

Oxidative Stress Induction Ability of Particles Emitting from Agricultural Open Burning in Japan

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Abstract

Oxidative stress due to exposure to PM_{2.5} that generates reactive oxygen species (ROS) is one of the pathways leading to morphologic changes and lung function decrements that are linked to exacerbation of asthma and chronic obstructive pulmonary disease. Biomass burning (BB) is one of the largest sources of PM with its expected induction of ROS through exposure. Open burning of agricultural residues is widely conducted in September and October in Japan, but it is unclear to what extent the oxidative stress induction ability (OSIA) is increased during the active BB season compared to other seasons, when the effects of BB are different. In this study we conducted heme oxygenase-1 assays on PM_{2.5} samples collected during the active BB season (October 2015) in Tsukuba, Japan, to clarify the influence of BB on OSIA. On average, BB particles were estimated to be responsible for 25% of PM_{2.5}, and 80% of OSIA in October 2015. At that time, PM_{2.5}, BB particles and OSIA were up to two times, eight times and seven times higher, respectively, than during seasons of low BB activity. Thus, elevated concentrations and OSIA are considered to arise mainly from BB particles. The results indicate that management of BB activity is important for good air quality and public health.

Key words: agricultural open burning, dithiothreitol assay, heme oxygenase-1 assay, oxidative stress

1. Introduction

Biomass burning (BB) is a major source of atmospheric aerosols and a cause of global and regional issues, including adverse effects on human health (Johnston, *et al.*, 2012) and climate system perturbations (Myhre, *et al.*, 2013). In Japan, agricultural open burning is often conducted during the harvest season in rice fields in September and October to reduce agricultural residues, such as rice straw and rice husks, as well as to alkalize fields. Estimates of domestic annual PM₁₀ and PM_{2.5} emissions (as of the year 2000) from open burning (not only field burning but also waste incineration) were 25 Gg and 18 Gg, representing 13% and 12% of total emissions in Japan, respectively (Kannari, *et al.*, 2007).

Respiratory tract oxidative stress after short term exposure to PM_{2.5} resulting in reactive oxygen species (ROS) generation is one pathway leading to morphologic changes and lung function impairment, which are linked to exacerbation of asthma and chronic obstructive

pulmonary disease (COPD) (U.S. EPA, 2019). Cells protect themselves against oxidative stress by generating antioxidant compounds such as glutathione and antioxidant enzymes such as heme oxygenase-1 (HO-1). The gene expression underlying this enzyme synthesis is the initial stage of oxidative stress (Hellack, *et al.*, 2017). Thus, oxidative stress induction ability (OSIA) can be assessed in vitro through an HO-1 cellular assay (Li *et al.*, 2003) and has been evaluated against PM samples. Acellular methods for determining oxidative potential (OP), such as a dithiothreitol (DTT) assay (Kumagai, *et al.*, 2002), have also been widely performed on PM samples as a proxy indicator of potential of oxidative stress in the last two decades (Shiraiwa, *et al.*, 2017; Bates, *et al.*, 2019). These PM assays have revealed the emission sources and chemical components of PM that cause oxidative stress. Previous studies have shown that BB emissions contain high levels of humic-like substances (Hoffer, *et al.*, 2006) which induce large amounts of ROS (Lin and Yu, 2011; Verma, *et al.*, 2012) and that, BB

particles contribute greatly to the OP of PM (Verma, *et al.*, 2015; Daellenbach, *et al.*, 2016; Weber, *et al.*, 2018; Fushimi, *et al.*, 2021) among various emission sources. In addition, BB organic aerosol (BBOA) has been shown to have higher OP per unit concentration (Verma, *et al.*, 2015) as well as higher OSIA per unit concentration than other sub-classes of organic aerosol such as secondary organic aerosols (Fujitani, *et al.*, 2023).

While chemical composition data have been accumulated historically with wide spatiality, in contrast, data on oxidative stress assessment are scarce. Fujitani, *et al.* (2023) showed that estimation of cellular OSIA and OP can be obtained from the chemical components of PM using concentration data of metals and subclasses of organic aerosol including BBOA. This would be useful in assessing oxidative stress from chemical composition data in areas where data on oxidative stress are not available and for comparison with previous epidemiological findings retrospectively. However, the predictability of OSIA or OP from chemical composition needs to be further validated with different seasonal data sets.

In this study, to assess the extent to which BB particle emissions affect OSIA and OP, evaluations were performed for PM_{2.5} collected during periods of high activity in open burning of agricultural residue in Tsukuba, Japan. The results were compared with those of samples collected during other seasons with different magnitudes of BB activity at the same sampling site. Furthermore, OSIA and OP were estimated from concentrations of chemical components such as metals and sub-classes of organic aerosol and compared to actual measurements.

