A semi-continuous study on the ecotoxicity of atmospheric particles using a versatile aerosol concentration enrichment system (VACES): development and field characterization

Xiaona Shang[1], Ling Li[1, 2], Xinlian Zhang[1], Huihui Kang[1], Guodong Sui[1], Gehui Wang[2], Xingnan Ye[1], Hang Xiao[3], Jianmin Chen[1, 2, 3*]

[1] Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP[3]), Department of Environmental Science & Engineering, Institute of Atmospheric Sciences, Fudan University, Shanghai 200438, China

[2] Institute of Eco-Chongming, 3663 N. Zhongshan Rd., Shanghai 200062, China

[3] Institute of Eco-Environment, University of Chinese Academy of Sciences, Beijing 100080, China

Correspondence to: Jianmin Chen (jmchen@fudan.edu.cn)

Abstract: Oxidative stress can be used to evaluate not only adverse health effects, but also adverse ecological effects, but limited research uses eco-toxicological assay to assess the risks posed by particle matters to non-human biomes. One important reason might be the concentration of toxic components of atmospheric particles is far below the high detection limit of ecotoxic measurement. To solve the rapid detection problem, we extended a versatile aerosol concentration enrichment system (VACES) for ecotoxicity aerosol measurement and firstly used VACES to provide a comparison of ecotoxicity between non-concentrated and concentrated aerosols in ambient air. In this study, the total concentration (number or mass), the concentration of chemical components, and the ecotoxicity were all increased by approximately 7 to 10 times in VACES, making the detection of ecotoxicity above the baseline. The comparison of ecotoxicity data and PM$_{2.5}$ concentration showed that low concentration was not matched with ecotoxicity, although high concentration corresponded to higher ecotoxicity. In addition, the higher saturation temperature in VACES caused a loss of particulate matter, of which nitrate accounted for about 18 %.

1 Introduction

Currently, most toxicological studies focus on discovering the relationship between particulate matter and the morbidity or mortality of organisms (e.g. Vincent et al., 2001; Cox et al., 2016; Miri et al., 2018), or exploring toxic mechanisms by

25   exposure experiments (e.g. Magnani et al., 2016; Huang et al., 2017; Rychlik et al., 2019). However, the measurement ecotoxicity data are rarely available because of technical limitations. For instance, it requires a long detection time due to the animal and plant reproduction or cell cultivation (National Research Council, 2006), but the concentration and chemical composition of particulate matter in the atmosphere continue to change over time, especially during severe pollution (Shang et al., 2018a, b). Thereby, a short analyzing time is quite important.

30   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 min; Hoover et al., 2005) and the cultivation period is only 5 min (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

35   luminescent bacteria test/). It had been reported that the photobacterium phosphoreum EC50 (median effective concentration) significantly correlated to rat and mouse LD50 (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 PM10. This study directly evaluated the overall ecotoxicity of particles

40   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 PM2.5 components analyzed by *Photobacterium Phosphoreum T3* bioassay was 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. However, the detection

45   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.,

50   2009).

In this respect, aerosol enrichment techniques have been developed and applied to increase aerosol concentrations to meet ecotoxicity detection limits. Among them, the versatile aerosol concentrator enrichment system (VACES) originally developed by Sioutas et al. (1999) is effectively used to concentrate ambient particles. Since then, it has been widely used for laboratory and field measurements of particulate matter (De Vizcaya-Ruiz et al., 2006; Steenhof et al., 2011; Plummer et al.,

55   2012; Loxham et al., 2013), because the physical and chemical properties do not change after concentration (Kim et al., 2001a, b; Wang et al., 2013). It has also been extended to combine various chemical and physical analysis of particulate matter (e.g. gases, water-soluble ions, heavy metals, polycyclic aromatic hydrocarbons, cloud condensation nuclei, etc.) (Jung et al., 2010; Freney et al., 2006; Pakbin et al., 2011; Zhao et al., 2005; Dameto et al., 2019). In addition, VACES has been applied to determine the relationship between particulate matter and health effects based on exposure experiments

60   (Klocke et al., 2017; Ljubimova et al., 2018). Nevertheless, although VACES was originally developed to provide technical support for ecotoxicity detection, there is no direct measurement data to show the change in ecotoxicity between ambient particles and VACES particles.

Therefore, according to the previous design, by optimizing technical parameters, we modified and further developed VACES to integrate into the ecotoxicity measurements, verified the enrichment effect on physio-chemical concentration and

65   ecotoxicity in laboratory and field studies, and also investigated the relationship between ecotoxicity and particulate masses.

2 Methodology

2.1 Design of VACES

VACES used a saturation and condensation system to rapidly grow particles into super-micron droplets, which were then concentrated by a virtual impactor (VI). Detailed description of the design of VACES is available in previous studies (e.g.

70   Kim et al., 2001a, b). Briefly, when the airflow was sucked into a water tank filled with deionized water (defined as a saturator) with a U-shaped heating tube inside, the particles became supersaturated. A tube was fixed above the outlet of the saturator, and a copper tube coil was tightly wound on the outside to provide fast condensation conditions. A chiller (Bilon,

China) filled with ethanol (80 %, Hushi, China) cooled through the coil. The condensed aerosols were drawn up to a virtual impactor, where particle concentration in sizes was concentrated to a desired level by changing the ratio of the major-to-minor air flow controlled by a mass flow controller (MFC, D08-4F, Sevenstar, China).

