



## Comparison of Oxidative Abilities of PM<sub>2.5</sub> Collected at Traffic and Residential Sites in Japan. Contribution of Transition Metals and Primary and Secondary Aerosols

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### ABSTRACT

Filter environmental samples of PM<sub>2.5</sub> were collected at a traffic intersection in Kawasaki, Japan, and at a residential site (Tsukuba, Japan) in summer and winter, and the chemical compositions of the samples and their oxidative abilities in the dithiothreitol (DTT) assay were determined. Laboratory-generated aerosols (diesel exhaust particles [DEPs], gasoline direct injection spark ignition particles, and secondary organic aerosols [SOAs] generated from various precursors) were also investigated. To assess the effects of transition metals in the filter samples, we also conducted DTT assays on solutions of metal compounds similarly to the filter samples. In addition, the samples were pretreated with chelating reagents to mask the effects of transition metals. The DTT consumption average values for the filter samples collected at the traffic site were 53 and 50 pmol min<sup>-1</sup> μg<sup>-1</sup> in summer and winter, respectively, and these values were 1.3 and 1.1 times the corresponding values at the residential site and were also higher than the values for the laboratory-generated aerosols. Transition metals (Cu, Fe, Ni, and Mn) in the environmental samples were considered to be major contributors to DTT consumption (more than 80%). After removal of the effect of these transition metals with the chelating reagents, the oxidative abilities of the environmental samples were correlated with the amounts of organic carbon, water-soluble organic carbon, and organic acids and were weakly correlated with the amounts of elemental carbon and inorganic ions. We also found that the oxidative abilities based on the amount of organic carbon after removal of the effects of transition metals for DEPs, photochemically generated SOAs, and environmental samples except in the case of the traffic site in summer were compatible.

**Keywords:** Dithiothreitol assay; Oxidative activity; Traffic intersection; Primary aerosol; Secondary organic aerosol.

### INTRODUCTION

Atmospheric air quality standards regulate particulate matter with a diameter of ≤ 2.5 μm (PM<sub>2.5</sub>) on the basis of mass concentration. However, PM<sub>2.5</sub> mass concentration is not necessarily the most appropriate indicator of the adverse health effects of inhalation exposure to aerosols. Such effects may also depend on chemical species present in the particles (e.g., organic compounds, metals, radicals), on particle shape (e.g., the aspect ratio of fibrous particles) (Stanton *et al.*, 1981; Poland *et al.*, 2008), and on particle surface area (for low-toxicity, low-solubility particles) (Oberdorster, 2000; Donaldson *et al.*, 2001; Wittmaack, 2007; Donaldson *et al.*, 2008; Sager and Castranova, 2009).

One recognized adverse health effect of aerosol inhalation is oxidative stress; aerosols can both directly produce reactive

oxygen species and stimulate cellular generation of reactive oxygen species (Delfino *et al.*, 2010; McWhinney *et al.*, 2013). Oxidative stress is an important mechanism leading to inflammation, which in turn plays a key role in airway diseases such as asthma and chronic obstructive pulmonary disease, as well as in heart disease. Studies have shown that chronic oxidative stress is also an important causative factor in lung cancer (Ayres *et al.*, 2008).

Various assays have been developed to assess the ability of aerosols to induce oxidative stress, which include dithiothreitol (DTT) assay, ascorbic acid assay, dichlorofluorescein diacetate assay, and profluorescent nitroxides assay. The ascorbic acid assay is used mostly in cellular studies and the dichlorofluorescein diacetate assay has been used widely in both cellular and acellular studies (Hedayat *et al.*, 2015). *In vitro* cell experiments were preferable to *in vitro* acellular experiments, but both have a potential major role to play and offer large cost advantages relative to human or animal inhalation studies and animal *in vivo* instillation experiments (Ayres *et al.*, 2008).

The DTT assay, developed by Kumagai *et al.* (2002), is an acellular method for assessing the oxidative ability of

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aerosols. Oxidative ability measured by the DTT assay correlates well with heme oxygenase-1 induction, which is an indicator of oxidative stress in living organisms (Li *et al.*, 2003). In the last decade, the DTT assay has been widely used to measure the oxidative abilities of various aerosols, including aerosols collected at emission sources (e.g., Shima *et al.*, 2006; Charrier *et al.*, 2015), ambient atmospheric aerosols (e.g., Li *et al.*, 2003), laboratory-generated aerosols such as those produced by reaction experiments (e.g., Li *et al.*, 2009; McWhinney *et al.*, 2011; McWhinney *et al.*, 2013; Jiang *et al.*, 2016; Kramer *et al.*, 2016), and aerosols in occupational settings (Sauvain *et al.*, 2015). Various DTT-consuming chemical species in aerosols have been identified, including polycyclic aromatic hydrocarbons (PAHs), quinones, transition metals, water-soluble organic carbon (WSOC), and humic-like substances (Fang *et al.*, 2015). However, there is no consensus on the relative contributions of such species to the overall DTT activity of ambient aerosols, as pointed out by Fang *et al.* (2015). If the relationship between DTT consumption and chemical species could be clarified, chemical speciation data which is already accumulated dataset might be useful for deducing the oxidative abilities of aerosols at various places and situations.

Epidemiological, *in vitro*, and *in vivo* studies have demonstrated that some metals in aerosols have various health effects (Schauer *et al.*, 2006, and reference therein), and soluble transition metals, have been shown to be important contributors to DTT consumption in DTT assays (Charrier and Anastasio, 2012). Also of interest are the relative contributions of aerosols from primary and secondary sources to the overall oxidative ability of atmospheric aerosols. The contributions of primary and secondary aerosols in environmental samples have been compared (Verma *et al.*, 2009a; Saffari *et al.*, 2014), and the oxidative ability of diesel exhaust particles (DEPs) and secondary organic aerosols (SOAs) have been directly compared (McWhinney *et al.*, 2013). Both primary and secondary particles possess high oxidative abilities (Verma *et al.*, 2009a), and the oxidative ability of naphthalene-derived SOAs was found to be higher than that of DEPs. Jiang *et al.* (2016) also compared the oxidative abilities of SOAs produced from various hydrocarbon precursors and found that oxidative ability depended on the precursor. The accumulation of additional data on the oxidative abilities of laboratory-generated aerosols can be expected to shed light on what occurs in the real world.

In this study, we compared the oxidative abilities of environmental PM<sub>2.5</sub> samples collected at a traffic intersection and with those of samples collected at a residential site. To clarify the species responsible for DTT consumption in the samples, we conducted concurrent chemical speciation analysis. For comparison, we also analyzed two primary aerosols (DEPs and particles generated by a gasoline direct injection spark ignition [DISI] vehicle), as well as various SOAs generated in laboratory chamber experiments. Furthermore, we carried out reagent-solution experiments to distinguish the effects of water-soluble transition metals from the effects of other chemical species.

## METHODS

### *Site Descriptions and Sampling Methods*

Environmental samples of PM<sub>2.5</sub> were collected at monitoring site near a traffic intersection in the city of Kawasaki, Kanagawa Prefecture, Japan, where a main highway (Industrial Road) intersects another road; this site is referred to hereafter as the traffic site. Kawasaki has a population of about 1.4 million, and its air is polluted by traffic emissions. Industrial Road is 30 m wide, has 7 traffic lanes, and it is situated approximately 5 m below the Metropolitan Expressway which run parallel to and above Industrial Road. The average weekday traffic volume at this intersection is approximately 52,000 vehicles, about 25% of which are heavy-duty diesel vehicles. Additional information about the measurement site is available in the literature (Fujitani *et al.*, 2012a). As a reference sampling site, we used a residential area in the city of Tsukuba (population, 0.2 million), Ibaraki Prefecture, Japan; this reference site is hereafter referred to as the residential site. There are no substantial sources of aerosols near this sampling site.

