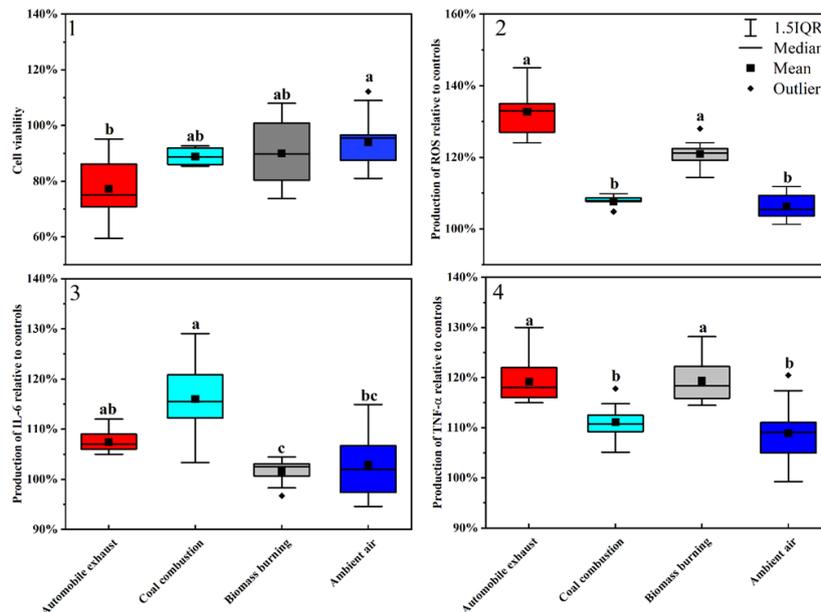


Figure 6. Cell viability, oxidative stress and inflammation levels of human alveolar epithelial cell lines (A549) exposed to PM_{2.5} suspension (80 mg L⁻¹) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air). The letters a, b and c are significant groups classified by Kruskal–Wallis test, p < 0.05.

Reviewer #2:

In this work, the authors studied the toxicological responses to PM_{2.5} from different combustion sources. They generated PM_{2.5} from a large variety of sources, including automotive engine exhaust, coal combustion and biomass burning. They concluded that PM_{2.5} in Nanjing is dominated by primary combustion sources, and PM_{2.5} generated from these sources are substantially more toxic than ambient PM_{2.5}. In general, the results are interesting and offers insights into PM toxicity. The authors also conducted a broad array of experiments and investigated multiple endpoints relevant to human health. However, the results can be analyzed and discussed in greater depth. The current version of the manuscript does not reflect hypothesis-driven research. The manuscript also contains many grammatical errors and awkward language that is not appropriate for scientific communication, to a point that it hinders reading of the manuscript. The manuscript should not be considered for publication until these substantial issues are fully addressed.

Reply and revision:

Thanks very much for your critical evaluation on our manuscript, which is an important guidance for us to improve the overall quality of this paper. We checked the whole paper carefully and correct the grammatical errors to make sure the language is accurate, clear and concise. We have revised the manuscript thoroughly according to your following advices on those substantial issues.

Currently, either the world air quality guidelines or the national air quality standards use the mass concentration of PM_{2.5} as the metric for PM_{2.5} pollution evaluation and management, in which all particles are treated as equally toxic, however, it is inconsistent with the scientific facts that particle toxicity are significantly related to their sources and chemical compositions (Shiraiwa et al., 2017; Kelly and Fussell, 2020). Therefore, to identify which component(s) and source(s) of ambient PM are most harmful to health, will be very helpful to optimize air quality guidelines/standards and prioritize targeted PM control strategies to more effectively protect public health.

The hypothesis of this study was that, dominated by the source and component effects, various anthropogenic combustions are very important environmental aerosol sources and contribute different hazardous compositions and thereby unequal toxicity effects to those of the urban ambient PM_{2.5}. Therefore, judging the most toxic source as priority emission to be targeted reduced preferentially for precise pollution control, might produce the greatest benefits for public health with improved environmental air quality.