2.2 Sampling

Sampling was conducted for several experiments, including laboratory performance test, field performance test, discontinuous sample collection and continuous sample collection. The performance test in this study used the enrichment factor (EF) defined as the ratio of concentrated (VACES) to non-concentrated (ambient) particle concentration, and the enrichment efficiency (EE) defined as the ratio of the concentrated concentration to ten times the non-concentrated concentration as a standard. The closer the EF and EE are to 10 and 100%, respectively, the better the enrichment effect of VACES. The instrument operating parameters (major air flow, minor air flow, condensation temperature and saturation temperature) were defined as the optimal parameters when the best enrichment effect was obtained. In the laboratory performance test, an atomizer (Model 9302, TSI, USA) was used to atomize polystyrene latex (PSL, Thermo Fisher Scientific, USA) to produce 200 nm, 300 nm, 500 nm and 700 nm particles respectively (Figure 1). On the one hand, after drying the generated PSL particles (Nafion tube, MD-700, Perma Pure, USA), set the corresponding voltage through a differential mobility analyzer (DMA, Model 3081, TSI, USA) for screening, and then entered the condensation particle counter (CPC, model 3775, TSI, USA) at a flow rate of 0.3 L min^{-1} for counting. On the other hand, PSL particles were introduced into VACES to obtain ten times the concentrations, and then the concentrations were calculated by the system of DMA and CPC after drying. Use four set data (number and mass) of PSL particles with and without enrichment to draw the EF calibration line. In comparison, field performance test was similar to laboratory test. The only difference was the replacement of PSL particles with ambient particles (<1.0 μm). In addition, during the field performance test, an aerosol filter (ETA Filters, USA) was installed at the inlet of the saturator to remove ambient particles to study the formation of particles in the VACES (Fig. 1; the dash line marked the filter location). Then, if no or few particles were observed in CPC, the impact of particle formation in VACES could be excluded or ignored.

In the performance test, we determined the optimal parameters (as defined above) of VACES. Then, we successively carried

out discontinuous and continuous VACES particle collection on the sixth floor of the Environmental Science and Engineering Department of Fudan University in Shanghai. We opened the inlet to the ambient air, in which particles were sucked into the saturator at a major flow rate and increased in concentration at a minor flow rate (Kim et al., 2001a). VACES particles were collected in 5 ml of deionized water through a biosampler (SKC, USA) for 30 min and 1 hour. In order to study the physio-chemical and ecotoxicity differences between VACES particles and environmental particles, we switched the inlet of the biosampler to ambient air after VACES particles were collected, that is, 30 min (1 hour) VACES samples, then 30 min (1 hour) environmental samples. From October 23rd to December 11st, 2019, we obtained a total of 10 sets of 30-minute samples and 10 sets of 1-hour samples. Therefore, due to time discontinuity, sampling was defined as discontinuous collection. In contrast, in the continuous sample collection process, we added a PM$_{2.5}$ sampler (PM-100, Wuhan Tianhong, China) to the inlet of VACES. To achieve continuous VACES particle collection, we added a peristaltic pump (BT100-4, HUXI, China) forward of the biosampler to pump in deionized water, and connected the outlet of biosampler to another peristaltic pump to evacuate the sample into an automatic fraction collector (BS-40A, HUXI, China). Pumping in and out were performed at a scheduled time (59-minute sleep mode and 1-minute work mode) and volume (5 mL). However, the continuous collection of ambient particles was performed by a PM$_{2.5}$ cyclone (Met one Instruments, USA) at a flow rate of 5 L min^{-1} and on a 47 mm Teflon filter (Whatman, USA). The filter sampling time is 8 h to meet the detection limit of water-soluble ions. In the end, we collected 88 VACES samples and 11 simultaneous ambient samples from December 18th to 31st, 2019. Note that the filter samples were extracted in 10 ml deionized water via 20-minute sonication and < 34 °C (< 34 °C within filter bottle and 45 °C out of filter bottle) heating condition.

2.3 Measurements

All samples were filtered using 0.22 μm pore size filters (Collins, China) and 10ml sterile syringes (KDL, China). Then, ecotoxicity assay and water-soluble ion measurement were conducted immediately. Regarding to the ecotoxicity assay, Jing et al. (2019) provided detailed information. In brief, 100 μL of the prepared bacterial suspension was pipetted into cuvettes to measure the luminous intensity as the baseline. After, the initial luminous intensity was recorded after adding 100 μL of sample. In 15 min, the luminous intensity was recorded again. After subtracting blank intensity tested using NaCl solution

(3 %), the light inhibition rate of VACES and ambient particles was calculated, respectively, according to the international standard (ISO 11348-1: 2007) procedures (Water quality, 2007). All samples were tested in triplicate and averaged in present study. To ensure the enrichment effect of VACES system, we also detected water-soluble ions of both ambient and VACES samples collected during continuous sampling period using an ion chromatography (940 Professional IC Vario, Metrohm, Swiss) integrated with an autosampler (863 Compact Autosampler, Metrohm, Swiss). Moreover, the atmospheric $PM_{2.5}$ concentration was monitored in a nearby state-controlled site (Liangcheng, Hongkou, Shanghai, China).

