SECONDARY FORMATION OF TOXIC NITROPOLYCYCLIC AROMATIC HYDROCARBONS PROMOTED ON MINERAL DUST AEROSOLS

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ABSTRACT

Atmospheric dust transported from desert areas contains large amounts of mineral particles that are known to adversely affect human health. Although the chemicals associated with dust have been recognized as a possible cause of negative health impacts, more research is required to pinpoint the exact underlying causes. According to the results of our monitoring studies of ambient aerosols, chemical reactions on mineral dust considerably increase the amount of nitropolycyclic aromatic hydrocarbons, a major class of toxic chemicals. This suggests that surface

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reactions on mineral dust should be considered as an atmospheric source of toxic organic chemicals and that they may play a significant role in adverse health effects induced by mineral dust aerosols.

Keywords: Polycyclic aromatic hydrocarbons, Nitropolycyclic aromatic hydrocarbons, Aeolian dust, Secondary formation

1. INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are a major class of toxic compounds found in ambient airborne particulates, some exhibiting stronger toxicological effects than their parent polycyclic aromatic hydrocarbons (PAHs) (Durant et al. 1996; Patton et al. 1986). NPAHs are produced from chemical reactions of PAHs in the atmosphere as well as from a wide variety of anthropogenic sources such as fuel combustion. Some NPAHs are formed via gas-phase reactions of semi-volatile PAHs, and subsequently deposit onto airborne particulates. For example, 2-nitropyrene is formed from the gas-phase reaction of pyrene (Py) with OH radicals in the presence of NO_2 (Atkinson and Arey 1994), and 2-nitrofluoranthene is formed via two pathways; that is, from either OH- or NO_3-radical initiated reactions in the gas phase (Atkinson and Arey 1994). By contrast, 1-nitropyrene (1-NP) and 1,8-dinitropyrene (1,8-DNP), which are probable carcinogens (IARC 2013), are representative NPAHs formed through combustion of fossil fuels (Schuetzle 1983). 1-NP can also be formed from gas-particle phase heterogeneous reactions (Nguyen et al. 2009; Ramdahl et al. 1984; Shiraiwa et al. 2009). However, the general conclusion from previous studies is that the impact of the heterogeneous formation on the amount of atmospheric 1-NP is negligible because the reaction rate and yield of 1-NP through this process are not sufficient to account for the ambient 1-NP concentrations (Nguyen et al. 2009; Ramdahl et al. 1984; Shiraiwa et al. 2009).

Recently, however, we reported that the heterogeneous nitration of PAHs by gaseous NO_2 on natural mineral particulates such as desert dust

aerosol is more important than previously thought, because natural mineral aerosols have more reactive surfaces than the model materials previously used (Kameda et al. 2016). This suggests that a combination of mineral dust aerosols and PAH-contaminated air could produce highly toxic chemicals in the atmosphere. Mineral dust is widely known to have a significant influence directly and/or indirectly on our respiratory health; for instance, possibly causing asthma (Kanatani et al. 2010), but the main factors affecting the respiratory system remain unclear. The heterogeneous processes which unexpectedly produce toxic organic chemicals such as NPAHs may be a key player in the adverse health effects induced by mineral dust aerosols.

In the present study, we sampled airborne particles at locations in Beijing (China) and Wajima (Japan), a remote background site on the Noto peninsula, during a heavy dust storm period, and measured the concentrations of particle-associated NPAHs (1-NP and 1,8-DNP), other typical PAHs, and gaseous atmospheric pollutants simultaneously in order to understand the secondary formation of atmospheric NPAHs on mineral dust aerosols.

Furthermore, we used the *umu Salmonella* mutagenicity assay for the soluble organic fractions (SOF) of ambient airborne samples to estimate the contribution of secondarily formed NPAHs to the total direct-acting mutagenicity of the SOF.