The key results were also analyzed and discussed further in depth, although still limited by the paper length considering large amount of data. Of course, more introduction and discussion about the mechanisms of PM_{2.5} toxicity to lung cells were added in the revised manuscript (also see a detailed answer to the hypotheses question below). The point-to-point replies and explanations for all revisions are listed below for easy reference.

General comments:

This study discusses risks, but is actually investigating hazard when measuring cytotoxicity of PM from specific sources. The exposure levels are all fixed at 80 mg/L. The discussion should better reflect this distinction.

Reply and revision:

Thanks very much for your important reminding. Yes, as you suggest, this study investigated the “toxic effects” based on the same mass concentration of PM_{2.5} exposure in body lung fluid system, which should be more precisely than using the word “risks” usually relating to the inhalation exposure concentration of PM_{2.5} in air. So, we unified these descriptions in the revised manuscript. Moreover, for comparing the cytotoxicity, the selected concentration of PM_{2.5} suspension is 80 mg L⁻¹ based on our pre-experiments covering lower and higher concentrations designed for the dose-response curves. Finally, under this dose, the oxidative stress and inflammation response sensitively, while the cell viability can keep sufficient for the successful toxicity tests.

What is the justification for using A549 cells? There have been some discussions about the limitations of A549 cells (<https://doi.org/10.1016%2Fj.bpj.2009.12.4289>) For example, A549 may be more resistant to exposure to external compounds (<https://doi.org/10.1158/1078-0432.ccr-08-2822>). Often BEAS-2B cells are preferred over A549 cells, even though A549 cells have been used in toxicology studies for many decades. A discussion of the limitations is needed.

Reply and revision:

Much thanks for your careful reminding and helpful comments.

Air pollution can harm lung alveoli and epithelial cells. The A549 cell line is derived from human lung cancer and has characteristics similar to alveolar epithelial cells. This cell line has long been used as a suitable epithelial alveolar model to investigate the

interactions between PM and lung epithelial cells (Park et al., 2018; Li et al., 2022b).

Yes, as you suggest, the human normal bronchial epithelial cell BEAS-2B is really preferred over the human lung adenocarcinoma epithelial cell A549, and we honestly selected A549 cell based on our lab's abundant experimental experiences before and also because it has been used popularly in *in vitro* toxicology studies to elucidate the cellular and molecular mechanisms of PM_s involved in lung for many decades. For instance, both cells were used in an aerosol study (Bonetta et al., DNA damage induced by PM_{0.5} samples in A549 and BEAS-2B human cell lines: Results of the MAPEC study. Toxicology Letters, 2017, 280, 1: S208), results of which highlighted the higher sensitivity of BEAS-2B cells respect to A549 also in samples with low level of pollutants, because the PM samples from Italian towns can induce genotoxicity in normal cells while cancer cells might be resistant to their adverse effects. Therefore, although our results are authentic and reasonable in current study, we added some limitations of A549 cells in discussion of the revised manuscript (a new Section 4.4 Limitations), and of course we will choose the generally more sensitive BEAS-2B cells in our subsequent studies.

One of the biggest weaknesses of this study is the lack of central hypotheses. It just reports on a number of endpoints without answering any specific research questions. The central point is that the toxicity measures are the highest for some sources, but there does not seem to be much attempt to answer why. The presentations on the endpoints are not discussed holistically. Why are the cell viability and different inflammatory markers not consistent with each other? What different physiological processes they represent? And how would those be associated or triggered by different trace components? The discussion in Section 4.3 seems to present a singular picture of ROS production, TNF-alpha and IL-6 expression, but the data are more nuanced.

Reply and revision:

Thank you very much for the critical comments. More introduction and discussion about the toxicological mechanisms of PM_{2.5} components to lung cells were added in the revised manuscript with significant improvements.