3 Results and discussion

3.1 Performance test of VACES

Optimization of VACES is to achieve 10-fold enrichment of ambient aerosol concentration mainly through modulating temperatures of saturator and chiller, the major air flow, the minor air flow and their flow ratio. By switching air pathways between ambient and VACES, and comparing their number and mass concentrations observed in scanning mobility particle sizer (SMPS: DMA+CPC), we established optimal parameters for the desired EF (10) and EE (100 %). Results showed that the EF of 10 could be achieved for particles larger than ~ 30 nm as setting the optimal parameters of –19 ± 1 °C condensation temperature, 45 ± 2 °C water bath temperature, 50 ± 1 L min^{-1} major air flow, and 1/10 minor-to-major air flow ratio, respectively. It should be noted that after mixing with ambient aerosol stream (ambient temperature was no more than 24 °C during experiment period), the saturation temperature was decreased to 31 °C or lower, thereby reducing the loss of volatile and semi-volatile compounds (Pirhadi et al., 2020). The corresponding EE ranged from 75 % to 100 % in different size ranges as listed in Table 1. The highest EE was obtained in size range of 30–100 nm, very close to 100%.

For laboratory performance test, the number concentrations of VACES and ambient PSL particles were alternatively measured six times in parallel. The EF calibration line was plotted by the number concentration in four sizes of VACES particles against ambient particles. It showed a quite high correlation coefficient (r^2 = 0.9999) and the EF of VACES was 10 approximately (Fig. 2).

Similarly, we measured the number and mass concentrations of particulate matter in field performance test. When the

concentration coordinate value of VACES was set to 10 times of that in ambient, the two curves almost coincided for particle size greater than 25 nm (Fig. 3a and 3b), indicating that the EE was close to 100 %. In addition, the investigation of particle formation in VACES showed that the maximum of newly formed particles in sizes was only ~ 1 % of total number and mass concentration (Fig. 3c and 3d), which could be neglected. Moreover, the mass peak always appeared in the same particle size with a similar concentration, which indicated that it was most likely to be a water vapor peak and not a newly formed particle in the system.

3.2 Ecotoxicity variation of VACES particles

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).

170   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).

175   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

180   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

185   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 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 $PM_{2.5}$

190   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.

195

Data availability: Data is available by contacting the corresponding author.

Competing interests: The authors declare that they have no conflict of interest.

Acknowledgments

This work was funded by National Natural Science Foundation of China (Nos. 21527814, 91843301, 91743202).

200 **References**

Akhtar, U. S., Rastogi, N., McWhinney, R. D., Urch, B., Chow, C. W., Evans, G. J., and Scott, J. A: The combined effects of physicochemical properties of size-fractionated ambient particulate matter on in vitro toxicity in human A549 lung epithelial cells, Toxicol. Rep., 1, 145–156, 2014.

Council, N. R.: Toxicity Testing for Assessment of Environmental Agents: Interim Report, The National Academies Press,
205 Washington, DC, 2006.

Cox, B., Gasparrini, A., Catry, B., Fierens, F., Vangronsveld, J., and Nawrot, T. S.: Ambient Air Pollution-related Mortality in Dairy Cattle: Does It Corroborate Human Findings?, Epidemiology, 27, 779–786, 2016.

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,
210 Atmos. Meas. Tech., 12, 4733–4744, 2019.

De Vizcaya-Ruiz, A., Gutiérrez-Castillo, M. E., Uribe-Ramirez, M., Cebrián, M. E., Mugica-Alvarez, V., Sepúlveda, J., Rosas, I., Salinas, E., Garcia-Cuéllar, C., Martínez, F., Alfaro-Moreno, E., Torres-Flores, V., Osornio-Vargas, A., Sioutas, C., Fine, P. M., Singh, M., Geller, M. D., Kuhn, T., Miguel, A. H., Eiguren-Fernandez, A., Schiestl, R. H., Reliene, R., and

Froines, J.: Characterization and in vitro biological effects of concentrated particulate matter from Mexico City, Atmos.
Environ., 40, 583–592, 2006.

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.

Freney, E. J., Heal, M. R., Donovan, R. J., Mills, N. L., Donaldson, K., Newby, D. E., Fokkens, P. H. B., and Cassee, F. R.: A
single-particle characterization of a mobile Versatile Aerosol Concentration Enrichment System for exposure studies, Part
Fibre Toxicol., 3, 8–8, 2006.

Hoover, K. R., Acree, W. E., and Abraham, M. H.: Chemical Toxicity Correlations for Several Fish Species Based on the
Abraham Solvation Parameter Model, Chem. Res. Toxicol., 18, 1497–1505, 2005.

Huang, Q., Chi, Y., Deng, J., Liu, Y., Lu, Y., Chen, J., and Dong, S.: Fine particulate matter 2.5 exerted its toxicological
effect by regulating a new layer, long non-coding RNA, Sci. Rep., 7, 9392, 2017.