PM<sub>2.5</sub> was concurrently collected both on 47-mm-diameter quartz fiber filters (2500QAT-UP, Pall) and on 47-mm-diameter Teflon filters (FP-500, Sumitomo Electric) by means of Federal reference method (FRM) samplers (Model 2000, Thermo Scientific) and on 8 in × 10 in Teflon filters (WP-500-50, Sumitomo Electric) by means of a high-volume air (HV) sampler (HV-1000R, Sibata). The sampling port of the FRM sampler was 3 m height from the ground, while the HV sampler was about 1 m above the ground. For the PM<sub>2.5</sub> cut, the FRM sampler was used the very sharp cut cyclone, while the HV sampler was used the impactor whose cut off curve was not clear.

Sampling was conducted for short periods during two summers (July–August 2014 and July–August 2015) and three winters (January 2014, January 2015, and January 2016). All sampling was conducted on weekdays, starting at 11 am and ending at 10:30 am the next day (that is, the sampling duration was 23.5 h). The sampling flow rates were 1000 and 16.7 L min<sup>-1</sup> for the HV sampler and the FRM samplers, respectively.

### *Laboratory-Generated Aerosols*

For comparison with the environmental samples collected at the traffic and residential sites, a number of laboratory-generated aerosols were also tested (Table 1). These aerosols were collected on 47-mm-diameter Teflon filters (FP-500, Sumitomo Electric) and 47-mm-diameter quartz fiber filters (2500QAT-UP, Pall) by means of a low-volume air sampler. DEPs were collected from an 8-L diesel engine located in the Nanoparticle Health Effect Laboratory of the National Institute for Environmental Studies (Fujitani *et al.*, 2009, 2012b). The engine was operated at a steady state at high idle (2000 rpm and 0 Nm), and DEPs were sampled in a dilution tunnel after dilution with air that had been filtered both chemically and with a HEPA filter. Particles emitted from a gasoline DISI vehicle were studied by means of a chassis dynamometer system (Nakashima *et al.*, 2010). Samples were drawn from a full-flow dilution tunnel, and

**Table 1.** Properties of tested aerosols.

Type of particle	Initial VOC conc. [ppm]	Initial O <sub>3</sub> conc. [ppm]	Initial NO conc. [ppm]	Reaction duration [min]	EC [%]	WIOC [%]	WSOC [%]	Ion [%]	Metal [%]	OA/OC
Particles generated by ozonolysis										
$\alpha$ -Pinene (pinene-rich conditions)	22.8	2.8	-	5	0.6	16.1	42.5	N.D.	N.D.	N.D.
$\alpha$ -Pinene (ozone-rich conditions)	1.9	12.5	-	5	0.9	1.1	53.3	N.D.	N.D.	N.D.
DEP + diesel exhaust	5.1	6.0	4.0	300	17.5	17.9	19.1	1.9	0.03	0.0011
Diesel exhaust	5.1	6.0	4.0	300	0.00	32.0	14.2	3.0	0.00	0.0018
Particles generated by photochemical reaction										
<i>m</i> -Xylene	5.3	-	2.4	180	1.7	31.7	0.0	N.D.	N.D.	N.D.
<i>m</i> -Xylene	5.3	-	2.4	300	3.8	4.1	35.0	N.D.	N.D.	N.D.
<i>m</i> -Xylene	5.3	-	2.4	900	1.0	15.4	24.0	N.D.	N.D.	N.D.
1,3,5-TMB	4.4	-	2.5	300	2.3	14.5	35.2	N.D.	N.D.	N.D.
Isoprene	5.2	-	2.5	300	7.5	8.3	20.4	N.D.	N.D.	N.D.
1,3-Butadiene	5.3	-	2.4	300	1.0	15.4	24.0	N.D.	N.D.	N.D.
DEP + Diesel exhausts <sup>a</sup>	5.1	-	4.0	60	39.7	24.2	12.4	1.6	0.07	0.0009
DEP + Diesel exhausts <sup>a</sup>	5.1	-	4.0	180	35.4	32.4	6.9	2.4	0.07	0.0013
DEP + Diesel exhausts <sup>a</sup>	5.1	-	4.0	300	31.3	22.0	14.2	1.9	0.06	0.0016
Diesel exhausts <sup>a</sup>	5.1	-	4.0	60	0.00	0.00	10.3	5.6	0.00	0.0083
Diesel exhausts <sup>a</sup>	5.1	-	4.0	180	2.58	27.4	17.7	4.6	0.00	0.0024
Diesel exhausts <sup>a</sup>	5.1	-	4.0	300	0.07	17.8	28.2	3.7	0.00	0.0017
Primary particles										
DEPs	-	-	-	-	19.5	66.2	1.4	0.20	0.04	0.0001
DISI particles	-	-	-	-	94.0 <sup>b</sup>	6.0 <sup>b</sup>	-	N.D.	N.D.	N.D.
Particles in Environmental samples										
Traffic site summer	-	-	-	-	14.4	9.1	5.6	58.9 (45.0) <sup>c</sup>	51.3 (11.7) <sup>c</sup>	0.0033
Traffic site winter	-	-	-	-	22.0	14.7	6.4	43.5 (30.6) <sup>c</sup>	18.2 (6.4) <sup>c</sup>	0.0062
Residential site summer	-	-	-	-	7.2	10.6	14.7	46.0 (42.8) <sup>c</sup>	4.0 (2.4) <sup>c</sup>	0.0072
Residential site winter	-	-	-	-	10.2	11.3	11.4	41.9 (31.1) <sup>c</sup>	5.4 (2.5) <sup>c</sup>	0.0068

Abbreviations: DEPs, diesel exhaust particles; DISI, direct injection spark ignition; EC, elemental carbon; OAs, organic acids; OC, organic carbon; N.D., not determined (chemical analysis was not conducted); 1,3,5-TMB, 1,3,5-trimethylbenzene; VOC, volatile organic compound; WIOC, water-insoluble organic carbon; WSOC, water-soluble organic carbon.

<sup>a</sup> H<sub>2</sub>O<sub>2</sub> (35.5 ppm) was added to generate OH radicals to promote photochemical reactions.

<sup>b</sup> Ratios of EC or OC to total carbon. On the basis of results for other DISI vehicles, metals and inorganic ions are expected to be minor fractions to particle mass. The data are from Fushimi *et al.* (2016).

<sup>c</sup> The values in parenthesis are corrected values.

Values in environmental samples are averages of all data for the specified sampling site during the specified season.

the vehicle was operated under Japanese transient mode (JC08 under hot start conditions).

Photochemical reactions and ozonolysis reactions of diesel exhaust (DE) with and without DEPs were conducted at the Nanoparticle Health Effect Laboratory. Specifically, unfiltered DE or DE from which DEPs had been removed with HEPA filters was introduced into an 8-m<sup>3</sup> reaction chamber made with 50- $\mu$ m-thick fluorinated ethylene propylene film. SOAs were generated either by ozonolysis or by photochemical reaction. In addition, SOAs were generated from individual volatile organic compounds (VOCs) in two small reaction chambers (0.2 or 1 m<sup>3</sup>) by means of the procedure described by Fujitani *et al.* (2012c). Specifically, SOAs were generated by ozonolysis of  $\alpha$ -pinene and by photooxidation of *m*-xylene, 1,3,5-trimethylbenzene (1,3,5-TMB), 1,3-butadiene, and isoprene in the presence of nitric oxide. The initial conditions for all these reactions are listed in Table 1.

#### DTT Assay and Aerosol Characterization

For the DTT assays of environmental samples, we used one-quarter of a 47-mm-diameter sample which was punched from 8 in  $\times$  10 in Teflon filters. For laboratory-generated aerosols, we used one-half or one-quarter of a 47-mm-diameter Teflon filter on which a sample had been collected by means of a low-volume air sampler. The filter portions were sonicated in mixture of 250 mM Tris base (Trizma base, pH 8.9, Sigma) and HCl buffer for 30 min at a temperature below 10°C, and then DTT (Wako) was added to a final concentration of 160  $\mu$ M. The solution was incubated at 37°C for 15 min and then centrifuged. A 1-mL aliquot of the supernatant was collected in a tube, and sufficient 5,5'-dithiobis(2-nitrobenzoic acid) (Wako) was added until a yellow color developed.