The central hypothesis of this study was that, depending on the source and component effects, various anthropogenic combustions are very significant environmental PM_{2.5} sources and contribute different hazardous compositions and thereby diverse toxicity

effects to those of the urban ambient PM_{2.5}. Therefore, by toxicity tests with detailed chemical analyses of these independent source samples, judging the most toxic source as priority emission to be targeted reduced preferentially for precise pollution control, might produce the greatest benefits for public health with improved environmental air quality. So we focused on comparing and quantifying the toxicity of various combustion sourced PM_{2.5} related to the possible mechanisms of toxic components. The toxicity indicated by the endpoints we measured was basic phenomena/discovery and also very valuable knowledge, moreover, we attempted to answer why by their relation with the measured chemical compositions, but it's really not much owing to the focus, and we also can't over-explain only based on statistical relations. We tried more explanation and discussion about the PM_{2.5} toxicological mechanisms in Section 1 (Introduction), Section 2.4 (Cell culture and cellular toxicity tests by in vitro PM_{2.5} exposure), Section 3.3 (Cell viability, oxidative stress and inflammation levels exposed to various mass-normalized PM_{2.5}), and Section 4.3 (PM_{2.5} toxicity related to specific sources by pivotal chemical components), with a new Limitation Section 4.4 of the revised manuscript.

The physiological mechanisms of PM-induced cell toxicity in respiratory system have been continuously investigated with some progresses (Kelly and Fussell, 2012, 2020; Shiraiwa et al., 2017; Mack et al., 2020; Li et al., 2022b), such as the metabolic activation, oxidative stress, inflammatory response, and apoptosis, focused on by current study. In brief, after inhalation and deposition onto the epithelium, redox-active materials in PM_{2.5} can induce the release of ROS, which cause oxidative stress (an imbalance between ROS and antioxidants, i.e., disequilibrium of the redox state of a cell) followed by inflammation and cell death. The ROS can mediate subsequent signaling pathways leading to biomolecule damage (e.g., DNA, lipid, and protein) and cellular injury, through mediating inflammatory responses including the release of pro-inflammatory cytokines like IL-6 and TNF- α by epithelial cells (Sabbir Ahmed et al., 2020; Landwehr et al., 2021). For instance, oxidative stress could trigger the induction of pro-inflammatory transcription factors, such as nuclear factor (NF)- κ B, via the mitogen-activated protein kinase (MAPK) signaling pathway. Components adsorbed on particle surface, such as redox-active metals (transition metals, Fe, Ni, V, Cr, Cu),

organic compounds (PAHs, quinones), or even carbonaceous core of particles, are responsible for oxidative stress (Cachon et al., 2014; Sabbir Ahmed et al., 2020). The non-redox active metals (Zn, Pb, Al) can also influence the toxic effects of transition metals by exacerbating or lessening the production of free radicals. The EC may not be a directly toxic component of PM_{2.5} but rather operate as a universal carrier of combustion-derived chemicals (semi-volatile organic fractions, transition metals) of varying toxicity. Inorganic soluble sulphates and nitrates are acidic and can interact with and influence the solubility other compositions like metal bioavailability (Kelly and Fussell, 2020).

In this study, multiple biological responses that facilitate identifying the specific PM triggering ROS and inflammatory responses leading to oxidative stress, and cell death were evaluated for source-specific PM_{2.5}. Cell viability (metabolic activity) evaluated the mitochondrial dehydrogenase activity of the living cells. Excessive intracellular ROS formation induced by PM_{2.5} is responsible for oxidative stress to the cells. Cytokines IL-6 and TNF- α were determined for the effect of PM_{2.5} on pro-inflammatory response in cells. However, the toxicity of PM_{2.5} may be the result of multiple components acting through different physiological mechanisms, with inconsistent relationships among endpoints (Park et al., 2018). For instance, in BEAS-2B cells, oxidative stress generated by H₂O₂ exposure often results in cytotoxicity rather than by stimulating cytokine/chemokine responses, sometimes no correlation between oxidative damage and cytokine/chemokine responses. Moreover, TNF- α gene was not detected in BEAS-2B cells exposed to atmospheric PM collected from Benin, but the gene expression of other inflammatory cytokines (IL-1 β , IL-6, and IL-8) were significantly induced, and decreasing cell viability was highly correlated with high secretion of all studied cytokines (Cachon et al., 2014). Therefore, in the present study, it was impossible to analyze all chemicals in PM_{2.5} and determine all related toxicological endpoints, unmeasured chemicals and endpoints might also play roles in the unexplained results.

