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.

(Note: Re-added or modified responses have been marked in blue below.)

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 gave a specific description of the scientific choice of the toxicological assay (photobacteria) in the following responses and also added 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 gave answers from four points: First, it remains a scientific issue in vitro experiments that there was 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 exposed animals and human simultaneously to PMs due to ethics. 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 focused 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 eco-toxicological assay, therefore, we changed 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 were 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) analyzed the ecotoxicity of size-fractionated particles using *Vibrio fischeri*. Such literature proved the feasibility of the photobacteria-based method in assessing the ecological toxicity of atmospheric particulate matter. The relevant descriptions 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.

All the replies mentioned above were already in the "Introduction" section or have been added. The blue font below shows better arrangements and revisions to the introduction:

To solve this problem, photobacteria (e.g. Photobacterium phosphoreum) are utilized in the ecotoxicity study of atmospheric particles, because the detection was rapid (e.g. within 15 minutes; Hoover et al., 2005) and the cultivation period is only 5 minutes (Jing et al., 2019). The method of measuring ecotoxicity using photobacteria bioluminescence inhibition bioassay has long been routinely applied and standardized for water and soil research (ISO 21338:2010: Water quality – Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of Vibrio fischeri /kinetic luminescent bacteria test/). It had been reported that the photobacterium phosphoreum EC_{50} (median effective concentration) significantly correlated to rat and mouse LD_{50} (the lethal dose for 50 percent of the animals tested) values, indicating the reliability of photobacteria-based ecotoxicity assay (Fort, 1992; Kaiser et al., 1994). Recently, photobacteria have also been often used to assess the ecotoxicity of particulate matter and chemical components in atmosphere. 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 was ecologically toxic. Eck-Varanka et al. (2019) analyze the ecotoxicity of size-fractionated particles using *Vibrio fischeri*. Such literature proved the feasibility of the photobacteria-based method in assessing the ecological toxicity of atmospheric particulate matter. However, the detection limit of ecotoxicity using photobacteria is high. For example, in Jing's research, samples with a light inhibitory rate of less than 20 % were considered to be non-toxic due to the impact of normal bacteria fluctuations. Whereas, the concentration of atmospheric aerosols is usually far lower than that required for eco-toxic assay in case of short sampling time (e.g. one hour), which means a longer sampling time is required. Nevertheless, long-time sampling may lead to a large loss of volatile substances or chemical reactions in the particles, subsequently resulting in large errors in ecotoxicity analysis (Weiden et al., 2009).

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 was the water temperature, which not only ensured 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 study the effects of atmospheric particles on human health, toxicity or ecotoxicity, our setting of 45±2 °C (the supersaturation temperature was 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 emphasized on the existing data and delete/re-write all health-related descriptions as given in response 1. The deeper discussion of the current data was shown in the next response.

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

Response 4: The deeper (in blue) discussion focusing on the current data was added in Discussion and Conclusion section as follows,

The study evaluated the ecotoxicity by the light inhibition rate of photobacteria, the higher the value, the higher the

ecotoxicity. The light inhibition rate was calculated by one hundred multiplying the ratio of the difference in fluorescence intensity between treated and untreated medium to the untreated medium, where untreated medium meant only bacteria in medium without particle samples, treated medium corresponded to a sample adding in bacteria medium. Discontinuous sampling was operated under $PM_{2.5}$ concentrations ranging from 21 to 187 μg m^{-3}. During the sampling period, the photobacteria light inhibition rate of almost all ambient samples was lower than the baseline (20 %). On the contrary, the rate of all VACES samples was higher than 20 % (Fig. 4a and 4b). Note that since bacteria is sensitive to environmental condition, it is difficult to determine whether the sample is non-toxic or toxic as inhibition rate is lower than the baseline. It implied that the increase in ecotoxicity caused by increase of particulate concentration could avoid the inaccurate assessment of particulate ecotoxicity in the range below the baseline. In addition, when the concentration was low, the change in the light inhibition rate of ambient particles did not match the concentration of environmental $PM_{2.5}$. However, under high concentrations of $PM_{2.5}$, they matched better. During continuous sampling period, $PM_{2.5}$ concentrations varied from 14 to 107 μg m^{-3}. The light inhibitory of both ambient and VACES particles exhibited similar trends with the change of $PM_{2.5}$ concentration (a strong positive correlation, $r^2 > 0.7$) (Fig. 5). Note that as removing the data points of low $PM_{2.5}$ concentration, the correlation coefficient increased. Both experiments indicated that as the concentration of $PM_{2.5}$ decreased, the impact of concentration on ecotoxicity might be weakened, and the ecotoxicity may be caused mainly by the toxic chemical components in the particles (Akhtar et al., 2014). In this regard, the effects of key toxic components on ecotoxicity changes need to be further studied using VACES.

The change of EF was roughly reversed to the trend of the light inhibition rate of the ambient and VACES particles (Fig. 4). The main reason was that the increase of the light inhibition rate of VACES particles was lower than that of the ambient at high $PM_{2.5}$ concentrations. Comparing the EF changes of chemical components, it was found that when the ratio of light inhibition rate of VACES to ambient particles decreased, the EF corresponding to nitrate also decreased (Fig. 6). It showed that under high $PM_{2.5}$ concentrations, the EE of VACES for high-concentration nitrate was reduced, which was probably attributed to the loss of nitrate in VACES at a higher saturation temperature (about 7 degrees higher than the ambient). According to calculations, among the VACES particle concentration loss (average of 20.1 % during entire experiment period), nitrate accounted for 18.0 %. Therefore, under the premise of providing sufficient water vapor, reducing the saturation temperature or reducing the deviation from the ambient temperature were an important way to improve the enrichment effect of VACES.

4 Conclusions and implications

To achieve detection limits for atmospheric particulate ecotoxicity, a versatile aerosol concentration enrichment system (VACES) was extended to be integrated with ecotoxicity measurement. The VACES was developed to increase particle concentrations by about 7–10 times under the conditions of chiller temperature (–19 ± 1 °C), saturator temperature (45 ± 2 °C, supersaturation temperature was less than 31 °C), major air flow (50 ± 1 L min^{-1}), and minor-to-major flow ratio (1/10). We conducted discontinuous and continuous sample collection to analyze the ecotoxicity of VACES and ambient particles in half-hour and one-hour time resolution, respectively. It was found that the ecotoxicity of almost all ambient samples below the detection baseline as ambient $PM_{2.5}$ concentration varied from 14 μg m^{-3} to 187 μg m^{-3}. After enrichment, however, the ecotoxicity was significantly detected for almost all samples, proving the feasibility of the integrated system on rapid ecotoxicity assay. In addition, by comparing the change of the ambient $PM_{2.5}$ concentration with the light inhibition rate of ambient and VACES particles, it was found that as the concentration of $PM_{2.5}$ decreased, the correlation between the $PM_{2.5}$ concentration and the light inhibition rate was significantly weakened. It meant that at low concentrations of particulate matter, the impact of concentration on ecotoxicity was greatly reduced, and the interference with ecotoxicity might be the

change of toxic components. Moreover, during the high $PM_{2.5}$ concentration period, the ecotoxicity of VACES particles and the EF were significantly weakened or reduced, due to the loss of nitrate in relatively high-temperature VACES system, which provided a guidance for improving the enrichment effect of VACES.