2. MATERIALS AND METHODS

2.1. Field Measurements

Airborne particulate matter was collected on the roof of a five-story building, approximately 20 m above ground level at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (116.34° E, 40.01° N, Beijing, China) in winter (November 20, 2009–January 15, 2010), spring (February 22, 2010–May 28, 2010, which included a heavy dust storm period), and summer (July 5, 2010–August 30, 2010). The

sampling site is located in northern Beijing and is primarily a residential and commercial area, where dominant PAH sources include vehicular traffic and fuel combustion for cooking and/or heating. Airborne particulate matter was also collected at Kanazawa University Noto Air Monitoring Station (136.54°E, 37.23°N, Wajima, Japan), which is located on the Noto Peninsula, 2.1 km south of the Japan Sea coast in winter (November 24, 2009–December 29, 2009), spring (February 22, 2010–June 6, 2010, which included a heavy dust storm period), and summer (July 28, 2010–August 31, 2010). No major emission sources of PAHs and NPAHs are located near the station. Samples were collected on quartz fiber filters using high-volume five-stage cascade impactors (Andersen air sampler; SIBATA, AH-600F) at a flow rate of 566 L min^{-1}. The collection periods were usually 2–3 days per sample in Beijing and 1 week in Wajima.

The filters before and after the sample collection were weighed after the equilibrium weight was attained for each filter stored in desiccators at constant relative humidity of ca. 30% under 295 ± 3 K. The mass of airborne particles was determined by subtracting the weight of the filter after sample collection from that before the collection. The filter samples were stored at 253 K until subjected to analysis. Concentrations of aeolian dust were obtained from the LIDAR (Light Detection and Ranging) DSS (Dust and Sandstorm) Observation Data Page managed by the Ministry of the Environment, Government of Japan (Ministry of the Environment 2010).

Daily concentrations of atmospheric NO_2 in Beijing were obtained from the Air Pollution Index (API), which was posted on the Beijing Public Net for Environmental Protection website (Beijing Public Net for Environmental Protection 2011). The obtained API values, that is, mean concentrations measured at 12 observation sites in Beijing, were converted to volume fractions using the methodology described in a previous study (Li et al. 2011).

Daily concentrations of atmospheric NO_2 in Wajima were provided by the Ishikawa Prefectural Institute of Public Health and Environmental Science.

2.2. Extraction of SOF from Airborne Particulates

The filter samples of ambient airborne particulates, onto which deuterated 1-NP and deuterated chrysene (1-NP-d_9 and Chry-d_{12}, respectively; internal standards) were added, were cut into fine pieces before extraction. SOF from the filter samples were extracted twice with 100mL of dichloromethane under sonication for 20min. The extracted solution was filtered with a cellulose acetate filter to remove solid residue, followed by the addition of 100μL of dimethyl sulfoxide (DMSO) into the filtrate to avoid complete drying out of the solvent during the concentration steps. After concentrating to ca. 5mL and filtering through a 0.45µm membrane filter, the samples were concentrated to ca. 100µL under a nitrogen stream to leave DMSO, and then 400 µL of methanol was added. An aliquot of each sample solution was subjected to quantification for PAHs and 1-NP using high-performance liquid chromatograph (HPLC).

2.3. Instrumentation for Chromatographic Analyses

An HPLC system with column-switching and chemiluminescence detection was employed for ambient particle-associated 1-NP and 1,8-DNP quantification as reported previously (Kameda et al. 2016). Briefly, the system consists of four HPLC pumps, a 6-port switching valve, a clean-up column (GL Sciences, Inertsil ODS-P, 3.0mm ID × 250mm), a separation column (GL Sciences, Inertsil ODS-3, 3.0mm ID × 250mm), a reducer column (Jasco, NPpak-RS, 4.0 mm ID × 10 mm), a trapping column (GL Sciences, Inertsil ODS-3, 4.0 mm ID × 30 mm), and a chemiluminescence detector (Soma Optics, S-3400).

The chemiluminescence reagent solution was an acetonitrile solution containing 0.03 mmol L^{-1} bis (2,4,6-trichlorophenyl) oxalate and 15 mmol L^{-1} H_2O_2. Mobile phases were methanol/water (3/1, v/v) for the clean up and reduction of NPAHs, and acetonitrile/imidazole-perchloric acid buffer (45/55, v/v) for the separation. The reduction of NPAHs into amino-PAHs, which is strongly fluorescent, was performed at 373 K in the reducer

column. HPLC analysis of the nine kinds of ambient particle-associated PAHs (Py, fluoranthene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and benzo[*ghi*]perylene) was performed with a Hitachi L-6200 pump, an F-1050 fluorescence detector, and an Inertsil ODS-3 column (3.0mm ID × 250mm, GL Sciences) as previously described (Kameda et al. 2016). Solutes were eluted isocratically using methanol/water (77/23, v/v) as the mobile phase at a flow rate of 0.45mL min^{-1}.