In toxicity assessments, cell vitality reflects the overall health of cells, encompassing factors such as cell membrane integrity, intracellular metabolic activity, and cell

proliferation capacity. Decreased cellular vitality may be associated with cell damage, toxic effects, or cellular apoptosis. Inflammation markers are employed to assess the extent and nature of inflammatory reactions, including the production of cytokines and inflammatory mediators, as well as the activation status of inflammatory cells. Inflammation is a complex physiological response, typically delineated by the immune and inflammatory reactions of the body to stimuli such as injury or infection. Alterations in inflammation markers can indicate the intensity and nature of the inflammatory response. Consequently, their variations may be incongruous.

Cachon, B. F., Firmin, S., Verdin, A., Ayi-Fanou, L., Billet, S., Cazier, F., Martin, P. J., Aissi, F., Courcot, D., Sanni, A., Shirali, P.: Proinflammatory effects and oxidative stress within human bronchial epithelial cells exposed to atmospheric particulate matter (PM_{2.5} and PM_{>2.5}) collected from Cotonou, Benin, *Environ. Pollut.*, 185, 340-351, <https://doi.org/10.1016/j.envpol.2013.10.026>, 2014.

Kelly, F. J., and Fussell, J. C.: Toxicity of airborne particles—established evidence, knowledge gaps and emerging areas of importance, *Phil. Trans. R. Soc. A*, 378, 20190322, <http://dx.doi.org/10.1098/rsta.2019.0322>, 2020.

Landwehr, K. R., Hillas, J., Mead-Hunter, R., Brooks, P., King, A., O’Leary, R. A., Kicic, A., Mullins, B. J., Larcombe, A. N.: Fuel feedstock determines biodiesel exhaust toxicity in a human airway epithelial cell exposure model, *J. Hazard. Mater.*, 420, 126637, <https://doi.org/10.1016/j.jhazmat.2021.126637>, 2021.

Li, T., Yu, Y., Sun, Z., and Duan, J.: A comprehensive understanding of ambient particulate matter and its components on the adverse health effects based from epidemiological and laboratory evidence. *Part. Fibre Toxicol.*, 19, 67, <https://doi.org/10.1186/s12989-022-00507-5>, 2022b.

Sabbir Ahmed, C.M., Yang, J., Chen, J. Y., Jiang, H., Cullen, C., Karavalakis, G., Lin, Y.-H.: Toxicological responses in human airway epithelial cells (BEAS-2B) exposed to particulate matter emissions from gasoline fuels with varying aromatic and ethanol levels, *Sci. Total Environ.*, 706, 135732, <https://doi.org/10.1016/j.scitotenv.2019.135732>, 2020.

In general, when examining the cell viability, ROS and inflammatory marker production data in the SI, I see significant variability within one source type, greater than between source types. Therefore, conclusions such as this one in the abstract: “The overall cytotoxicity of PM_{2.5} was automobile exhaust > coal combustion > biomass burning, with different toxicity pathways and triggers” is very problematic without examining the statistical significance. I also have some questions about weighting (see comment below) and whether the weighting is reflecting of relative contributions in the atmosphere.

Reply and revision:

Thanks for the reminding. We performed a significance analysis based on the Kruskal-

Wallis test to modify the statistical figures and summarisation in the revised manuscript.