Jing, W., Liu, Q., Wang, M., Zhang, X., Chen, J., Sui, G., and Wang, L.: A method for particulate matter 2.5 (PM2.5)
biotoxicity assay using luminescent bacterium, Ecotox. Environ. Safe., 170, 796–803, 2019.

Jung, H., Arellanes, C., Zhao, Y., Paulson, S., Anastasio, C., and Wexler, A.: Impact of the Versatile Aerosol Concentration
Enrichment System (VACES) on Gas Phase Species, Aerosol Sci. Tech., 44, 1113–1121, 2010.

Kaiser, K. L., McKinnon, M. B., and Fort, F. L.: Interspecies toxicity correlations of rat, mouse and Photobacterium
phosphoreum, Environ. Toxicol. Chem., 13, 1599–1606, 1994.

Kim, S., Jaques, P. A., Chang, M., Barone, T., Xiong, C., Friedlander, S. K., and Sioutas, C.: Versatile aerosol concentration
enrichment system (VACES) for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and coarse
ambient particles Part II: Field evaluation, J. Aerosol Sci., 32, 1299–1314, 2001.

Kim, S., Jaques, P. A., Chang, M., Froines, J. R., and Sioutas, C.: Versatile aerosol concentration enrichment system (VACES)
for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and coarse ambient particles Part I:
Development and laboratory characterization, J. Aerosol Sci., 32, 1281–1297, 2001.

Klocke, C., Allen, J. L., Sobolewski, M., Mayer-Pröschel, M., Blum, J. L., Lauterstein, D., Zelikoff, J. T., and Cory-Slechta,

D. A.: Neuropathological Consequences of Gestational Exposure to Concentrated Ambient Fine and Ultrafine Particles in the

240   Mouse, Toxicol. Sci., 156, 492–508, 2017.

Ljubimova, J. Y., Braubach, O., Patil, R., Chiechi, A., Tang, J., Galstyan, A., Shatalova, E. S., Kleinman, M. T., Black, K. L., and Holler, E.: Coarse particulate matter (PM2.5–10) in Los Angeles Basin air induces expression of inflammation and cancer biomarkers in rat brains, Sci. Rep., 8, 5708, 2018.

Loxham, M., Cooper, M. J., Gerlofs-Nijland, M. E., Cassee, F. R., Davies, D. E., Palmer, M. R., and Teagle, D. A. H.:

245   Physicochemical Characterization of Airborne Particulate Matter at a Mainline Underground Railway Station, Environ. Sci. Technol., 47, 3614–3622, 2013.

Magnani, N. D., Muresan, X. M., Belmonte, G., Cervellati, F., Sticozzi, C., Pecorelli, A., Miracco, C., Marchini, T., Evelson, P., and Valacchi, G.: Skin Damage Mechanisms Related to Airborne Particulate Matter Exposure, Toxicol. Sci., 149, 227–236, 2016.

250   Miri, M., Alahabadi, A., Ehrampush, M. H., Rad, A., Lotfi, M. H., Sheikhha, M. H., and Sakhvidi, M. J. Z.: Mortality and morbidity due to exposure to ambient particulate matter, Ecotoxicol. Environ. Saf., 165, 307–313, 2018.

Pakbin, P., Ning, Z., Eiguren-Fernandez, A., and Sioutas, C.: Modification of the Versatile Aerosol Concentration Enrichment System (VACES) for conducting inhalation exposures to semi-volatile vapor phase pollutants, J. Aerosol Sci., 42, 555–566, 2011.

255   Plummer, L. E., Ham, W., Kleeman, M. J., Wexler, A., and Pinkerton, K. E.: Influence of Season and Location on Pulmonary Response to California's San Joaquin Valley Airborne Particulate Matter, J. Toxicol. Environ. Health Sci. A, 75, 253–271, 2012.

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.

260   Rychlik, K. A., Secrest, J. R., Lau, C., Pulczinski, J., Zamora, M. L., Leal, J., Langley, R., Myatt, L. G., Raju, M., Chang, R. C., Li, Y., Golding, M. C., Rodrigues-Hoffmann, A., Molina, M. J., Zhang, R., and Johnson, N. M.: In utero ultrafine particulate matter exposure causes offspring pulmonary immunosuppression, Proc. Natl. Acad. Sci., 116, 3443–3448, 2019.

Shang, X., Lee, M., Han, J., Kang, E., Kim, S. W., Gustafsson, Ö., and Chang, L.: Identification and Chemical

Characteristics of Distinctive Chinese Outflow Plumes Associated with Enhanced Submicron Aerosols at the Gosan Climate

265   Observatory, Aerosol Air Qual. Res., 18, 330–342, 2018.

Shang, X., Zhang, K., Meng, F., Wang, S., Lee, M., Suh, I., Kim, D., Jeon, K., Park, H., Wang, X., and Zhao, Y.: Characteristics and source apportionment of fine haze aerosol in Beijing during the winter of 2013, Atmos. Chem. Phys., 18, 2573–2584, 2018.