2. Experimental Methodology

2.1 Site Descriptions and Sampling Methods

The observation site was located at the National Institute of Environmental Studies (NIES) center in Tsukuba (36.05°N, 140.12°E; population, about 200,000), Japan, about 50 km northeast of Tokyo. The main harvest season for rice tends to span September and October, and open burning of rice husks and rice straw are often conducted in these two months. During the year of observation, 2015, rice harvesting and the burning of rice husks peaked in the middle of September and the burning of rice straw peaked in October (Tomiyama, *et al.*, 2017). Atmospheric observations were conducted between 7 October and 30 October 2015. Observations were intermittent from 16 to 20 Oct because of power outages.

PM_{2.5} was concurrently collected both on 20.32 cm × 25.4 cm Teflon filters (TFH-R, Horiba, Kyoto, Japan) and on quartz fiber filters (2500QAT-UP, Pall, New York, USA) by means of a high-volume air samplers (HV-1000R, Sibata Scientific Technology, Soka, Japan) with PM_{2.5} impactor (Kaneyasu, 2010). All sampling was conducted from around 10 a.m. on weekdays to 10 a.m. two days later (i.e., 48 hours of sampling) and on

weekends from around 10 a.m. on Friday to 10 a.m. on Monday (i.e., 72 hours of sampling), with a sampling flow rate of 740 l min⁻¹. During sampling, the aerosol species in PM_{1.0} were concurrently measured with a high-resolution time-of-flight particle aerosol mass spectrometer (AMS, Aerodyne Research Inc., Billerica, USA) and the sub-classes of organic aerosol were determined by positive matrix factorization (PMF) analysis. In PMF analysis, the measured mass spectrum is decomposed for several factors, and the concentration and mass spectrum is obtained for each factor by determining the residual matrix become minimum. Each PMF factor can be assigned an emission source and particle type (sub-class of organic aerosol) by its diurnal pattern, mass spectrum comparison of PMF factors obtained in previous studies, and correlation of time variation with external tracers. Atmospheric pollutants (i.e., PM_{2.5}, NO, NO₂, etc.) and meteorological data were obtained continuously from the NIES Air Quality Research Station. These PM_{1.0} and atmospheric pollutant data are discussed in detail by Fujitani, *et al.* (2020).

2.2 Chemical Analysis of PM_{2.5} Samples

The Teflon filter samples were analyzed for particle mass, water soluble ions and elements. Particle mass was obtained by incrementing the mass of the collection media after sampling. Before weighing the Teflon filter samples, the Teflon filters were placed in the chamber at 25°C and 50% humidity for at least 24 hours. An electronic balance (minimum reading 0.1 mg, LA130S-F, Sartorius AG, Göttingen, Germany) was used for weighing. Each sample was weighed at least twice, and the results were averaged if the difference was within 1 mg; the mean value was adopted as the weight of the sample. The water-soluble ion component (F⁻, Cl⁻, NO₂⁻, NO₃⁻, SO₄²⁻, oxalate ion (C₂O₄²⁻), Na⁺, NH₄⁺) was analyzed by ion chromatography (IC850, Metrohm, Herisau, Switzerland). For the analysis, one-third of the Teflon filter was placed in a 15 ml polypropylene container, 10 ml of ultrapure water was added, and ultrasonic extraction using an ultrasonic bath (US-5A, ASONE, Osaka, Japan) was performed for 10 min two times. The extract was filtered through a disposable membrane filter (hydrophilic PTFE) with a pore size of 0.45 μm. Elements (Be, Na, Mg, Al, Si, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Ba, Tl, Pb, Th, U) were analyzed by inductively coupled plasma–mass spectrometry (7700x, Agilent Technologies, Santa Clara, USA). For the analysis, samples were prepared using 1/32 of the Teflon filter, and pretreatment was performed by the ultrasonic-heat block method. Carbon components (elemental carbon, organic carbon and total carbon) were analyzed using quartz fiber filter samples. For the analysis, 8 mmΦ of the filter was punched out from two locations and analyzed using a thermal optical carbon analyzer (Model 2001 Carbon Analyzer, Desert Research Institute, Reno, USA) by

means of the IMPROVE protocol and the two analyses were averaged. Pyrolysis of the organic carbon during the analysis was corrected using reflected light.

2.3 Protocols for HO-1 and DTT Assays

OSIA and OP were determined by means of HO-1 assay and DTT assay according to the protocol of Fujitani, *et al.* (2023). Briefly, for the HO-1 assay, a rat alveolar epithelial cell line (SV40-T2 cells) was used. The samples were extracted in two separate processes using distilled deionized water (DDW) and dichloromethane (DCM) for water-soluble and water-insoluble components of particles, respectively, and the cells were exposed for three hours to 1 ml of the solution at PM concentrations of 50 $\mu\text{g-PM ml}^{-1}$ for each solution. For OP, the samples were extracted by Tris-HCl buffer at pH 8.9 or DCM, and the solution concentration was then adjusted to 30 $\mu\text{g-PM ml}^{-1}$ and

250 $\mu\text{g-PM ml}^{-1}$, respectively. DTT was added to each sample at a concentration of 160 μM . The solution was incubated at 37°C for 15 min to complete the reaction.