Yes, there were really significant variability of toxicological indicators within one PM_{2.5} source type, that is exactly why we selected these 30 representatively specific combustion source samples for mass-normalized investigation independently. These combustion PM_{2.5} samples are different to each other in raw biomass characteristics like compositions, and all the original data of each independent source type were provided by the Figures S8-S19 in the Supplementary Materials. Focusing on the differences among the three combustion source groups, their grouped statistical results for general comparisons were showed in the Figures 2-7 of main text. Considering the multi-endpoints measured and the PM_{2.5} toxicity mechanisms mentioned above, based on the cell viability first, and then ROS followed by inflammatory markers, together with the significantly related toxic chemical composition contents (Park et al., 2018), we put forward a general sequence of overall mass-normalized toxicity for these three combustion sources PM_{2.5} to readers and managers.

As to the weighting contribution, the profiles were averaged with equal weights from each source in Figure 6, which showed the general statistics of the three source groups compared with ambient air, while the independent values of detailed specific types for each source group were showed in Figures S16-S19, respectively. This study investigated the unequal “toxic effects” based on the same mass concentration of PM_{2.5} exposure in body lung fluid system, while the “risks” usually relating to the inhalation exposure concentration of PM_{2.5} in air were not calculated and evaluated much in this paper.

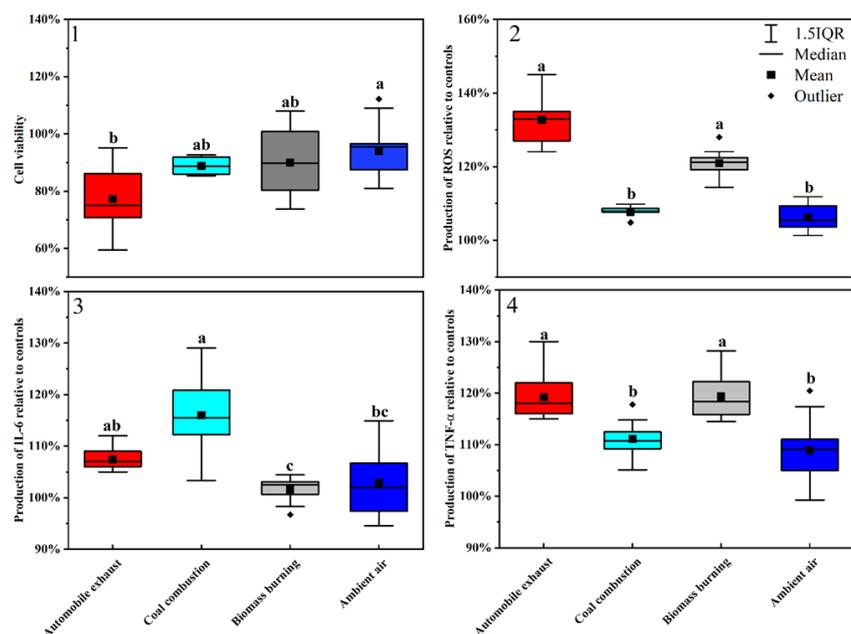


Figure 6. Cell viability, oxidative stress and inflammation levels of human alveolar epithelial cell lines (A549) exposed to PM_{2.5} suspension (80 mg L⁻¹) from various specific sources (n=10 for each combustion source and n=16 for urban ambient air). The letters a, b and c are significant groups classified by Kruskal–Wallis test, $p < 0.05$.

There has not been any direct evidence that sulfate itself is toxic (or linked with oxidative stress). However, it seems that increase in sulfate is associated with stronger acidity and greater solubility and bioavailability of redox active metals (see for example <https://pubs.acs.org/doi/10.1021/acs.est.6b06151>). Therefore the conclusion about water soluble ions being a source of toxicity might be too simplistic and should be discussed in more detail.

Reply and revision:

Thanks for the comment on helping explaining the roles of sulfate in PM_{2.5} toxicity. We involved these discussion and reference in the revised manuscript.