Sioutas, C., Kim, S., and Chang, M.: DEVELOPMENT AND EVALUATION OF A PROTOTYPE ULTRAFINE PARTICLE

270   CONCENTRATOR, J. Aerosol Sci., 30, 1001–1017, 1999.

Steenhof, M., Gosens, I., Strak, M., Godri, K. J., Hoek, G., Cassee, F. R., Mudway, I. S., Kelly, F. J., Harrison, R. M., Lebret, E., Brunekreef, B., Janssen, N. A. H., and Pieters, R. H. H.: In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential - the RAPTES project, Part Fibre. Toxicol., 8, 26, 2011.

275   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.

Vincent, R., Kumarathasan, P., Goegan, P., Bjarnason, S. G., Guenette, J., Berube, D., Adamson, I. Y., Desjardins, S., Burnett,

280   R. T., Miller, F. J., and Battistini, B.: Inhalation toxicology of urban ambient particulate matter: acute cardiovascular effects in rats, Res. Rep. Health Eff. Inst., 2001. 5–54; discussion 55–62, 2001.

von der Weiden, S. L., Drewnick, F., and Borrmann, S.: Particle Loss Calculator – a new software tool for the assessment of the performance of aerosol inlet systems, Atmos. Meas. Tech., 2, 479–494, 2009.

Wang, D., Kam, W., Cheung, K., Pakbin, P., and Sioutas, C.: Development of a Two-Stage Virtual Impactor System for High

285   Concentration Enrichment of Ultrafine, PM2.5, and Coarse Particulate Matter, Aerosol Sci. Technol., 47, 231–238, 2013.

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.

Water quality: Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent

bacteria test) — Part 1: Method using freshly prepared bacteria, ISO 11348–1:2007, 2007.

290 Zhao, Y., Bein, K. J., Wexler, A. S., Misra, C., Fine, P. M., and Sioutas, C.: Field evaluation of the versatile aerosol concentration enrichment system (VACES) particle concentrator coupled to the rapid single-particle mass spectrometer (RSMS-3), J. Geophys. Res.–Atmos., 110, 2005.

Table 1 Enrichment efficiency of ambient aerosols in VACES at different size ranges.

Particle size	Condensation (−19 ± 1 °C)	Saturation (−45 ± 2 °C)	Major flow (50 ± 1 L min^{-1})	Minor-to-Major flow ratio (1/10)	Total
30–50 nm	99 % ± 22 %	85 % ± 10 %	97 % ± 13 %	97 % ± 13 %	98 % ± 8 %
50–100 nm	100 % ± 12 %	85 % ± 5 %	99 % ± 9 %	99 % ± 9 %	91 % ± 6 %
100–200 nm	85 % ± 11 %	82 % ± 3 %	78 % ± 12 %	80 % ± 12 %	79 % ± 3 %
200–1000 nm	NA	NA	NA	NA	75 % ± 10 %