A 200- $\mu$ L sample of the solution was placed in one well of a 96-well plate, and the optical density was measured at 414 nm with a microplate reader (POLARstar OPTIMA,

BMG LABTECH) to determine the amount of residual DTT in the solution. Standard solutions at various DTT concentrations were analyzed simultaneously with each sample, and the relationship between DTT concentration and optical density was determined. Operating blanks without a filter were also analyzed with the samples, and the DTT consumption for the blanks was subtracted from the results obtained for the samples. A blank filter was also measured and found to make only a minor contribution to DTT consumption.

The filter samples were also analyzed for particle mass, elemental carbon (EC), organic carbon (OC), WSOC, organic acids (OAs), inorganic ions, and metals as follows. Particle mass was obtained by determining the difference in the mass of each 47-mm-diameter Teflon filter before and after sampling by means of an electric microbalance (UMX 2, Mettler–Toledo; readability 0.1 µg) equipped with a charge neutralizer (Am-241) in an air-conditioned room maintained at a constant temperature of  $23 \pm 0.2^\circ\text{C}$  and a relative humidity of  $50 \pm 1\%$ . The EC and OC contents of samples collected on quartz fiber filters were quantified by means of a carbon analyzer (model 2001, Desert Research Institute) using the IMPROVE protocol (Chow *et al.*, 2001). The usage for analysis were 8-mm-diameter samples which were punched from quartz fiber filter. For determination of WSOC, 4 pieces of 8-mm-diameter samples were punched from quartz fiber filter and they were sonicated in distilled deionized water of 400 µL for 30 min at a temperature below  $10^\circ\text{C}$ , and then the solution was filtered. The filtrate was applied to a baked quartz fiber filter and analyzed by means of the IMPROVE protocol. Water-insoluble organic carbon (WIOC) was determined by subtracting WSOC from OC.

Samples collected on the Teflon filters were used whole of 47-mm-diameter sample for OA analysis and one-half of 47-mm-diameter samples for inorganic ions and metal analysis, respectively. For environmental sample, 47-mm-diameter samples were punched from 8 in  $\times$  10 in Teflon filter. The amounts of OAs (succinic acid, methylsuccinic acid, maleic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, undecanedicarboxylic acid, dodecanedicarboxylic acid, 3,4-dimethylbenzoic acid, *o*-toluic acid, *m*-toluic acid, *p*-toluic acid, and 3,4-dimethylbenzoic acid) were determined by gas chromatography–mass spectrometry (7890A, Agilent; JMS1000GC MkII, JEOL) after preparation as follows. Samples spiked with an internal standard were subjected to ultrasonic extraction with 3 mL of 1:1 dichloromethane/methanol for 15 min. Following the extraction, the solution was filtered, and the filtrate was dried. BSTFA (50 µL), pyridine (10 µL), and 1:1 dichloromethane/hexane (140 µL) were added to the residue. The resulting solution was allowed to react at  $70^\circ\text{C}$  for 2 hours, and the target materials were trimethylsilylated. Then hexane (400 µL) was added to the solution, which was analyzed by gas chromatography–mass spectrometry.

Inorganic ions ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{2-}$ , and  $\text{SO}_4^{2-}$ ) were analyzed by ion chromatography (ICS-1000, DIONEX) after samples were extracted with sonication for 15 min in a mixture of 9.5

mL distilled deionized water and 0.5 mL ethanol. Metals (Li, Be, Na, Mg, Al, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Mo, Ag, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, W, Ti, Pb, Bi, Th, and U) were analyzed by inductively coupled plasma–mass spectrometry (7500iG1833A, Agilent) after samples were extracted with microwave-assisted extraction for 30 min in a mixture of 5 mL nitric acid, 2 mL hydrofluoric acids, and 1 mL hydrogen peroxide.

All solvents used were analytical grade. Only some of environmental samples were also extracted with distilled deionized water, and the extract was analyzed for Fe, Ni, and Cu. The water-soluble fractions of these metals were determined, and the fractions were assumed to be the same for the samples that were not analyzed.

### ***Assessment of the Effects of Water-Soluble Transition Metals on DTT Assay Results***

Because DTT consumption is enhanced by the presence of transition metals (Charrier and Anastasio, 2012), we also conducted DTT assays on solutions of metal compounds similarly to the filter samples to assess the effects of water-soluble transition metals in the filter samples. We used the following metal compounds: vanadium(V) oxide (Wako special grade, 99%, Wako), chromium(VI) oxide (Wako special grade, 98%, Wako), manganese(II) chloride tetrahydrate (JIS special grade, 99.0%, Wako), iron(III) chloride (99.4%, Wako), nickel(II) sulfate hexahydrate (JIS special grade, 99.0–102.0%, Wako), and copper(II) sulfate (JIS 1st grade, 97.50%, Wako). These compounds were selected on the basis of the results of Charrier and Anastasio (2012) and on the fact that these metals were detected in the environmental samples. In addition, zinc(II) sulfate (practical grade, 95.0%, Wako), ammonium sulfate (JIS special grade, 99.5%, Wako), and azelaic acid (> 98%, Tokyo Chemical Industry) were also tested. The concentrations of the solutions of the metal compounds were chosen on the basis of the concentrations found in the environmental samples.

To mask the effects of transition metals and assess the contributions of other chemical species to DTT consumption, we pretreated the samples with Chelex 100 Resin (sodium form, 100–200 mesh, BIORAD) either alone or in combination with 4H (EDTA free acid) (ethylenediaminetetraacetic acid, Dojindo Molecular Technologies) at various concentrations.

## **RESULTS AND DISCUSSION**

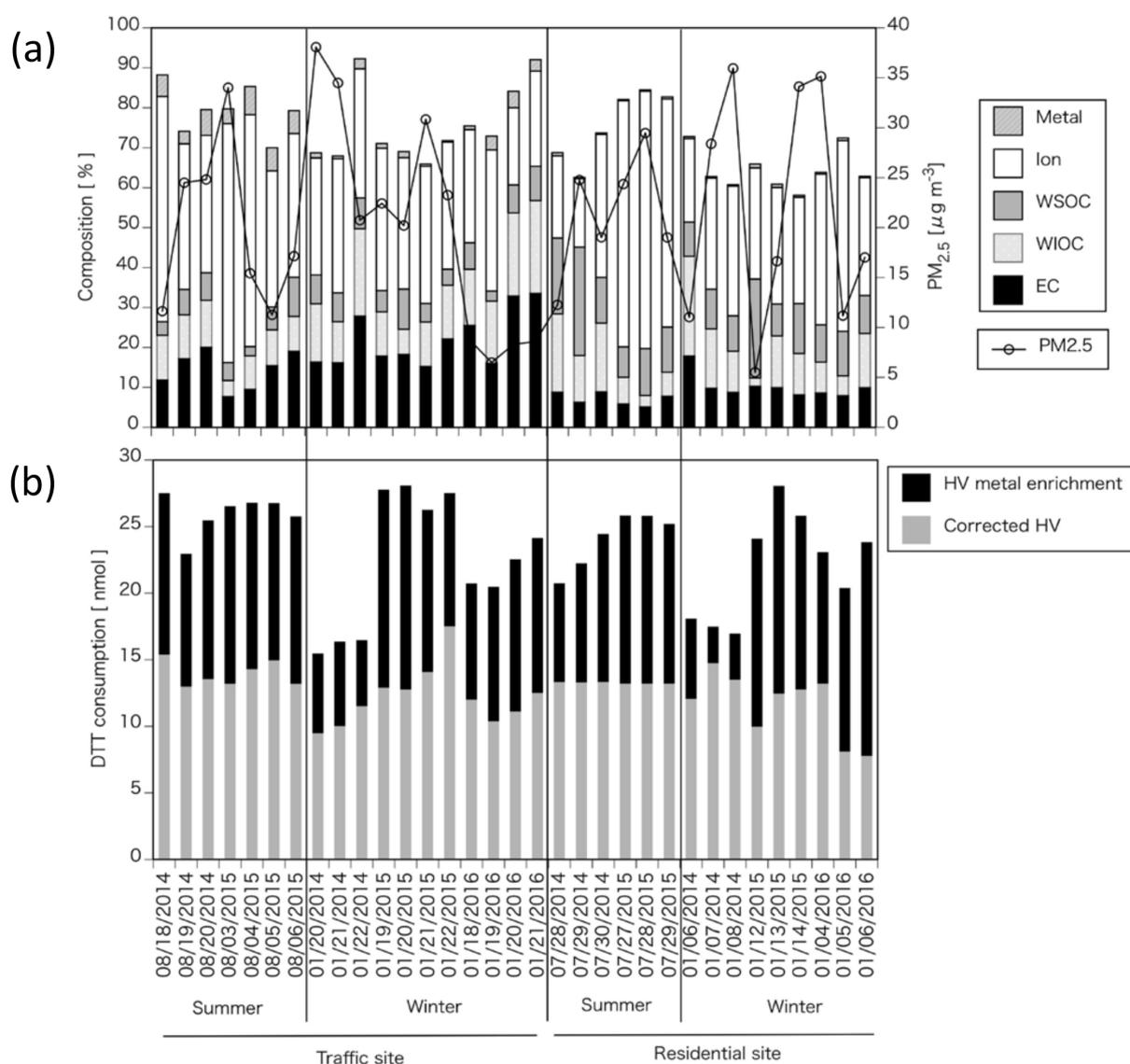
### ***PM<sub>2.5</sub> Concentration and Chemical Composition and DTT Consumption***