Fang et al., Highly acidic ambient particles, soluble metals, and oxidative potential: a link between sulfate and aerosol toxicity. *Environ. Sci. Technol.* 2017, 51, 5, 2611–2620.

Specific comments:

Abstract: the abstract should be rewritten with the following considerations. First, the abstract should convey global information, and some of the statements are specific to this study (or geographical area), such as the source apportionment results. These source apportionment results are not applicable to other areas. Furthermore, the abstract does not place the conclusions from the study in the appropriate context. It is unclear how the toxicological results affect PM_{2.5} pollution control policy, which are already targeting PM_{2.5} from major sources, such as coal combustion and vehicle exhaust.

Reply and revision:

Thanks very much for your detailed comments. We reorganized the Abstract in the revised manuscript.

Introduction:

I believe these papers are relevant to the investigation of source toxicity and should be discussed in the introduction as part of literature review:

<https://doi.org/10.1038/s41598-018-35398-0>

<https://doi.org/10.1016/j.envpol.2018.09.074>

Reply and revision:

Much thanks for your reminding. Yes, we read them before but sorry for missing citing. We have added these papers to the introduction and also for result comparisons.

Park, M., Joo, H. S., Lee, K., Jang, M., Kim, S. D., Kim, I., Borlaza, L. J. S., Lim, H., Shin, H., Chung, K. H., Choi, Y.-H., Park, S. G., Bae, M.-S., Lee, J., Song, H., and Park, K.: Differential toxicities of fine particulate matters from various sources, *Scientific Reports*, 8, 17007, [10.1038/s41598-018-35398-0](https://doi.org/10.1038/s41598-018-35398-0), 2018.

Borlaza, L. J. S., Cosep, E. M. R., Kim, S., Lee, K., Joo, H., Park, M., Bate, D., Cayetano, M. G., and Park, K.: Oxidative potential of fine ambient particles in various environments, *Environ. Pollut.*, 243, 1679-1688, <https://doi.org/10.1016/j.envpol.2018.09.074>, 2018.

The term biomass burning is quite broad and could be better defined. Biomass burning can include biofuel burning (for cookstoves or home heating), agricultural crop burning, wildfires or burning for land use change. It seems that this study is focused on crop burning, based on the type of fuel used. I suggest replace the use of “biomass burning” with “crop burning”.

Reply and revision:

Thanks for your meticulous comment. Yes, biomass burning can include biofuel burning (both solid, liquid, or gas), agricultural crop burning, and wildfires of plants mainly trees. In this study, we typically burned 8 types of crop (straws of rice, wheat, corn, soybean, peanut, rape, and sesame, corncob), and also 2 types of firewood (branches of peach and pine), so “crop burning” may not cover all biomass investigated. But “plant biomass burning” or “solid biomass burning” might be more accurate and we unified as “plant biomass burning”.

Line 33 and others: Many terms (such as particulate matter, consumption) should not be plural

Reply and revision:

Corrected overall.

Line 83: I am not sure if “big duty” is the proper term. Maybe “heavy duty”?

Reply and revision:

Yes, “heavy duty” should be better and revised.

Line 105: how long were the samples collected for? Are they 24 hour samples?

Reply and revision:

Detailed information added. As the actual mixture of various source particles in real environment, totally 16 representative ambient air PM_{2.5} samples (each time lasting 23h) covering a year monthly were collected from December 2019 to October 2020 in an urban site surrounded by traffic, residential and commercial quarters of Nanjing city, Yangtze River Delta of eastern China, using a high-volume air sampler (800 L min⁻¹) with quartz microfiber filters.

Line 113: what is the digestion efficiency? What is the digestion method? (I suspect that some of these methodological details are covered in a previous paper, but relevant details should be included in this manuscript.)

Reply and revision:

Yes, most methods applied for sample analyses were experienced and published. Detailed information was added. The PM_{2.5} samples were digested by concentrated HNO₃-HClO₄ acids with a progressive heating program. The recoveries for standard reference material ranged from 90-110 %.