3. Results and Discussion

3.1 State of Open Burning of Agricultural Residue and its Contribution to PM in Tsukuba

Figure 1 shows the PM_{2.5} concentration and major chemical composition of the PM mass in the filter samples, organic aerosol-PMF fraction to organic aerosol, OSIA and OP. The PM_{2.5} concentrations were below the Japanese environmental standard (24-hour value of 35 $\mu\text{g m}^{-3}$) for all samples. Note, however, that the 1-hr PM_{2.5} concentration (according to continuous measurement) increased to a maximum of 100 $\mu\text{g m}^{-3}$ on October 10 due to intensive BB and then declined sharply

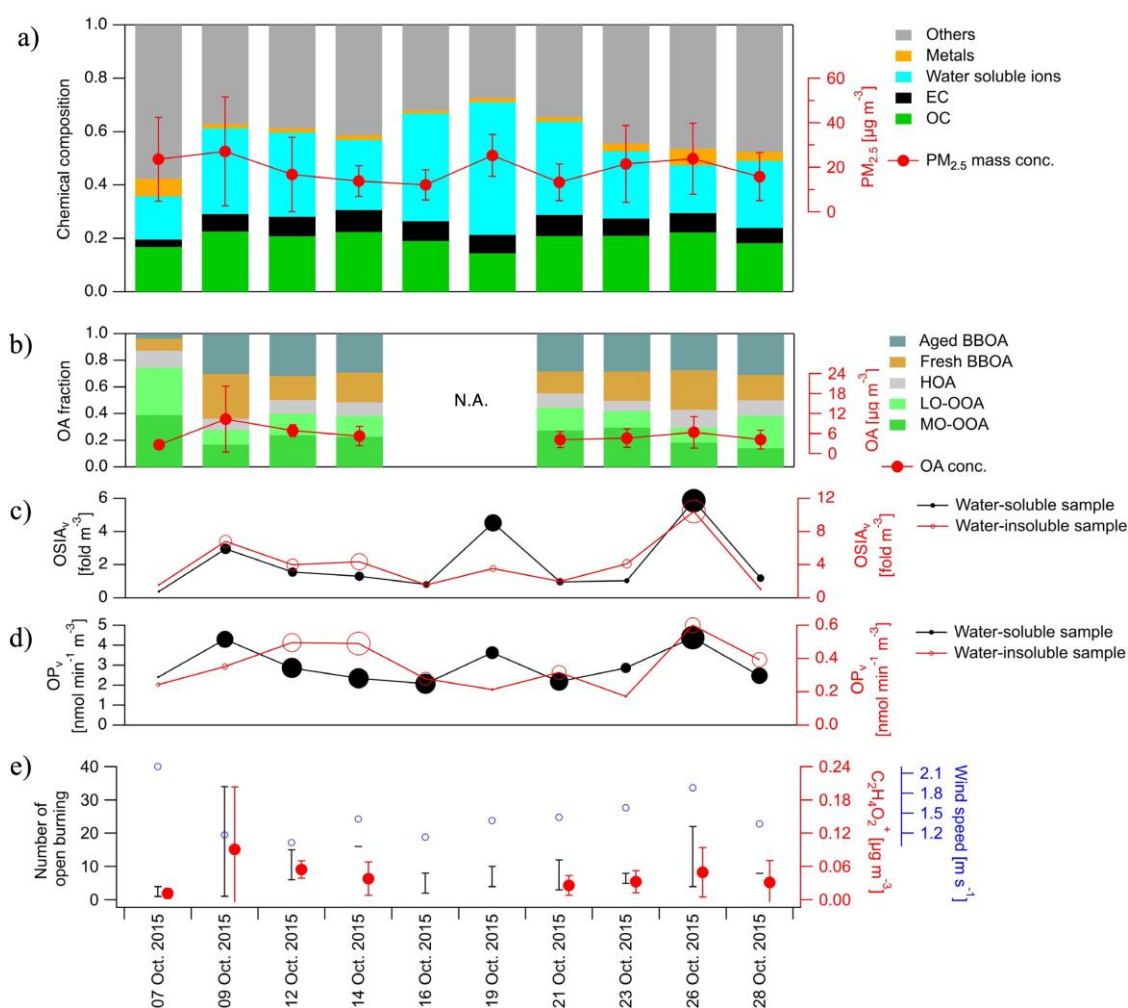


Fig. 1 Overview of the field campaign at the Tsukuba observation site for a) PM_{2.5} concentrations and chemical compositions of filter samples; b) organic aerosol (OA) concentration and OA sub-fractions determined by positive matrix factorization (PMF) analysis, c) oxidative stress induction ability (OSIA), d) oxidative potential (OP), and e) the maximum and minimum of the daily number of open burning events during each sampling period in the visual inspection area of Tsukuba City according to Tomiyama, *et al.* (2017), the $\text{C}_2\text{H}_4\text{O}_2^+$ fragment ion ($m/z = 60.0211$), which is a fragment ion of levoglucosan and tracers of biomass burning, and the wind speed. The OA fraction and $\text{C}_2\text{H}_4\text{O}_2^+$ ion are averaged over the filter sampling period. The x-axis label is the filter ID and indicates the sampling start date. The y axis in c) and d) for OSIA and OP indicate air volume basis (OSIA_v , OP_v), while the size of the plots indicates the relative magnitude of induction fold change relative to control per PM mass (OSIA_m or OP_m). Error bars in PM_{2.5}, OA and $\text{C}_2\text{H}_4\text{O}_2^+$ represent standard deviation of the continuous measurement 1-hr averaged data during the sampling period. EC: elemental carbon; OC: organic carbon; DMSO: dimethyl sulfoxide; MO-OOA: more-oxygenated oxygenated OA; LO-OOA: less-oxygenated OOA; HOA: hydrocarbon-like OA; BBOA: biomass-burning OA.

late in the evening of October 11 due to rain (Fujitani, *et al.*, 2020). As a result, the three-day average value was lower than the environmental standard; the larger standard deviations of PM mass and organic aerosol concentrations in ID 09Oct.2015 compared to the other samples resulted from this variability.

For organic aerosol-PMF, each of the five factors is associated with an emission source following Fujitani, *et al.* (2020): freshly emitted BBOA (fresh BBOA), aged BBOA, hydrocarbon-like organic aerosol (HOA), more-oxygenated oxygenated organic aerosol (MO-OOA), and less-oxygenated oxygenated organic aerosol (LO-OOA). The difference between fresh BBOA and aged BBOA is a feature of the difference in intensity of the fragment ion of $C_2H_4O_2^+$ ($m/z = 60.0211$) which is a fragment ion of levoglucosan. Aged BBOA has a lower signal intensity for $C_2H_4O_2^+$ than fresh BBOA due to atmospheric aging through atmospheric evolution due to OH radical reactions occurring either in the aqueous phase or gas phase, or heterogeneous reactions at the gas-particle interface. It is estimated that the aged BBOA had been aged for several hours in the atmosphere. HOA is an indicator of vehicle tailpipe emissions, and MO-OOA and LO-OOA are indicators of secondary organic aerosols.