The summation of concentrations of major chemical components (carbon component from the FRM sample and inorganic ions and metals from the HV sample) were compared to gravimetric PM<sub>2.5</sub> from the FRM sample, which largely exceeded gravimetric PM<sub>2.5</sub> especially in traffic site samples (Table 1). We compared concentrations of inorganic ions and metals that collected by the HV sampler to those concurrently collected by the FRM sampler for traffic site in Jan 2015, Aug 2015, and Jan 2016. The concentrations of sulfate which might be belong to smaller size range in

PM<sub>2.5</sub> agree well (differences were within 10% from average values) from both samplers. On the other hand, such as Ca<sup>2+</sup> and all metals, which expected in relatively larger size range in PM<sub>2.5</sub> generated from such as road dust were much higher (about 2–33 times) in the HV samples in spite of equipping with a 2.5 μm 50% cutoff impactor. There are two possibilities. One is the height of sampling port was different between them. The second one is cut off curves were likely different for both samplers. Therefore, we also showed the results of corrected HV samples by adjusting to FRM samples using the differences between FRM and HV samplers for concentrations of inorganic ions and metals, and all environmental samples were applied the constant correction factors in each chemical component. Both raw

data and corrected HV samples for chemical compositions as well as DTT results are shown.

Fig. 1 shows PM<sub>2.5</sub> concentrations and chemical compositions after correction of the HV sample, as well as DTT consumption values, for the environmental samples collected from the two sites in summer and winter. Although PM<sub>2.5</sub> concentration varied among the samples, the average concentrations for the two seasons and the two sampling sites were similar (about 20 μg m<sup>-3</sup>). Inorganic ions were the major species in all the samples, accounting for 40–60% of the PM<sub>2.5</sub>, and still dominant 31–45% after correction of the HV samples (Table 1). The PM<sub>2.5</sub> compositions at the two sampling sites differed; specifically, EC and metals were more abundant at the traffic site than at the residential site.



**Fig. 1.** (a) PM<sub>2.5</sub> concentrations (right y-axis) and chemical compositions of PM<sub>2.5</sub> (left y-axis) in environmental samples. EC, elemental carbon; WIOC, water-insoluble organic carbon; WSOC, water-soluble organic carbon; inorganic ions, and metals. Metals and inorganic ions are showed in corrected results of the HV samples. (b) Consumption of dithiothreitol (DTT) by environmental samples and the contributions of DTT consumption by enriched metals by the HV sampler. The date indicates the date of start of samplings. The unit is nanomoles of DTT consumed by 200 μL of sample solution. Because 200 μL of each solution contained 32 nmol of DTT, the theoretical maximum DTT consumption was 32 nmol.

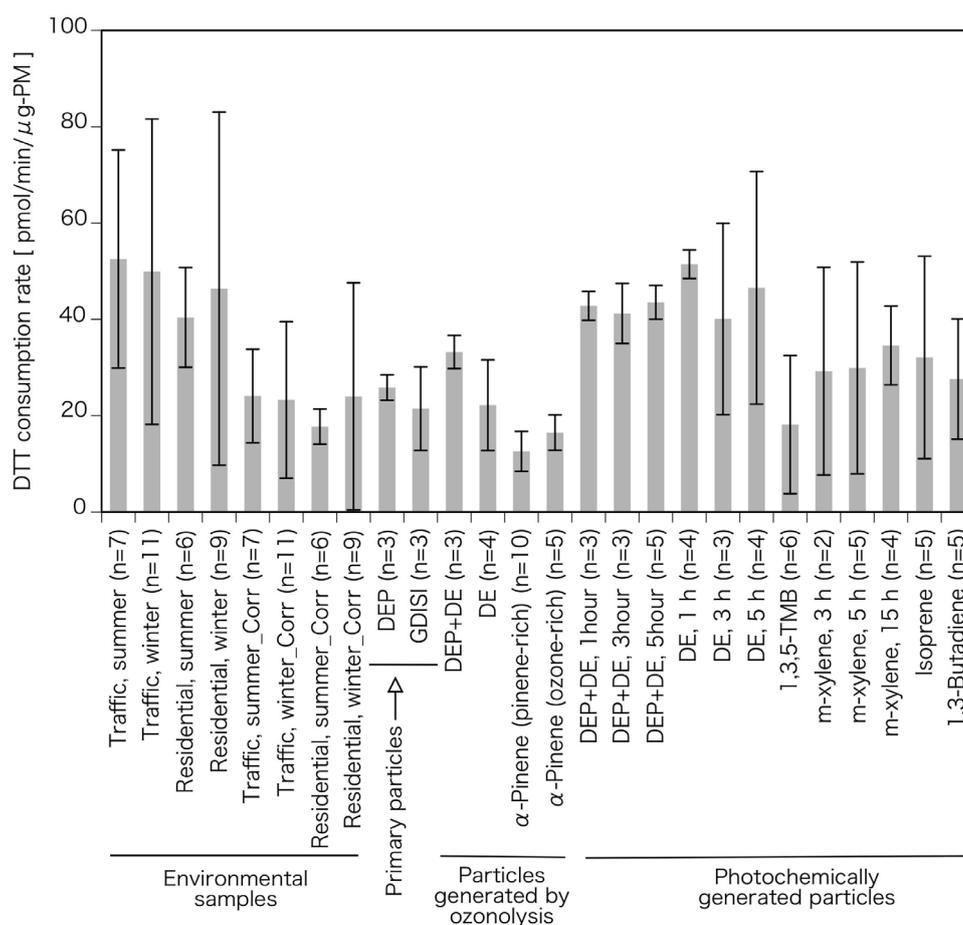
After correction of the HV samples, abundance of metals in the traffic site was largely reduced, but still more abundant at the traffic site than at the residential site. There was little seasonal variation in composition at residential site.

Composition data for the laboratory-generated aerosols are also listed in Table 1. Inorganic ions and metals were minor constituents of the laboratory-generated aerosols. The OA/OC ratios in Table 1 are indicators of the sources of the aerosols. OC is generated by both primary and secondary sources, whereas OAs are present at much higher contents in SOAs. Therefore, the ratios in the table indicate the extent to which SOAs predominated in the environmental aerosols. In this study, the OA/OC ratio for the DEPs was 0.0001, and both ozonolysis and photochemical reaction of diesel exhaust, increased the ratio by at least 10 fold. In both seasons, the OA/AC ratios at the residential site were higher than those at the traffic site, which suggests that primary sources predominated at the traffic site and that the contribution of secondary sources was higher at the residential site than at the traffic site.