Line 124: ultrasonication has been shown to impact ROS. (<https://doi.org/10.1080/02786826.2014.981330>) Potential artifacts should be discussed.

Reply and revision:

Thanks for the reminding. We cited your recommended paper for reference and made corresponding limitation discussion in the revised manuscript. Because ultrasonication treatment is the most commonly used and even might only efficient method to peel off the particulate matter (PM) from sample filters, the potential impact on ROS of PM can't be completely eliminated at 0 °C and was ignored as a systematic error. Moreover, the experimental indicators of this study did not measure ROS in the PM but cellular ROS.

Miljevic, B., Hedayat, F., Stevanovic, S., Fairfull-Smith, K. E., Bottle, S. E., Ristovski Z. D.: To sonicate or not to sonicate PM filters: reactive oxygen species generation upon ultrasonic irradiation, *Aerosol Sci. Tech.*, 48, 1276-1284, DOI: 10.1080/02786826.2014.981330, 2014.

Lines 143-145: there is an assumption of normality for Pearson correlation test. In many cases the distribution is not necessarily normal, and transformation may be needed (such as a log transform).

Reply and revision:

Thank you very much for the nice reminding. According to your comment, we checked the data of normality again and found that Spearman correlation test was more suitable for this study. We also revised the relevant results and discussion in the revised manuscript.

Line 157-159: Secondary organic aerosol often shows a greater toxicity than primary aerosol, and its fraction is still 34% (which is not small). Also, secondary does not mean it is not anthropogenic. Classifying the combustion sources are primary sources (rather than anthropogenic sources) would be more appropriate.

Reply and revision:

Yes, you are right. secondary aerosols can't be ignored, and part of them are also from anthropogenic sources. Our meaning here is exactly that, secondary aerosols (some also from combustions as anthropogenic sources) contribute (34%) to the urban air PM_{2.5}, but the primary sources of combustions as anthropogenic sources contribute more (66%). Sorry for the confusion.

Line 173-174: this sentence is incomprehensible.

Reply and revision:

Sorry for the confusion. We have made clear revisions.

Line 180-183: are these results related to differential uptake of metals in different parts of the plant?

Reply and revision:

Yes, different plant species and even different plant parts differ significantly in their ability to uptake and accumulate metals from soil (Zhao et al., 2020), that has been explained in the Discussion of Section 4.2.

Meifang Zhao, Suping Zeng, Shuguang Liu, Zhiqiang Li, Lei Jing, 2020. Metal accumulation by plants growing in China: Capacity, synergy, and moderator effects. *Ecological Engineering*, 148, 105790, <https://doi.org/10.1016/j.ecoleng.2020.105790>.

Line 190: why does automotive exhaust contain so much Na and Ca? On road studies would point to these alkali metals coming from resuspended road dust instead, but the experiments here sampled engine exhaust directly.

Reply and revision:

Thank for the reminding. Yes, this experiment sampled engine exhaust directly. The contents of Ca^{2+} and Na^+ might be related to additives in lubricant oil for anti-wear and anti-corrosion of the engine.

Line 214: why does increased inflammatory injury lead to greater probability of apoptosis? As far as I know, TNF-alpha and IL-6 are not markers of apoptosis, and one cannot distinguish apoptosis from the cell viability results. If the authors are assuming that association based on literature, relevant papers should be cited. Otherwise, none of the results in this study is pointing to apoptosis.

Reply and revision:

Thanks for your reminding. We added the following relevant papers cited for reference:

Wang Y, Cao M, Liu A, Di W, Zhao F, Tian Y, Jia J. Changes of inflammatory cytokines and neurotrophins emphasized their roles in hypoxic-ischemic brain damage. *Int J Neurosci.* 2013,123(3):191-5.

Victor, F. C., and Gottlieb, A. B.: TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis, *J. Drugs Dermatol.*, 1, 264-275, 2002.