Cases of open burning of agricultural residues were observed by visual inspection around the observation site, with 10 October having the highest number of cases and 26 October having the second-highest number of cases (Tomiya, *et al.*, 2017). On these days, concentrations of $PM_{2.5}$, fresh and aged BBOA, and the fragment ion of $C_2H_4O_2^+$ which is BB tracer were relatively high. A high correlation coefficient (0.82) was found between the number of burning events and the fresh BBOA fraction with respect to organic aerosol for the entire observation period data. These results indicate that BB had an impact on PM mass and organic aerosol, and that BB caused significant air quality perturbation. Averaged over the observation period, the sum of fresh and aged BBOA accounted for about 50% of organic aerosol (OA) ($0.5 = BBOA / OA_{environment}$). Considering that organic aerosol accounted for about 29% of PM mass ($0.29 = OA_{environment} / PM_{environment}$) for the observation period, and that organic aerosol from rice straw burning accounted for about 57% of the PM mass in the emission source experiment ($0.57 = BBOA / PM_{BB}$) (Fujitani, *et al.*, 2020), which resulted in a contribution of BB aerosols (including organic aerosol, elemental carbon and other species) to PM mass during the observation period, which was estimated to be about 25% ($0.25 = PM_{BB} / PM_{environment} = OA_{environment} / PM_{environment} \times BBOA / OA_{environment} / BBOA / PM_{BB} = 0.29 \times 0.5 / 0.57$). Metals constituted less than 6% of PM mass in most samples, and their fraction was smallest among the major components, but some metals exhibit OP and OSIA and are thus noteworthy components.

OSIA can be represented as the induction fold change relative to the control per unit air volume (denoted as $OSIA_v$: [fold change m^{-3}] = [fold change $\mu g-PM^{-1}$] \times [$\mu g-PM m^{-3}$]) and OP can be represented as the DTT consumption rate per unit air volume (OP_v : [nmol-DDT $min^{-1} m^{-3}$] = [nmol-DDT $min^{-1} \mu g-PM^{-1}$] \times [$\mu g-PM m^{-3}$]). The size of the plots in Figs. 1c and 1d indicates the relative magnitude induction fold change relative to the control per unit PM mass [$OSIA_{rc}$: fold change μg^{-1}] or DTT consumption rate per unit PM mass [OP_m : nmol-DDT $min^{-1} \mu g^{-1}$]. Sample ID 26Oct.2015 exhibited the highest values for all indices among the samples; OSIA and OP were highest per unit air volume as well as per unit PM mass for both solutions.