DTT consumption and  $PM_{2.5}$  concentration did not seem to be correlated with each other (Fig. 1(b)). If the chemical compositions of all the samples were the same, then DTT

consumption would be linearly related to  $PM_{2.5}$ . However, the chemical compositions of the environmental samples varied, and the oxidative abilities of the various chemical species differed. Fig. 1(b) also showed the contribution of DTT consumption by enriched metals by the HV sampler. The enriched metals were calculated by the difference of the metal concentrations between the HV and the FRM samples, and DTT consumptions by enriched metals were calculated by using the results of reagent experiments shown in later. Fig. 2 shows the DTT consumption rates per microgram of PM for all the samples used in this study. DTT consumption by the environmental samples was relatively higher than that of the laboratory-generated aerosols, and the major cause of the higher oxidative abilities of the environmental samples might be the presence of transition metals. The oxidative abilities of the environmental samples collected at the traffic site were relatively higher than those of the samples collected at the residential site, by factors of 1.3 in the summer and 1.1 in the winter (1.4 in the summer and 1.0 in the winter after correction of the HV samples).

We compared the DTT consumption rates that we determined to those reported in previous studies. Verma *et al.* (2012) and Fang *et al.* (2015) reported DTT consumption



**Fig. 2.** Rates of dithiothreitol (DTT) consumption per microgram of particulate matter for the various types of particles analyzed in this study. The corrected environmental HV samples are also shown in the figure. DE, diesel exhaust; DEP, diesel exhaust particles; GDISI, gasoline direct injection spark ignition; 1,3,5-TMB, 1,3,5-trimethylbenzene. Error bars indicate the standard deviations of multiple measurements.

rates of 23 and 35  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ , respectively, in an urban environment in Atlanta. Fang *et al.* (2015) summarized rates from previous studies (Cho *et al.*, 2005; Ntziachristos *et al.*, 2007; Hu *et al.*, 2008; Verma *et al.*, 2009b; Charrier and Anastasio, 2012), which ranged from 13 to 76  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  at residential sites. Janssen *et al.* (2014) reported a rate of 81  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  for an urban background site in the Netherlands. Rates at traffic sites have been reported to be 34  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  (Fang *et al.*, 2015), 133  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  (Janssen *et al.*, 2014), and 8–62  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  (Cho *et al.*, 2005; Ntziachristos *et al.*, 2007; Hu *et al.*, 2008; Verma *et al.*, 2009b; Charrier and Anastasio, 2012). The rates we determined for the traffic and residential sites in this study fell in the middle of the range of rates reported in the literature. The rate of oxidative ability decrease to about one-half after correction of the HV sample, but still within the reported values.

For laboratory-generated primary aerosols, the rate of DTT consumption by DEPs was higher than the rate for DISI particles, and both rates were lower than the rates for most of the SOAs. SOAs consist entirely of organic components, DISI particles consist mostly of EC, and DEPs contain both organic components and EC, which suggests that the oxidative ability of organic components is higher than that of EC. McWhinney *et al.* (2013) analyzed DEPs emitted from a vehicle equipped with an oxidation catalyst at various engine loads and found that the DTT consumption rate of the DEPs ranged from 23 to 60  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ . The oxidative ability of the DEPs obtained in this study was below the lower limit reported by these investigators. Geller *et al.* (2006) used a chassis dynamometer facility to test a diesel vehicle (Euro 4) and a gasoline port fuel injection (PFI) vehicle (Euro 3). These investigators reported DTT consumption rates of 39  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  for diesel vehicle under transient operation and 25  $\text{pmol min}^{-1} \mu\text{g}^{-1}$  for gasoline PFI vehicle under transient operation, and they showed that the oxidative ability of DEPs was higher than that of particles emitted from the gasoline vehicle, which is consistent with the results of the current study. However, note that the chemical composition of the particles emitted from the gasoline PFI vehicle differed substantially from that of the DISI particles investigated in this study; specifically, OC predominated in emissions from the PFI vehicle (Geller *et al.*, 2006), whereas EC predominates in DISI particles (Fushimi *et al.*, 2016).

The oxidative abilities of the SOAs depended on the precursor: specifically, the oxidative abilities of the SOAs generated by ozonolysis were lower than those of the SOAs generated by photochemical reaction, and this was true for both of the diesel SOAs and the  $\alpha$ -pinene SOA. For photochemical reaction, the oxidative ability of the diesel SOAs were higher than the oxidative abilities of the SOAs generated from the individual VOCs. Because DE contains various kinds of VOCs, intermediate-volatility organic compounds, and semivolatile organic compounds, which can be precursors of SOAs (Donahue *et al.*, 2012), it is likely that the DE contained precursors that generated SOA that was more toxic, which is other than VOCs that conducted in the single VOC experiment in this study.

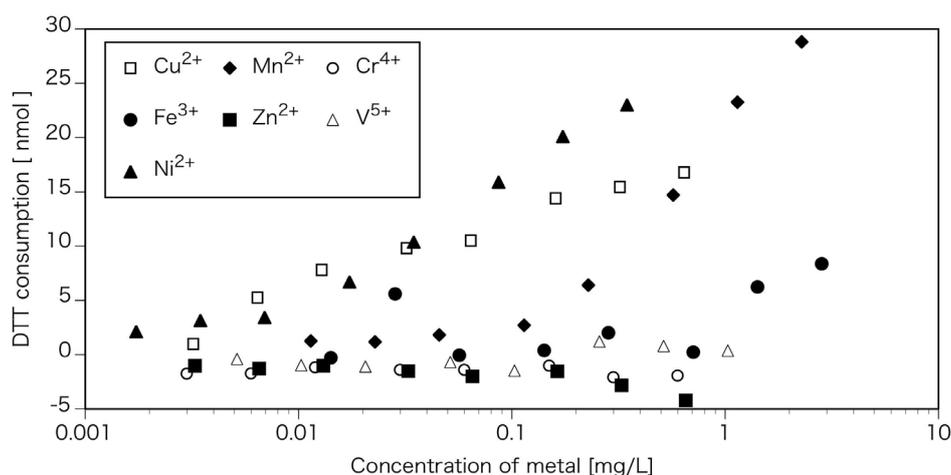
We also conducted reaction chamber experiments using DE combined with DEPs. Comparison of the results obtained DEPs with the results obtained after reactions of DE combined with DEPs revealed that oxidative ability was increased both by ozonolysis and by photochemical reactions. These results suggest that not only DEPs but also SOAs generated by reaction of DE contributed to the oxidative ability of the environmental samples.

When we conducted photochemical experiments at various reaction times, we observed no correlation between oxidative ability and reaction time for the diesel SOAs. It is possible that the reaction did not progress substantially after a certain time, as indicated by the OA/OC values; in the DEP+DE experiments, OA/OC increased with increasing reaction time, but the opposite was true for the experiments with DE alone. Note, however, that the oxidative ability of the *m*-xylene SOA increased slightly with increasing reaction time.

Jiang *et al.* (2016) have reported that  $\alpha$ -pinene SOA has the lowest DTT consumption rate (5–9  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ ) relative to 1,3,5-TMB SOA (14–23  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ ) and isoprene SOA (31–62  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ ). We observed the same trend in this study, and our DTT consumption rate for the 1,3,5-TMB SOA was identical to theirs. McWhinney *et al.* (2011) conducted ozonolysis reactions of exhaust from a two-stroke gasoline engine and found that the oxidative ability before the reaction was 0.71  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ , whereas that after the reaction was 8.6  $\text{pmol min}^{-1} \mu\text{g}^{-1}$ . Li *et al.* (2009) reported that the SOA generated by ozonolysis of exhaust consisting of DEPs and particles after ozonolysis had higher oxidative ability than DEPs alone. Rattanavara *et al.* (2011) studied the aging of exhaust consisting of DEPs and DE under sunlight and indicating that aged DEPs have higher oxidative ability than fresh DEPs. The results we obtained in the current study are consistent with the results of these previously reported experiments involving reactions of automobile exhausts; that is, oxidative ability is increased by both photochemical reactions and ozonolysis.