*NA: not available

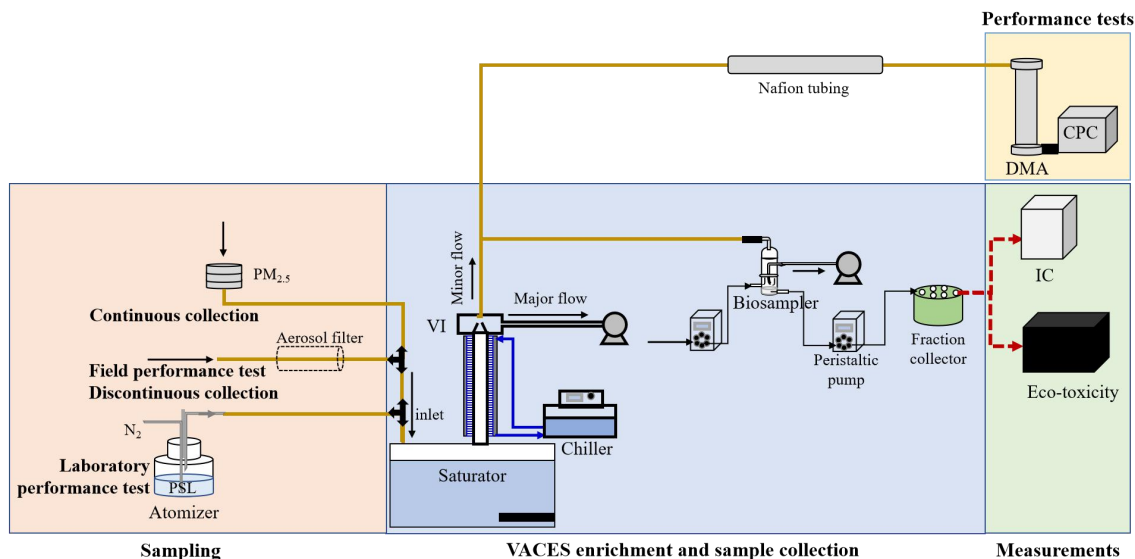



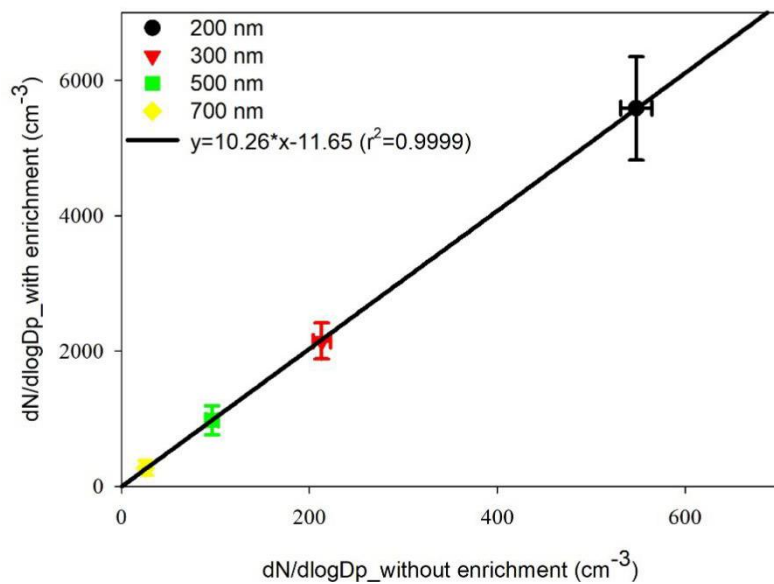
Figure 1 Set-up for performance test and field sample collection. The experiments referring to VACES including Laboratory and field performance tests, discontinuous and continuous sample collection and their measurements. In laboratory performance test, air flow passed through atomizer, VACES (saturator-condensation tube-virtual impactor), nafion tubing, DMA, and CPC successively; For two field performance tests, in the first one air flow passed through VACES, nafion tubing, DMA, and CPC successively, and in the second one air flow passed through aerosol filter, VACES, nafion tubing, DMA, and CPC successively; During discontinuous sample collection, particles followed the flow line of VACES-to-biosampler; For continuous sample collection, particles were collected from $PM_{2.5}$ sampler, VACES, biosampler, to fraction collector. Both types of collected samples were used for online ionic measurement by an ion chromatography and online eco-toxic assay by a photobacterium acute toxicity analyzer (an integrated instrument with automatic operation controlling by a programming).

305

Figure 2 Calibration of enrichment factor of VACES system using Polystyrene Latex (PSL) aerosol reagent at the size of

200 ~ 700 nm, where error bars are standard deviation of six parallel measurements.

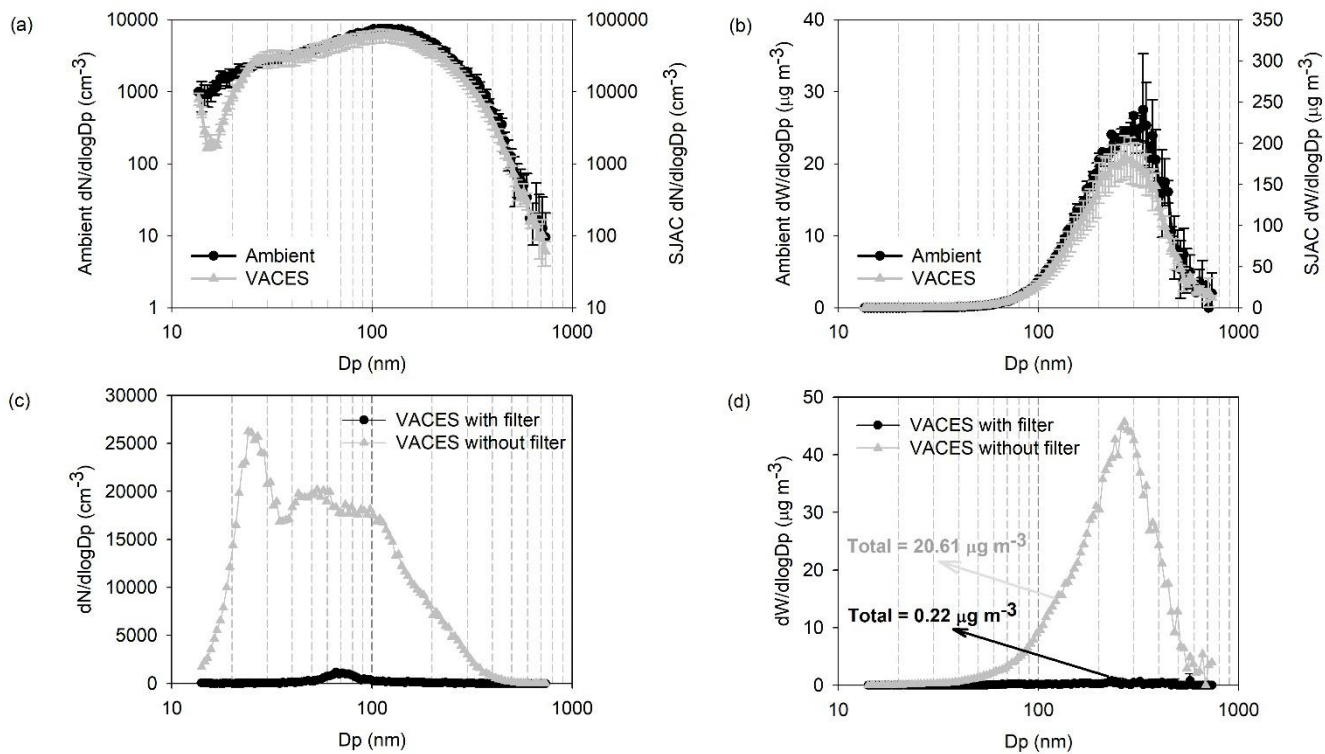



Figure 3 Particle (a and c) number and (b and d) mass size distribution in ambient and VACES system, error bar is standard

310   deviation of three parallel experiments.