3.2 Comparison with Other Seasonal Samples with Different BB Impacts on OSIA and OP

The impacts of BB on OSIA and OP are discussed by comparing the results of other seasons with different degrees of BB impact. Fig. 2 shows comparisons of three seasons; January 2017 and August 2017 where the periods of observations were conducted in winter and summer, respectively, at the same sampling site. Each observation was conducted for about three weeks and the observation protocols were mostly identical to those in this study. According to the results of a year-round survey of BB frequency in Tsukuba in 2016, BB was also observed in January and August (Fig. 2b). In those months, however, monthly BB frequency was low and the contribution of fresh BBOA to organic aerosol was as small as less than 10%. Comparing the three periods, not only fresh and aged BBOA but also $PM_{2.5}$ and organic aerosol were highest during October 2015. In particular, the increase in organic aerosol concentration during October 2015 was mostly identical to the increase in fresh and aged BBOA concentration.

Boxplots of PM components exhibiting OSIA or OP are shown in Fig. 2c. Since the predominant source varies with the season and the chemical compositions of each source differ, the concentration of each component varies with the season and is characteristic. In addition to fresh and aged BBOA, Cu, Pb and Zn tended to be higher in October 2015 than other periods, but the origins of these metals are not clear (see the next section). January 2017 (winter) was the period with the highest contributions of vehicle tailpipe emissions (HOA and elemental carbon) and soil (Fe and Mn). Fe and Mn are considered to be predominantly of soil origin at this location, given the behavior of Al, an indicator of soil composition, in combination with their high abundance in the soil particles. Winter in Tsukuba has drier weather compared to the other seasons, and the wind speeds are higher, so soil particles tend to aerosolize. This may have contributed to the high winter concentrations of Fe and Mn. August 2017 (summer) was the period with the highest contribution of heavy oil combustion and ship