#### **Reagent-Solution Experiments and Oxidative Abilities of Metals in Environmental Samples**

DTT consumption and the amounts of water-soluble transition metals in the environmental samples were strongly correlated ( $R = 0.80$ ), whereas DTT consumption and the amounts of other chemical species (OC, WSOC, OA, EC, and inorganic ions) showed little or no correlation. Considering the results of Charrier and Anastasio (2012), we expected transition metals to be the predominant contributors to DTT consumption by the environmental samples collected in this study. Because aerosol samples were extracted by Tris-HCl buffer, which has extractive properties similar to those of water. We estimated the contributions of transition metals to DTT consumption by measuring the DTT consumption of solutions of transition metal compounds, as well as solutions of ammonium sulfate and azelaic acid, as a function of solute concentration (Fig. 3). We found that solutions of Zn, Cr, and V did not consume DTT, and neither did a solution of ammonium sulfate or azelaic (data not shown). In contrast, solutions of Ni, Cu, Mn, and Fe



**Fig. 3.** Dithiothreitol DTT consumption by liquid solutions of metal compounds. The theoretical maximum consumption was 32 nmol.

did consume DTT, and DTT consumption increased with increasing metal concentration.

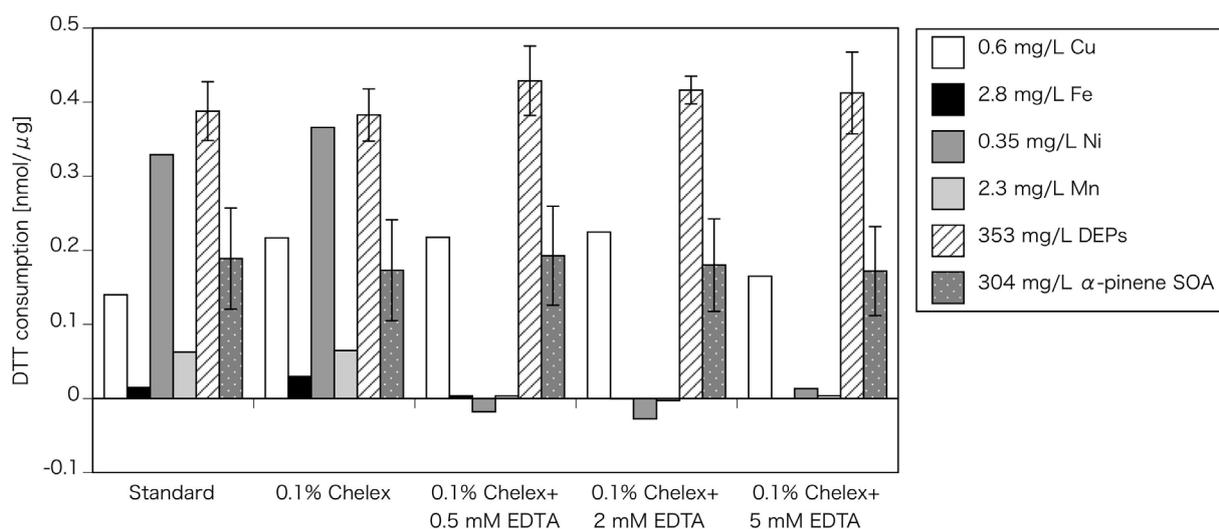
Compare the consumption of DTT with amount of transition metal, the Ni(II) solution consume the most DTT (136 times in average for various concentration tests), and Cu(II) (94 times), Mn(II) (11 times), and Fe(III) (7 times) within the concentration range tested in this study, and thus these four transition metals may act as catalysts. Kachur *et al.* (1997) proposed  $\text{Cu}^{2+}$ -DTT complex is formed and catalyzes oxidation of free DTT via formation of an oxygen containing intermediate. DTT consumption per amount of transition metals is different with different transition metals, which may depend on the complex formation constant for metal-DTT complex and its catalytic potency. Charrier and Anastasio (2012) showed Cu(II) was the high reactivity and Fe(III) was low reactivity, which is consistent with our finding, but order of Ni(II) and Mn(II) was inconsistent with our finding. Further studies are needed to clarify the oxidative potential and mechanism of DTT consumption.

To further evaluate the effects of metals on DTT consumption, we conducted chelation experiments on solutions of Ni, Cu, Mn, and Fe in the DTT assay. We showed the results at the highest concentration tested, as well as on filter samples of DEPs and  $\alpha$ -pinene SOA (Fig. 4). Addition of Chelex+EDTA completely eliminated DTT consumption by all of the metal solutions except the Cu solution; DTT consumption by the Cu solution decreased slightly with increasing EDTA concentration. Charrier and Anastasio (2012) reported that DTT consumption by a 1  $\mu\text{M}$  Cu(II) solution was substantially reduced when 1 mM EDTA was added. Our test concentration of Cu (about 10  $\mu\text{M}$ ) was higher than theirs, owing to the high Cu concentration in the environmental samples; therefore the amount of EDTA we used may have been insufficient to completely mask the effect of Cu.

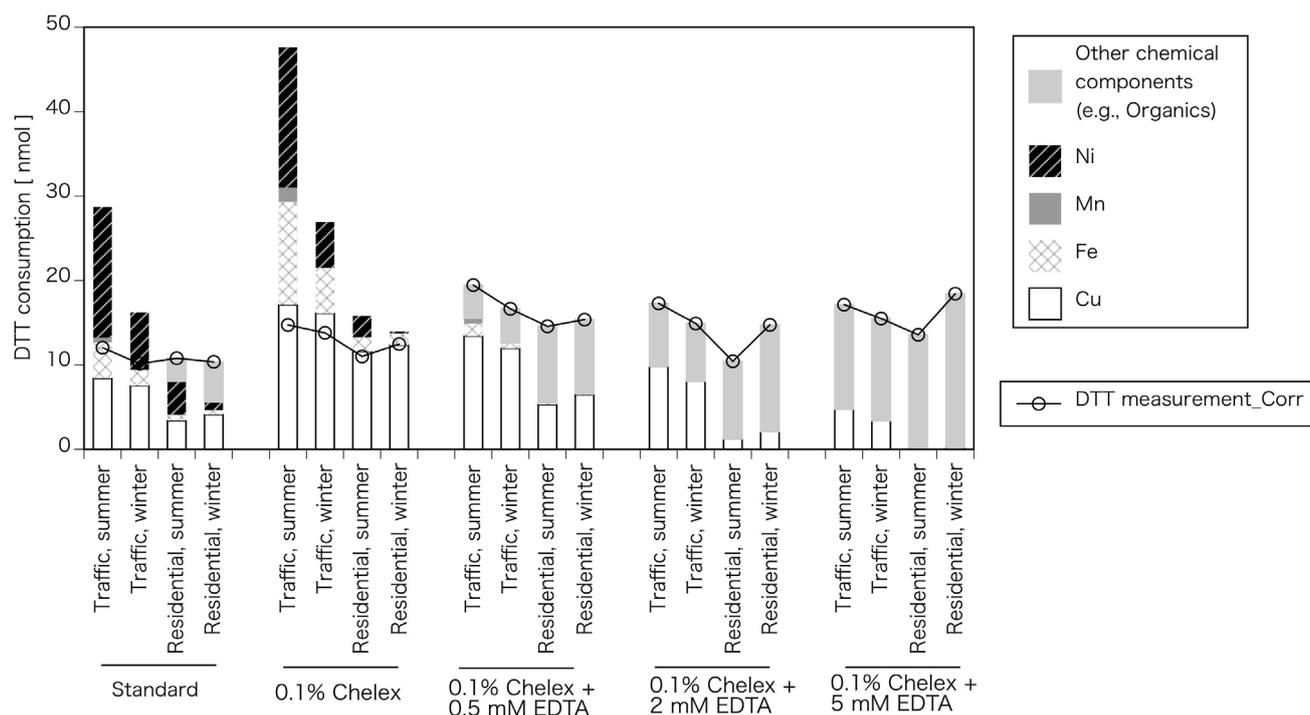
Charrier and Anastasio (2012) also noted that DTT consumption by phenanthrenequinone decreased with increasing EDTA concentration up to 1 mM, indicating that the oxidative abilities of compounds other than transition metals can also be affected by EDTA. In this study, we

found that DTT consumption by DEPs and the  $\alpha$ -pinene SOA was insensitive to EDTA concentration (within the standard deviation for multiple experiments). DEPs contain trace metals but at very low concentrations (the highest concentration was 0.002  $\text{mg L}^{-1}$  for Ni), and no transition metals are expected to be present in the  $\alpha$ -pinene SOA; thus four transition metals were insensitive to the addition of EDTA. Further, although DEPs contain quinones such as 9,10-phenanthrenequinone (0.07  $\text{ng } \mu\text{g}^{-1}\text{-PM}$ ) and 1,2-naphthoquinone (< 0.04  $\text{ng } \mu\text{g}^{-1}\text{-PM}$ ), the contributions of organic species in filter sample to DTT consumption may have been minor in our study.