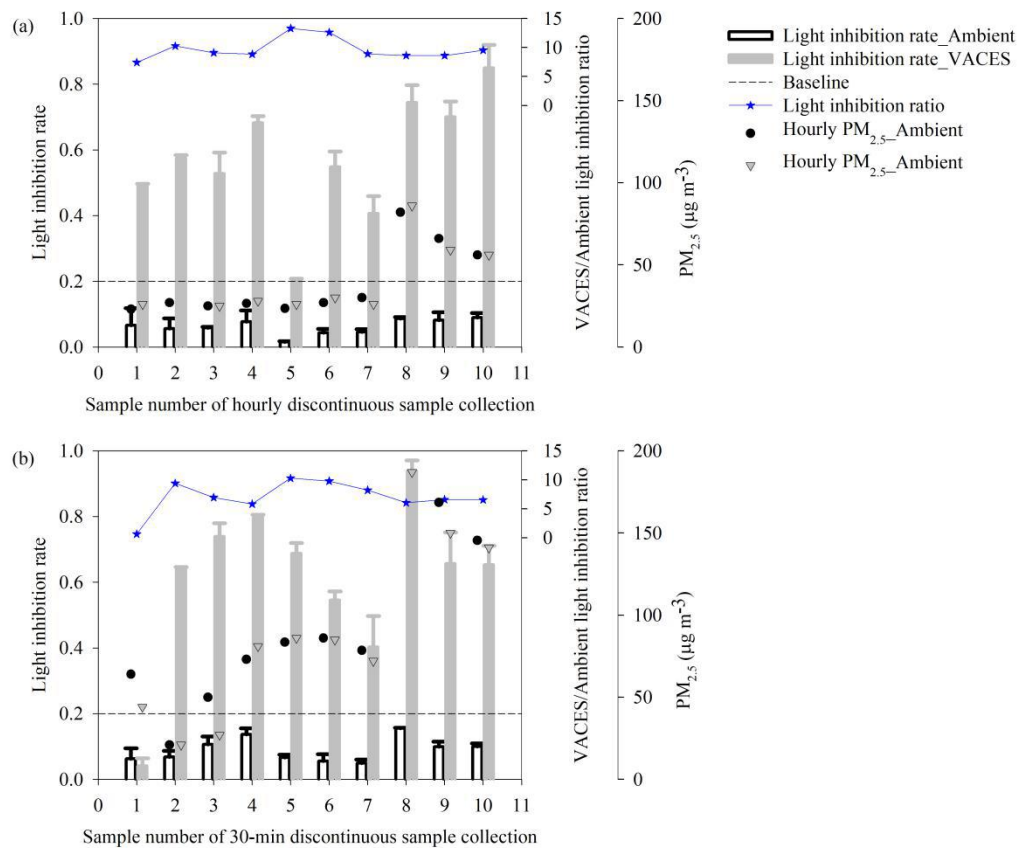



Figure 4 Comparison of light inhibition rate and ratio of ambient and VACES particles with ambient PM$_{2.5}$ concentration based on (a) hourly and (b) 30-min discontinuous sample collection during October 23rd–December 11st, 2019 in Shanghai, China. Baseline reflected the accuracy of photobacteria based ecotoxicity assay method and below the baseline, the accuracy is low.