Line 235: why is high NO₃⁻ a marker of automotive exhaust? Similar to my previous comment. The Zhang 2022b paper cited in this manuscript only refers to diesel vehicles. Would it be appropriate to apply this finding to all motor vehicles? Without understanding the emissions of these compounds, it would be very difficult to use them as markers confidently.

Reply and revision:

Thanks for the reminding. Because the concentration of NO₃⁻ in PM_{2.5} from automotive exhaust in this study was the highest among the three typical combustion sources. We corrected the reference by the following relevant paper:

Hao, Y., Gao, C., Deng, S., Yuan, M., Song, W., Lu, Z., and Qiu, Z.: Chemical characterisation of PM_{2.5} emitted from motor vehicles powered by diesel, gasoline, natural gas and methanol fuel, *Sci. Total Environ.*, 674, 128-139, <https://doi.org/10.1016/j.scitotenv.2019.03.410>, 2019.

Line 239: road dust is not a natural source

Reply and revision:

Yes, we corrected as “fugitive soil dust”.

Line 244: are the profiles averaged with equal weights from each source, or are they weighted by abundance? For example, there are likely more light duty vehicles than heavy duty vehicles.

Reply and revision:

Sorry for confusion. Yes, the profiles were averaged with equal weights from each source in Figure 2, which showed the general statistics of the three source groups

compared with ambient air, while the values of detailed specific types for each source group were showed in Figure S4, S5, and S6, respectively. To avoid ambiguity, we have already made clear revisions.

Line 261: heavy metals are not necessarily linked with oxidative stress. More accurately, it should be the “redox-active” metals that are linked with oxidative stress.

Reply and revision:

Thanks for the correction. Revised accordingly.

Line 329: the toxicity of PAHs are linked with mutagenicity and not necessarily connected to oxidative stress. Rather, oxidation products of PAHs (such as quinones) are the redox active components. This point here seems quite arbitrary and not necessarily linked with the results shown in this study.

Reply and revision:

Yes, you are wright. To avoid ambiguity, we deleted that inappropriate point and re-organized this paragraph.

Line 345: how is this city typical of a megacity in eastern China? In terms of population, or relative source contributions, or climate?

Reply and revision:

We revised “typical” as “representative”. The three universally typical combustion sources are the most key objects of this study, while the city investigated just provide representative ambient urban air samples. Of course, Nanjing city is a typical megacity in Yangtze River Delta of eastern China, either considering population, socio-economic conditions, air quality, or climate, and the sampling urban site surrounded by traffic, residential and commercial quarters is also very common in China.

Figure 2: how are outliers defined?

Reply and revision:

In box plots, outliers are usually defined as data points that are outside 1.5 times the interquartile range (IQR) away from the upper and lower quartiles. In this study, the outliers also reflect significant variations within a pollution source type to some extent. Therefore, they were kept in this figure.

Supplementary: Figures S8-S19 would be better presented if the data were grouped by species or endpoints rather than by sources. For example, it would be easier to compare ROS production for different sources if all the ROS data from different sources were right next to each other.

Reply and revision:

Thank you very much for the kind suggestion to reclassify the samples by species or endpoints rather than by sources in Figures S8-S19 of the Supplementary Materials. Because all the 30 specific combustion source samples we investigated are selected representatively and are different to each other in raw characteristics like compositions, we provide all the original data of each specific source type showed by the Figures S8-S19 in the Supplementary Materials, and the grouped statistical results for comparisons were showed in the Figures 2-7 of main text. Considering the paper length already with so many figures, and to avoid confusion of too much information in a figure, focusing on the general differences among the three combustion source groups, we finally didn't show the statistical results of sub-groups among each source group in Figures S8-S19, and the specific component was not combined with specific source into a figure.

Of course, we fully understand the information value might be provided by these statistical ways, and we did these analyses for self-use supporting the results and discussion.