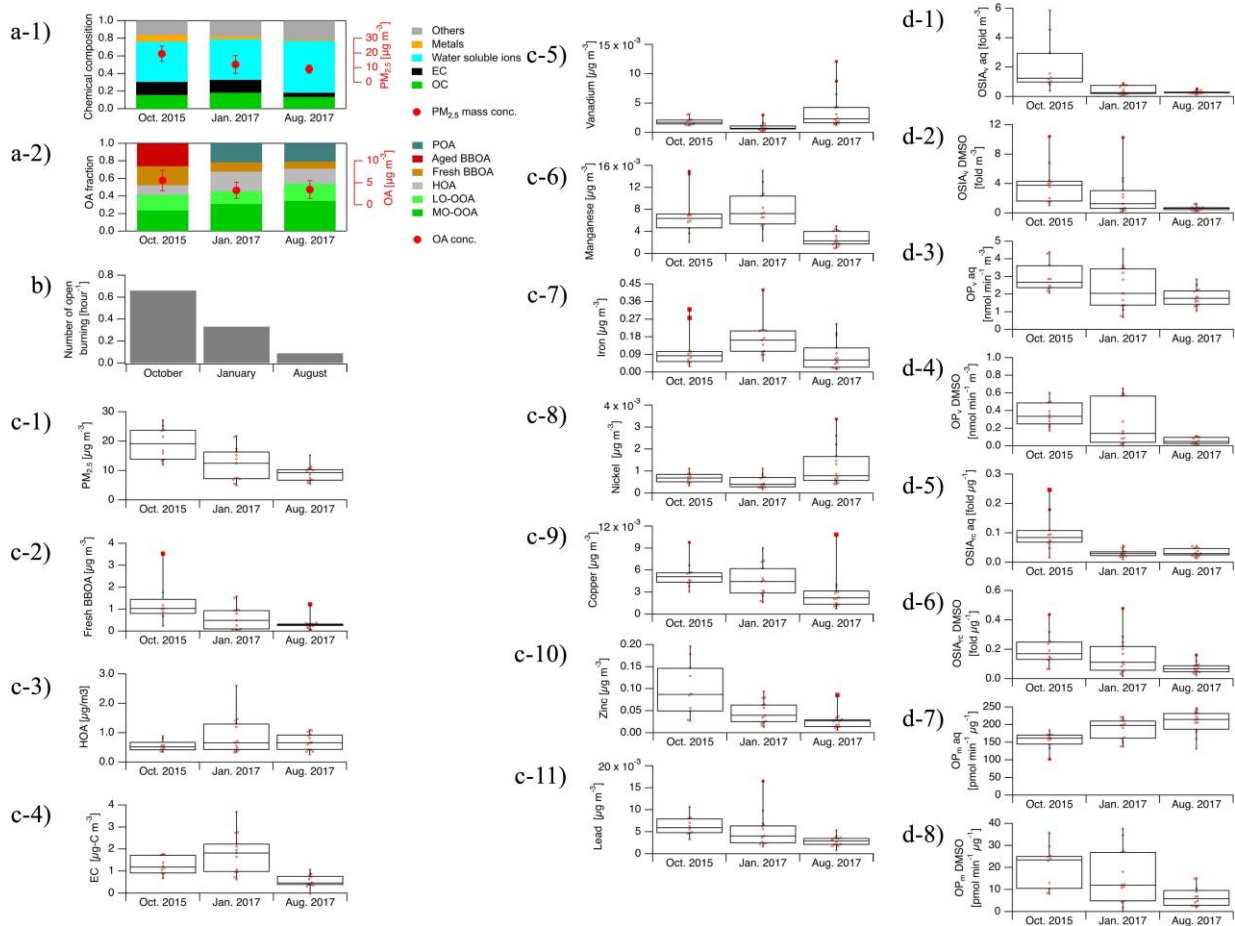


Fig. 2 a) Concentrations and fractions of chemical compositions during each observation period at the Tsukuba sampling site, b) Numbers of open burning events for different month, obtained through annual observations in 2016 in Tsukuba (Tomiya, *et al.*, 2017), and boxplots for each observation period; c) Chemical components exhibiting OSIA or OP, d) Oxidative stress induction ability (OSIA) and oxidative potential (OP) expressed in per unit air volume and in per unit PM mass. Error bars in a) represent the standard deviation of the data during the sampling period. The horizontal bar in the boxes represents the median value. The upper and lower bounds of the boxes represent the 25th and 75th percentiles, with whiskers extending to the highest and lowest data values. POA in a-2) indicates primary emitted OA other than HOA and BBOA.

exhaust (V, Ni) and secondary organic aerosols (Fig. 2a). Southerly winds tend to prevail during the summer, resulting in influences from sources in industrial areas of the coastal bay south of the measurement site (Fujitani, *et al.*, 2021). The OSIA and OP per unit air volume and per unit PM mass (Fig. 2d) for both extracted samples were mostly highest in October 2015. In the next section, the relationship between OSIA or OP and the chemical components is discussed.

3.3 Influences of Chemical Components on OSIA and OP

Figure 3 shows OSIA and OP values, the contribution fraction of each component to OSIA and OP, and compositional fraction of each chemical component exhibiting OSIA or OP for each sample in October 2015 and the average values of each season. The fraction of each component to OSIA or OP was determined by the concentrations of the chemical species responsible for OSIA or OP and their unit values of OSIA [$\text{fold change } \mu\text{g-component}^{-1}$] or OP [$\text{nmol-DDT min}^{-1} \mu\text{g-component}^{-1}$], the values of which were obtained by

Fujitani *et al.* (2023). The OSIA of each component was summed assuming that a linear relationship was valid to obtain the total OSIA of all components, and the fraction of each component to the total OSIA was determined. The fraction of each component to the total OP was obtained by the same procedure. Water-soluble metals and PMF-organic aerosol were considered to exhibit OSIA or OP in the calculations for the water-soluble samples, while PMF-organic aerosol and elemental carbon were considered for the water-insoluble samples. Furthermore, among the metals, Mn, Fe, Ni and Cu, which showed OP in reagent solution experiments, were considered, and for showing OSIA, Zn, Pb, V and Cr were considered in addition to the OP-exhibiting metals. For each metal component, the concentration of water-soluble components was calculated using the measured total concentration and the average water-soluble fraction obtained from the summer and winter samples. Metals and elemental carbon derived from vehicle tailpipe emissions and BB emissions were calculated assuming an emission source profile, using those used in the Chemical Mass Balance model (Takahashi *et al.*, 2011) and the

results of agricultural residue combustion experiments (Fushimi *et al.*, 2017). Soil-derived metals were calculated using Al as an indicator, and the soil profile of the average earth crust was used (Mason, 1966). The remaining concentrations of metals and elemental carbon that were not assigned to vehicle tailpipe and BB emissions and soil, were assumed to originate from sources other than those. As a result, it was estimated that most of the metal in terms of amount was from other sources not considered in this study and most of the elemental carbon by amount was attributable to vehicle tailpipe emissions (Fig. 3e).

Averaged over the samples in each season, the OSIA for the water-soluble samples was dominated by BB (fresh and aged BBOA) in October 2015, which contributed nearly 80% (Fig. 3a). In other seasons, the contribution of OOA and other metals increased and the contribution of BB decreased slightly, to around 40%–50%. The contribution of BB to OSIA in the

water-insoluble samples was about 50% in October 2015 and about 20% in the other seasons (Fig. 3b). In the other seasons, vehicle tailpipe emissions (HOA, vehicle tailpipe emission-elemental carbon) was predominant (70%–80%). The trend of the OP contribution for each solution was generally consistent with the trend of OSIA (Fig. 3c, d). These results indicate that OSIA and OP significantly increased during the active open burning season, and the majority of the increase was attributable to BB aerosols.