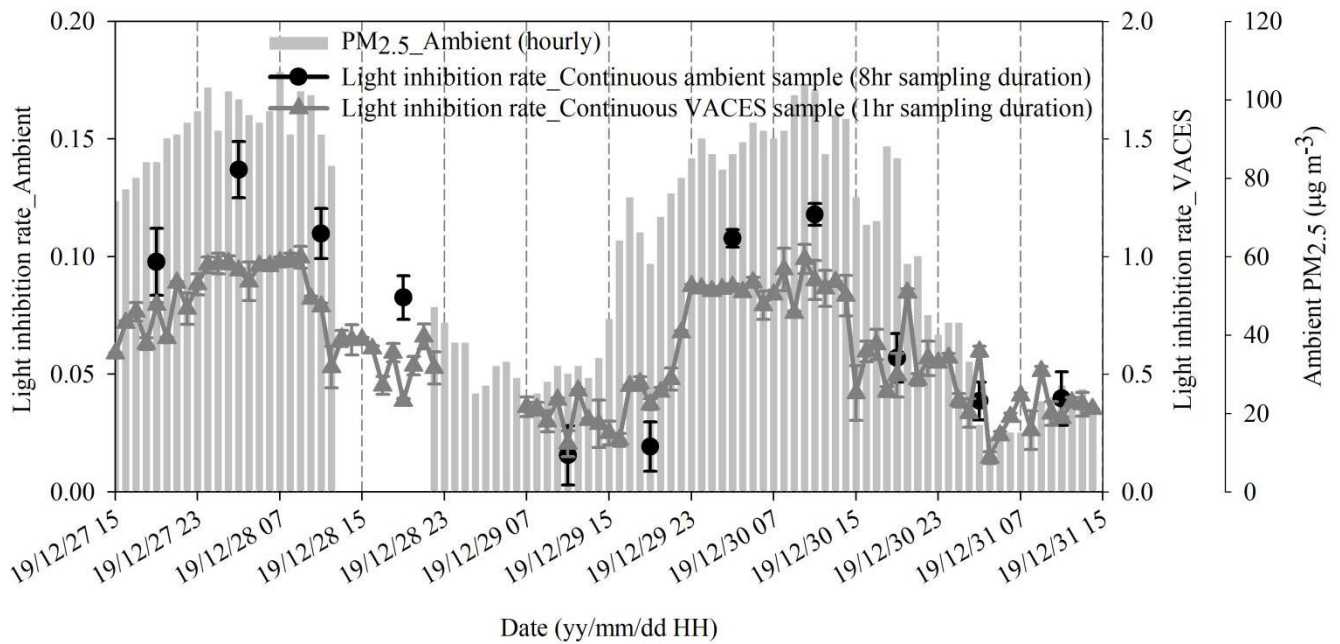



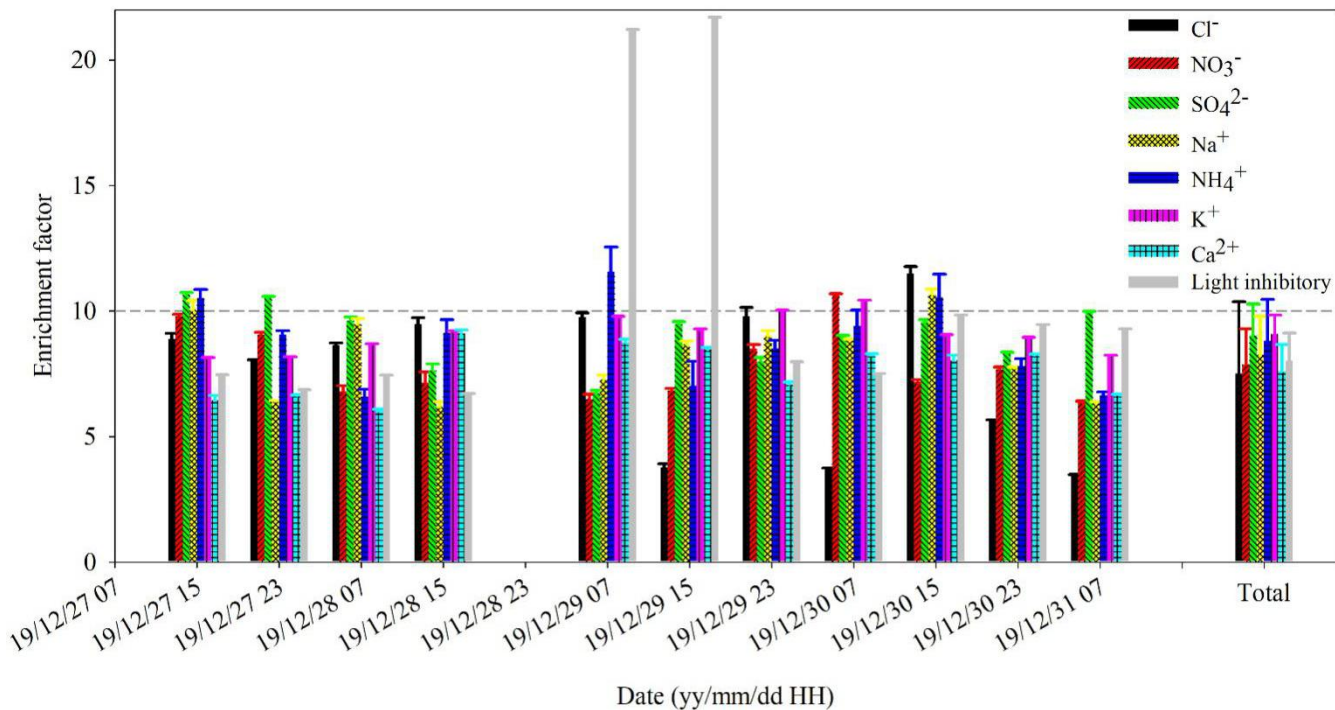
Figure 5 Comparison of light inhibition rate between ambient and VACES particles based on continuous sampling of VACES and ambient. VACES samples were collected hourly and ambient filter samples were collected every eight h. The PM$_{2.5}$ concentration data was collected hourly from a nearby monitoring center (online data).

320

Figure 6 Enrichment factors of chemical compositions and light inhibitory of PM$_{2.5}$ during continuous sampling period. The EF was calculated by the ratio of chemical concentrations of VACES to ambient particles. The component concentration of VACES particles was one hour per sample, and the concentration of ambient particles was 8 h per sample. For the ratio, we averaged the concentrations of VACES samples every 8 h to correspond to that of ambient samples.