We also treated our environmental samples with chelating reagents to mask the effects of the transition metals (Fig. 5). The contributions of the metals to DTT consumption after the HV sample corrections were calculated from the metal concentrations in the buffer solution and the results of the reagent-solution experiments. The metal concentrations in the buffer solution were determined from the metal contents in the filter samples and in the water-soluble fractions of Fe (0.23), Ni (0.73), Cu (0.66), and Mn (0.54), respectively. Note that the water-soluble fraction of Mn was used average value of fractions of Fe, Ni, and Cu. In a standard experiment with no chelating reagents, more than 54% of the DTT consumption (more than 80% without the HV sample corrections) was considered to be due to the transition metals in buffer solution. Cu and Ni contributed substantially to DTT consumption in all the environmental samples. For the traffic site samples, estimation of DTT consumption by the four transition metals was higher than the DTT measurement. This inconsistency is due in part to the hypothesized constant value of water-soluble fraction in each environmental sample. Other possibilities are heterogeneously of particle mass loading in filter sample of HV sampler. All four transition metals were enriched in the environmental samples compared to crustal soil; the enrichment factor for Cu was 186 (HV sample correction), and that for Ni exceeded approximately 172 in the samples collected at the traffic site. Davis *et al.* (2001) estimated that atmospheric deposition of Cu generated from brake abrasion accounted for 47% of



**Fig. 4.** Dithiothreitol (DTT) consumption by metal compound solutions, diesel exhaust particle (DEPs) filter samples, and  $\alpha$ -pinene secondary organic aerosol (SOA) filter samples in standard experiment and treated with Chelex 100 Resin either alone or in combination with ethylenediaminetetraacetic acid (EDTA) at various concentrations. Data for DEPs and the  $\alpha$ -pinene SOA are means ( $n = 2-10$ ), and error bars indicate standard deviations.



**Fig. 5.** Dithiothreitol (DTT) consumption by environmental samples in standard experiment and treated with Chelex 100 Resin either alone or in combination with ethylenediaminetetraacetic acid (EDTA) at various concentrations. These results are shown in the corrected results of the HV samples. The bars indicate the cumulative contributions of four transition metals and other chemical components to DTT consumption.

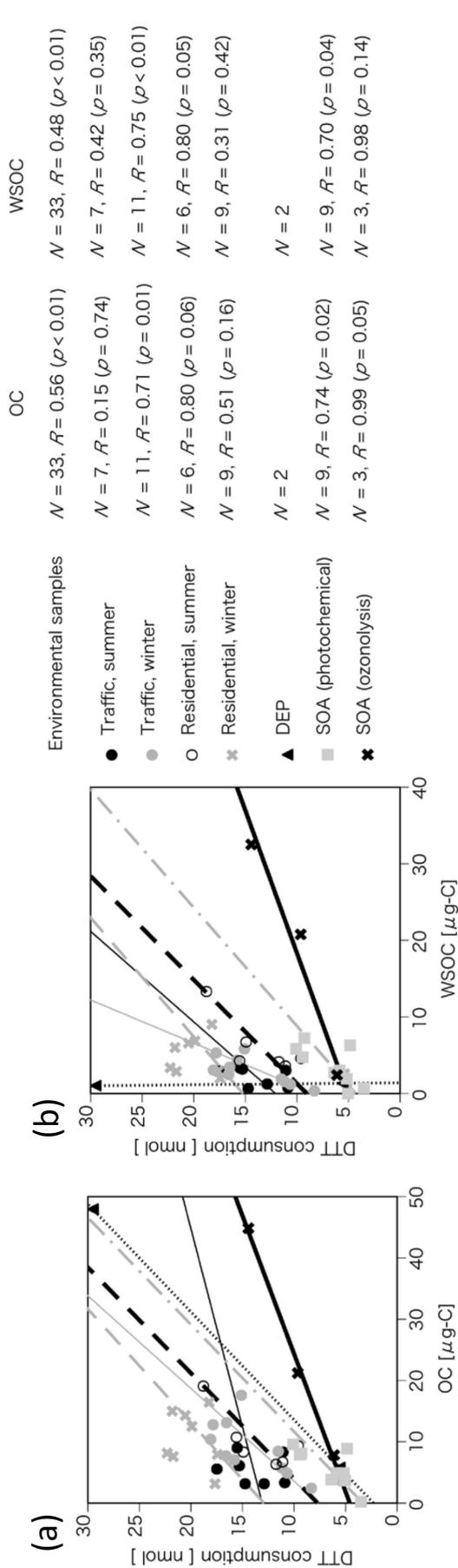
the Cu in urban runoff. Okuda *et al.* (2007) reported that in the Tokyo metropolitan area, heavy oil combustion is a major source of Ni in fine particles such as  $PM_{2.5}$  and that Ni is distributed mostly in relatively water-soluble fractions.

Owing to the masking of the effects of the transition metals by the chelating reagents, the contributions of other chemical species increased as the amount of EDTA was

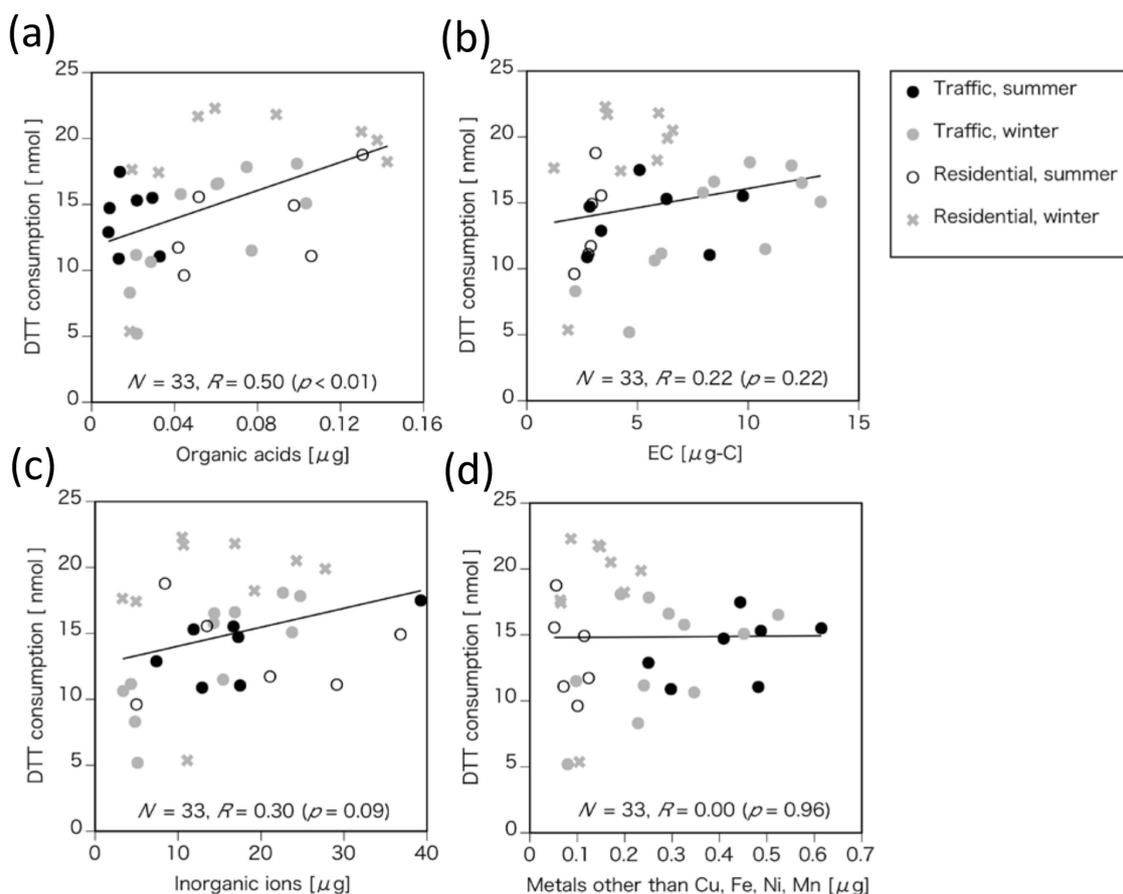
increased (Fig. 5). The other contributors to DTT consumption after the effects of the transition metal were masked are discussed in the next section.