The contributions of components with larger contributions to OSIA and OP (Fig. 3a–d) differed from their mass-based contribution (Fig. 3e). In particular, the contribution of metals was greater in the water-soluble samples, because the unit values of OSIA and OP for metals are orders of magnitude higher than those of organic aerosol. However, BB had low metal content according to the source profiles, and the BB-derived metals present contributed little to OSIA or OP. Therefore, in BB, organic matter rather than metals was found to be

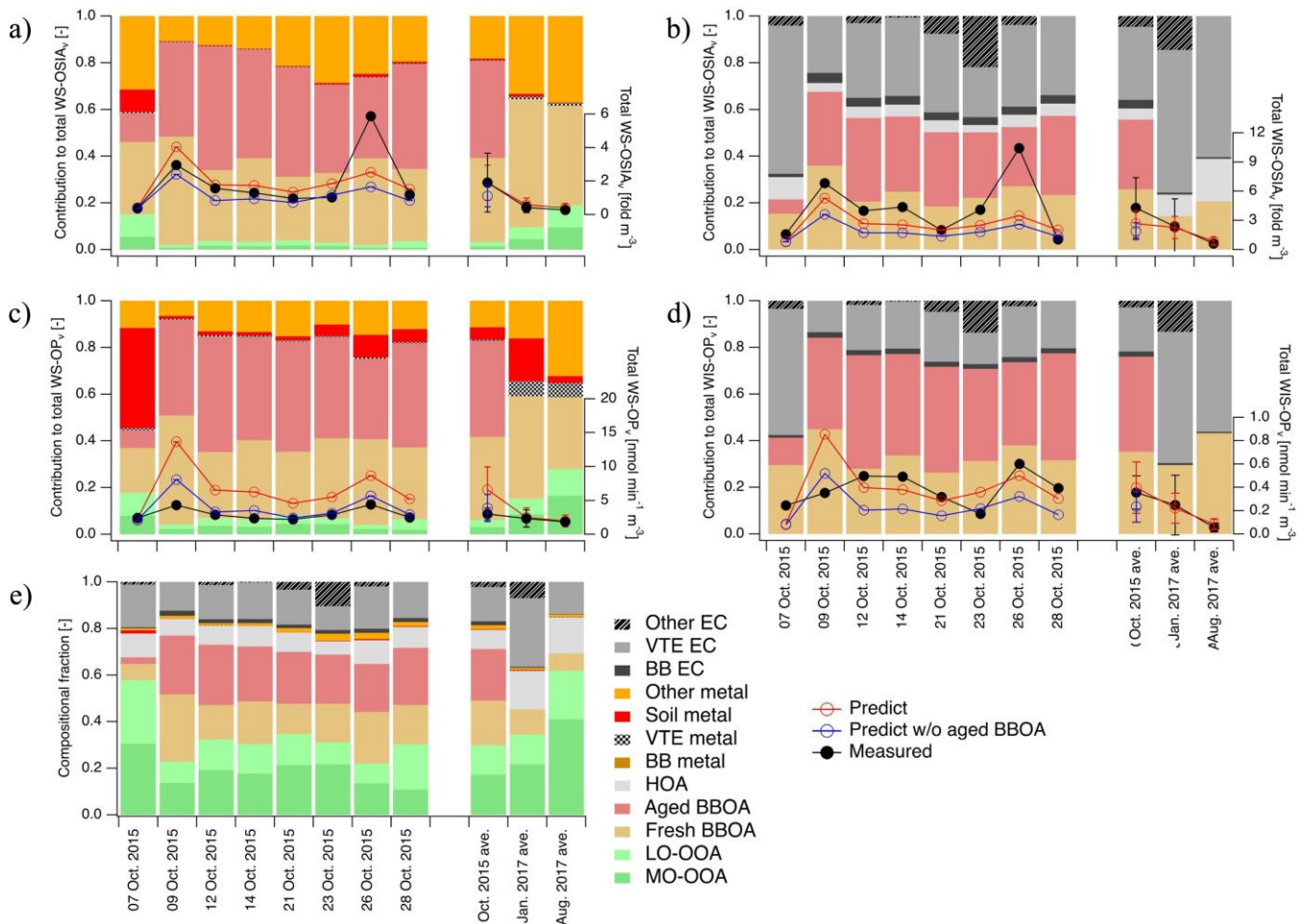


Fig. 3 a) Oxidative stress induction ability (OSIA) and contribution of water-soluble (WS) chemical components to OSIA of WS samples, b) OSIA and contribution of sub-classes of organic aerosols (OA) and elemental carbon (EC) in water-insoluble (WIS) samples, c) Oxidative potential (OP) and contribution of chemical components to OP of WS samples, d) OP and contribution of sub-classes of OA and EC of WIS samples, and e) Composition of chemical species that exhibit OSIA or OP. Predicted values of total OSIA or OP were determined by multiplying the concentration of each chemical component and the unit value of OSIA or OP (per unit of chemical component mass) and the sum of each OSIA or OP value. The predicted values for cases without aged BBOA were determined by assuming the unit value of OSIA or OP was zero for aged BBOA. The error bars indicate the standard deviation of the samples in each sampling campaign. VTE indicates vehicle tailpipe emission.

the main causative component.

