Correspondence to Review 1

Thank you very much for your thorough and constructive comments on our manuscript amt-2020-10, entitled "A semi-continuous study on the toxicity of atmospheric particles using a versatile aerosol concentration enrichment system (VACES): development and field characterization". We made all corrections and revised the manuscript according to your comments. The response is given to each comment. In the revised manuscript, changes including some technical corrections are colored in blue.

General comments: This work investigated a semi-continuous system intending to online monitor the toxicity of atmospheric particles. An existing device, the VACES (versatile aerosol concentration enrichment system) was extended and enhanced for this purpose. This study provided a comparison between the measurement of the "toxicity" through using concentrated aerosols in the VACES system and non-concentrated from ambient air. The manuscript is generally well-written and is straightforward (maybe too much). Within the framework of analyses presented in this work, the results appear to be sound. However, there are a number of major issues, and among them, the scientific choice of the toxicicological assay. These should be considered and addressed before the manuscript can be considered for publication.

Response: As you pointed out, we give a specific description of the scientific choice of the toxicological assay (photobacteria) in the following responses and also add relevant explanations in manuscript.

Specific concerns:

Comment 1: A major issue is that throughout the manuscript, the authors emphasized that PM health effects, may be measured by photobacteria assay and to my knowledge, this bioassay has not been shown in any study to be associated with adverse PM health effects (if this is incorrect, please provide relevant citations). The study relies on the assay developed by Jing et al, 2019 which is in fact an ecotoxicological assay, not a toxicological assay. This ecotoxicological assay is based on the light inhibition of photobacteria which is sensitive to most of environmental toxics. This assay is not

specific and responds to many toxics when it's known that PM health effects rely mainly on oxidative stress. And finally this ecotoxicological assay (called biotoxic assay by their authors Jing et al., 2019) has not been compared to any toxicological assay. It does not quite make sense that the undertone of the manuscript hinges on the ability of VACES to permit monitoring aerosol toxicity when there is currently no link between photobacteria inhibition and adverse health effects from PM (if not relevant, please provide citation or comparison between photobacteria answer and toxicological results towards atmospheric particles)

Response 1: According to your questions mentioned in Comment 1, we give answers from four points:

First, it remains a scientific issue in vitro experiments that there is a lack of direct data support of the relationship between toxicity (e.g., cytotoxicity and ecotoxicity) and adverse PM health effects. Even for the exposure experiments (e.g., fish and mammalian), to our knowledge, no study exposes animals and human simultaneously to PMs. In fact, the definition of health effects are changes in health resulting from exposure to a source, which does not specifically refer to humans. However, in toxicology research, once health effects are mentioned, many people first think of human health effects. Therefore, in the absence of studies on the correlation between ecotoxicity and human health effects, we adopted your comments to remove or modify all discussions on health effects, and focus on the existing ecotoxicity results of PMs in order to avoid from misunderstanding.

Second is the use of the "toxicity" in the manuscript. It's absolutely right that the photobacteria experiment is an ecotoxicological assay, therefore, we change all "toxicity" words to "ecotoxicity" in manuscript as typically used in previous studies.

Third, there are several studies reported significant correlations between the Microtox (Photobacterium phosphoreum) EC_{50} and rat/mouse LD_{50} values (e.g., Fort, 1992; Kaiser et al., 1994), indicating the feasibility of photobacteria-base method on evaluating toxicity (exactly ecotoxicity).

Finally, the method of measuring ecotoxicity using photobacteria has long been routinely applied for water and soil research. This method has been standardized by the International Standards Organization (ISO 21338:2010: Water quality - Kinetic determination of the inhibitory effects of sediment, other solids and colored samples on the light emission of *Vibrio Fischeri*/ kinetic luminescent bacteria test. Photobacteria are also often used to assess the ecotoxicity of particulate matter and chemical components in ambient air. For instance, Turóczi et al. (2012) used *Vibrio fishcer* to

study the ecotoxicity of PM_{10}. This study directly evaluated the overall ecotoxicity of particles from different sources and seasons. Tositti et al. (2018) developed an ecotoxicity detection method using *Vibrio fishcer*, and found that ecotoxicity was closely related to the compositions of PM_{10}. Wang et al. (2016) demonstrated that the $PM_{2.5}$ components analyzed by *Photobacterium Phosphoreum T3* bioassay is ecologically toxic. Eck-Varanka et al. (2019) analyze the ecotoxicity of size-fractionated particles using *Vibrio fischeri*. Such literatures prove the feasibility of the photobacteria-based method in assessing the ecological toxicity of atmospheric particulate matter. The relevant description of the ecotoxicity assay of PMs in previous studies were also summarized and added in manuscript (Introduction section).