#### **Contributions to DTT Consumption by Species Other than Water-Soluble Transition Metals in environmental Samples**

Figs. 6 and 7 show the relationships between DTT



**Fig. 6.** Dependence of dithiothreitol (DTT) consumption on amounts of (a) organic carbon (OC) and (b) water-soluble organic carbon (WSOC) for environmental samples and laboratory-generated particles after removal of the contributions of four transition metals (Cu, Fe, Ni, Mn). The data are shown in after corrections of the HV samples for environmental samples. The secondary organic aerosol (SOA) data are for SOAs generated from individual precursors and diesel exhaust diesel exhaust (DE) alone. The lines indicate the following fits: thin black line, traffic site in summer; thin gray line, traffic site in winter; bold black dashed line, residential site in summer; bold gray dashed line, residential site in winter; dotted gray line, DEPs; dot-dashed gray line, SOAs generated by photochemical reactions; bold black line, SOA generated by ozonolysis. Number of samples and correlation coefficients are shown.



**Fig. 7.** Dependence of dithiothreitol (DTT) consumption on amounts of (a) organic acids, (b) elemental carbon (EC), (c) inorganic ions, and (d) metals other than the four transition metals (Cu, Fe, Ni, Mn) in environmental samples after removal of the contributions of the four transition metals. The data are shown in after corrections of the HV samples. Number of samples, correlation coefficients, and fitting lines for all environmental samples are shown.

consumption and the amounts of various chemical components in the environmental samples after removal of the contributions of Cu, Fe, Mn, and Ni by treatment with Chelex and EDTA (5 mM). The data are shown in after corrections of the HV samples for environmental samples. Fig. 6 also shows DTT consumption by DEPs, by SOAs generated single VOCs and DE alone. The results for the DEPs were obtained in the presence of the chelating reagents (Chelex and 5 mM EDTA), and the SOA results were obtained in the absence of chelating reagents. The amounts of metals other than the four transition metals showed no correlations with DTT consumption (Fig. 7(d)), indicating that the contributions of these other metals to DTT consumption were negligible after the samples had been treated with the chelating reagents. Relatively positive correlations were observed for OC (Fig. 6(a)), WSOC (Fig. 6(b)), and OAs (Fig. 7(a)), whereas the correlations for EC (Fig. 7(b)) and inorganic ions (Fig. 7(c)) were relatively weak. The correlations between DTT consumption and the amounts of these chemical species were stronger in the presence of the chelating reagents than in their absence. In previous studies, inorganic ions were reported not to consume DTT (Ntziachristos *et al.*, 2007; Biswas *et al.*, 2009), and we observed no DTT consumption by ammonium sulfate

in reagent-solution experiment. Thus, the weak correlations of inorganic ions to DTT consumption was likely due to covariant of inorganic ions and chemical components that have oxidative ability (i.e., OC, WSOC).

It is interesting that the oxidative ability on the basis of OC (that is, the slope of the dependence of DTT consumption on the amount of OC) for the DEPs was compatible with the oxidation abilities of SOAs generated photochemically, as well as the oxidative abilities of the environmental samples, except in the case of the traffic site in summer (Fig. 6(a)). This result suggested that organic composition of either DEPs or SOAs generated from photochemical reactions, or both particles resembled to those of PM<sub>2.5</sub> collected at traffic and residential sites except the traffic site in summer.

Although the oxidative ability of aerosols at the traffic site in summer was the highest on the basis of mass of particulate matter (Fig. 2), the oxidative ability was insensitive to OC amount. Number of samples for the traffic site in summer was small ( $n = 7$ ) and if the excluding outlier, the slope become similar to other environmental samples. Another possible explanation for this is that the concentration of OC at the traffic site in summer was affected by road dust, including tire dust (Hildemann *et al.*, 1991), and therefore the environmental sample from that site in summer contained

different organic composition to the other environmental samples. Actually, OA/OC ratio was the lowest among the environmental samples. On the basis of WSOC, DTT consumption at the traffic site in summer was similar to that at the residential site in summer.

In this study, the filter samples were extracted with Tris-HCl buffer, and insoluble particles in the buffer were not removed by means of a syringe filter; therefore WIOC and EC may also have contributed to DTT consumption in our experiments. In particular, even though WIOC and EC were the major constituents of the DEP samples used for this study (Table 1), these samples did consume some DTT. Previous investigators also reported that the amounts of OC and PAHs were highly correlated with DTT consumption by a water extract of DEPs (Geller *et al.*, 2006) and by environmental samples collected with a water impinger (Li *et al.*, 2003; Ntziachristos *et al.*, 2007). Although PAHs themselves do not contain functional groups capable of catalyzing DTT oxidation, PAHs are markers for the presence of organic compounds that do contribute to DTT oxidation, including quinones, oxy-PAHs, and nitro-PAHs, which are redox active (Cho *et al.*, 2005; Verma *et al.*, 2009a). Furthermore, the oxidative activity of methanol extracts of an ambient aerosol sample has been shown to be strongly correlated with WIOC ( $R = 0.94$ ), and this result emphasizes the importance of water-insoluble organic aerosol components in toxicological studies (Verma *et al.*, 2012).

In addition, EC (black carbon) is also a candidate for the consumption of DTT. McWhinney *et al.* (2013) used phosphate buffer to extract a filter sample of DEPs and found that the sample retained little of its initial oxidative ability once insoluble material had been removed by filtration; they attributed this result primarily to reactions occurring at the black carbon surface. However, these investigators also discussed other possibilities, including noncatalytic mechanisms of loss, such as slow adsorption or reaction with the DEP surface.

These previous results suggest that in our study, WIOC and EC also contributed to DTT consumption, even in the buffer. Further studies are needed to elucidate the relationships between DTT consumption and various chemical species, especially organic compounds. In addition, the results of DTT assays of various aerosol types should be compared with the results of *in vitro* cell-based assays such as the heme oxygenase-1 assay to understand more realistic health effects of atmospheric aerosols.

## CONCLUSIONS

We conducted DTT assays and chemical analyses of environmental samples collected at a traffic site and a residential site, as well as laboratory-generated aerosols (DEPs, gasoline DISI particles, and SOAs generated from various precursors). We made the following observations:

1. Among the environmental aerosols samples we collected, the aerosols from the traffic site during summer had the highest oxidative ability.
2. The oxidative abilities of the environmental aerosol samples were higher than those of laboratory-generated

aerosols, owing to the presence of transition metals in the environmental samples. Four transition metals (Cu, Fe, Ni, and Mn) in the environmental samples accounted for most of the DTT consumption ( $\geq 80\%$  for original results and  $\geq 54\%$  for the HV correction results), and the contribution of these metals was particularly high at the traffic site.

3. After the effect of transition metals in the environmental samples were removed by means of chelating agents, DTT consumption was correlated with the amounts of OC, WSOC, and OAs and weakly correlated with the amounts of EC and inorganic ions.
4. The oxidative ability of the DEPs was typically lower than that of SOA generated from photochemical reaction on a particle mass basis, whereas the oxidative abilities were compatible on the basis of the amount of OC. The oxidative abilities on the basis of the amount of OC for the environmental samples (except for the sample from the traffic site in summer) after removal of the effect of transition metals were also compatible with the oxidative abilities of the DEPs and SOA generated from photochemical reaction.

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