We focused on October 2015 samples in detail. As described in Section 3.1, ID 09Oct.2015 and ID 26Oct.2015 were samples collected during especially active open burning, while ID 07Oct.2015 and ID 26Oct.2015 were samples collected during windy ($> 1.9 \text{ m s}^{-1}$) days when it was largely soil particles that tended to aerosolize. Accordingly, the contribution of soil and BB to OSIA and OP increased in ID 07Oct.2015 and ID 09Oct.2015, respectively. For ID 26Oct.2015, both emissions exhibited simultaneous influence, likely resulting in the highest OSIA and OP per unit air volume and per unit PM mass (Fig. 1).

Figure 3 also shows the predicted values of total OSIA and OP. For aged BBOA, no unit values of OSIA or OP were available. Therefore, we determined the predicted values of total OSIA and OP for the case in which the unit values of OSIA and OP for aged BBOA were assumed to be identical to those of fresh BBOA, or for the case in which they were ignored (i.e., unit values of OSIA and OP assumed zero). The predicted values generally followed the inter-sample variability trend of the measured values in October 2015, and most of the samples were predicted to be close to the measured values, except for ID 26Oct.2015 for OSIA and ID 09Oct.2015 for OP. This result indicates that OSIA and OP values can be estimated retrospectively for most samples using concentration data for chemical components such as metals and sub-organic aerosol classes, and unit values of OSIA and OP for those chemical components.

By comparing these two cases in which unit values of OSIA and OP for aged BBOA are either identical to values for fresh BBOA or zero, it is possible to estimate whether unit values of OSIA and OP in aged BBOA are greater or lesser relative to their values in fresh BBOA. Namely, the unit values of OSIA and OP of aged BBOA for water-soluble samples are expected to be lower than those of fresh BBOA, since the total predicted values of water-soluble OSIA and water-soluble OP for most samples exceed the measured values. On the other hand, the unit values of OSIA and OP for aged BBOA of water-insoluble samples are expected to be higher than those for fresh BBOA, since the total predicted values of water-insoluble OSIA and water-insoluble OP for most samples are lower than the measured values. Accordingly, the results indicate that oxidative stress induction changes with aging, but the amount of substances that induce oxidative stress or their unit values may have decreased in the water-soluble samples and increased in the water-insoluble samples as a result of aging. For example, although not an oxidative stress inducer, water-soluble components such as levoglucosan have also been suggested to decrease with aging (Fujitani *et al.*, 2020). This hypothesis should be validated in laboratory experiments in future challenges.

Underestimation of the predicted value of OSIA compared to the measured value (ID 26Oct.2015) is similar to the tendency seen in a previous study (Fujitani *et al.*, 2023): underestimation OSIA when it is high. This can be explained by the following hypotheses. 1) The high actual value is due to the presence of substances such as endotoxins, rather than of chemical components; 2) The HO-1 gene is expressed by the oxidation and degradation of Kelch-like ECH-associated protein 1 (Keap1). In addition, several other antioxidants such as glutathione coordinate metals in the cell. In samples with high OSIA, antioxidants such as glutathione alone may not be able to counteract oxidatively active substances, resulting in these greater quantity of oxidatively active substances reacting with Keap1, leading to higher HO-1 expression.

4. Conclusions

To assess the extent to which BB particles affect OSIA and OP, an evaluation was conducted of OSIA, OP and chemical components in $\text{PM}_{2.5}$ environmental samples collected during periods of high BB activity, that is, the burning of agricultural residue in Japan. Not only were $\text{PM}_{2.5}$ concentrations higher, but also OSIA and OP per unit air volume and per unit PM mass in periods of high open burning activity than in other seasons. The results indicate that management of BB activity is important toward maintaining good air quality and protecting public health.

Acknowledgments

This study received financial support from the National Institute for Environmental Studies (2015–2017), JSPS KAKENHI (Grant Number 17K00590), and the Environment Research and Technology Development Fund (Grant Number JPMEERF20165005) administered by the Ministry of the Environment, Japan. The authors gratefully acknowledge the technical assistance provided by Dr. Y. Kondo, Ms. M. Ihara, Ms. E. Shimaya, Mr. Y. Sugaya, and Ms. M. Chiba, Mr. A. Togashi, Ms. S. Takahashi, Ms. K. Higo, and Ms. M. Kobayashi (NIES), Mr. T. Fujii and Mr. H. Konno (Horiba Techno Service), and Mr. M. Oikawa and Mr. M. Nakamura (Green Blue Corp.). Meteorological data was obtained from the Air Quality Research Station at NIES.

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(Received 4 July 2023, Accepted 26 December 2023)