References

Eck-Varanka, B., Hubai, K., Horváth, E., Kováts, N., Teke, G., and Tóth, Á.: Assessing Ecotoxicity of Size-fractionated Airborne Particulate Matter, E3S Web Conf., 99, 2019.
Fort, F.: Correlation of Microtox EC, with mouse LD, Toxicol. In Vitro, 5, 73–82, 1992.
Kaiser, K. L., McKinnon, M. B., and Fort, F. L.: Interspecies toxicity correlations of rat, mouse and Photobacterium phosphoreum, Environ. Toxicol. Chem., 13(10), 1599-1606, 1994.
Tositti, L., Brattich, E., Parmeggiani, S., Bolelli, L., Ferri, E., and Girotti, S.: Airborne particulate matter biotoxicity estimated by chemometric analysis on bacterial luminescence data, Sci. Total Environ., 640, 1512-1520, 2018.
Turóczi, B., Hoffer, A., Tóth, Á., Kováts, N., Ács, A., Ferincz, Á., Kovács, A., and Gelencsér, A.: Comparative assessment of ecotoxicity of urban aerosol, Atmos. Chem. Phys., 12, 7365–7370, 2012.
Wang, W., Shi, C., Yan, Y., Yang, Y., and Zhou, B.: Eco-toxicological bioassay of atmospheric fine particulate matter (PM2.5) with Photobacterium Phosphoreum T3, Ecotox. Environ. Safe., 133, 226-234, 2016.

Comment 2: Another point is the comparison of the “ecotoxicity” between non-concentrated and concentrated aerosols in ambient air. During both experiments are done for a temperature of 45◦±2 ◦C in the saturator and then results are compared for ambient samples and samples through VACES. I guess that this temperature is not physiologically relevant when aiming at monitoring human health. The system shouldn’t overpass 37.5◦C since at 45◦C, many semi-volatile components may disappear and influence the answer of the system.

Response 2: Yes, you are absolutely correct. A current publication showed that as the temperature increased to 50 °C, the concentration of particle number, mass, semi-VOCs, and volatile ions in the VACES system was lost by 50% (Pirhadi et al., 2020). However, in our study:

On one hand, the water in saturator was heated to 45±2 °C, but after mixing with ambient aerosol stream (ambient temperature was no more than 24 °C during experiment period), the temperature of the saturator decreased to 31 °C or lower. In Dameto de España's study, they emphasized that "this saturator temperature difference has a strong influence on the outlet temperature at the exit of the condenser and consequently on the actual supersaturation experienced by the particles". In our research, the temperature difference was also found, and the actual supersaturation temperature (vapor temperature) of the ambient particles was only 31 °C (after mixing with the ambient air). Since the temperature of the aerosol stream at the outlet of the saturator cannot be controlled, the only temperature that can be controlled is the water temperature, which should not only ensure the enrichment factor of the particulate concentration (~ tenfold), but also maintain a temperature similar to the ambient temperature (just as you mentioned, when conducting human health studies, the temperature should be below 37.5 °C). Therefore, even when studying the effects of atmospheric particles on human health, toxicity or ecotoxicity, our setting of 45±2 °C (the actual supersaturation temperature is 31 °C) is reasonable. Moreover, at the current temperature, the enrichment efficiency of PM in VACES reached 75-98% (size-dependent) as shown in Table 1, which was comparable with those studies setting temperature at ~ 35 °C.

On the other hand, the ambient filter samples were sonicated in an ultrasonic bath set at 45°C. In order to fix the bottle (with filters) in water and maintain a temperature similar to the supersaturation temperature in VACES, we put the bottle in a plastic box, the temperature of the water in the box did not exceed 34 °C.

The above details were not explained in the manuscript, which caused readers to misunderstand. Therefore, we added the above descriptions to the manuscript (Section 2.2 and 3.1).

References

Pirhadi, M., Mousavi, A., Taghvaee, S., Shafer, M. M., and Sioutas, C.: Semi-volatile components of PM2.5 in an urban environment: Volatility profiles and associated

oxidative potential, Atmos. Environ., 223, 117197, 2020.
Dameto de España, C., Steiner, G., Schuh, H., Sioutas, C., and Hitzenberger, R.: Versatile aerosol concentration enrichment system (VACES) operating as a cloud condensation nuclei (CCN) concentrator: development and laboratory characterization, Atmos. Meas. Tech., 12, 4733-4744, 2019.

Comment 3: Finally, the data presented are good, but the manuscript should be modified/re-written to emphasize on the measurements and data and not over-extrapolate the impacts and implications of the results to human health.

Response 3: We completely agree with your opinion. As you figured out in Comment 1, we have no further data to explain the relationship between ecotoxicity and health effect (It's a work of our follow-up research), thereby we emphasize on the existing data and delete/re-write all health-related descriptions as given in response 1.

Comment 4: All the more, analysis and VACES performances should be deeper.

Response 4: As a technical paper, we aimed to simply provide the technical parameters of the VACES-ecotoxicity system and clarify its feasibility in preliminary laboratory and field measurements. The feasibility of the system was mainly clarified from two aspects: 1) The system steadily concentrated the concentration of PM to nearly 10 times; 2) Even if the concentration of PM was very low (a common case in the atmosphere), PM via concentration can meet the detection limit of ecotoxicity. These two points are very important for further online measurement of PM ecotoxicity in ambient air. In this study, we obtained a stable enrichment effect, and when the environmental PM concentration was below the detection limit, the ecological toxicity of PM (0.5 h or 1 h sampling) was much higher than the detection limit. Then, the feasibility of the system was verified.

These summary descriptions were added to the manuscript (Conclusion section) to emphasize the key points and make the system performance analysis more in-depth and clearer. In addition, as you suggested, in future research, we will conduct a more in-depth analysis, focusing on the application of VACES-ecotoxic system in atmosphere and combining with other factors (such as chemical composition, gaseous precursors and meteorological parameters) to investigate the characteristics of PM ecotoxicity (semi-continuous or even online).