2.4. Mutagenicity Assay

Mutagenicity of the samples collected during the sampling periods was assayed with a *Salmonella typhimurium* MN2009 strain following the *umu* test developed by Oda et al. (1995) in the absence of S9 mix. 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) was used as a positive control for the assay. The filters after sample collection were cut into fine pieces and the SOF was extracted with 100 mL of dichloromethane by sonication for 15-20 min. The pieces of the filter or insoluble solid substance were removed from the extract solution of SOF with a cellulose acetate filter (Advantec MFS, No.2), followed by the removal of solvent with a rotary evaporator and nitrogen stream. The residue thus obtained was re-dissolved in 1 mL of DMSO, providing the sample solution.

2.5. Materials

1-NP and Chry-d_{12} were purchased from Wako Pure Chemical Industries. 1-NP-d_9 was obtained from C/D/N isotopes. The PAH standard mixture (EPA 610 Polynuclear Aromatic Hydrocarbons Mix) and 1,8-DNP were purchased from Supelco and Accu Standard, respectively. All solvents and other chemicals used were of HPLC or analytical grade, purchased from Wako Pure Chemical Industries.

3. RESULTS AND DISCUSSION

3.1. Atmospheric Concentrations of NPAHs and Dust

Variations in concentrations of airborne particles, gaseous NO_2, particle-bound PAHs, particle-bound 1-NP, and particle-bound 1,8-DNP in Beijing are shown in Figures 1–4. The concentrations of the particle-bound 1-NP during the heavy dust storm period (March 19–22) was significantly higher than those during non- or low-dust periods (Figure 3), although concentrations of NO_2 (Figure 1) and PAHs (Figure 2) were not unusually different. Anthropogenic emissions, such as those from fossil fuel combustion, have been regarded as dominant sources of 1-NP, 1,8-DNP, in addition to PAHs (Schuetzle 1983). To determine whether 1-NP and 1,8-DNP were secondarily formed on dust particles, we evaluated their concentrations relative to benzo[*k*]fluoranthene (BkF), a fairly unreactive and non-volatile PAH (Figure 5) (Finlayson-Pitts and Pitts 2000).
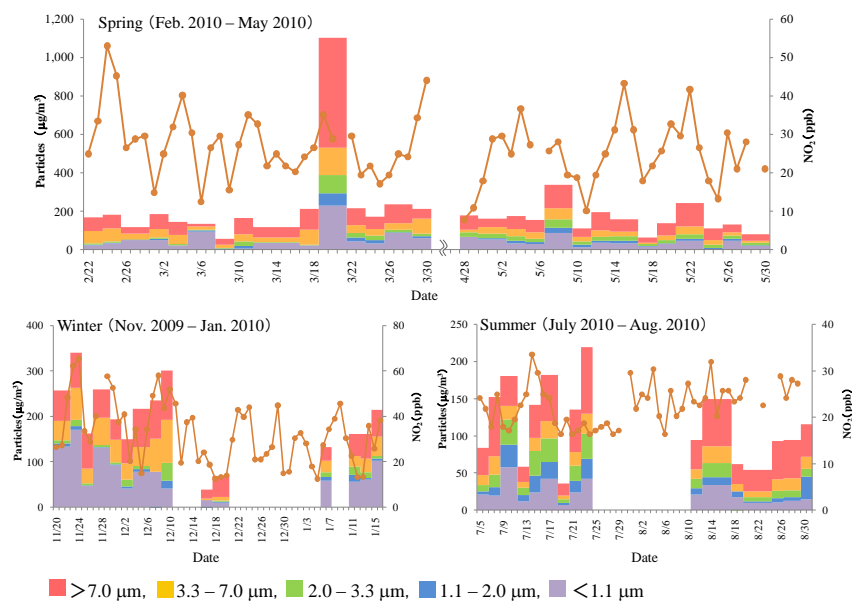
Figure 1. Concentrations of ambient airborne particles (bars) and NO_2 (circles) in Beijing.
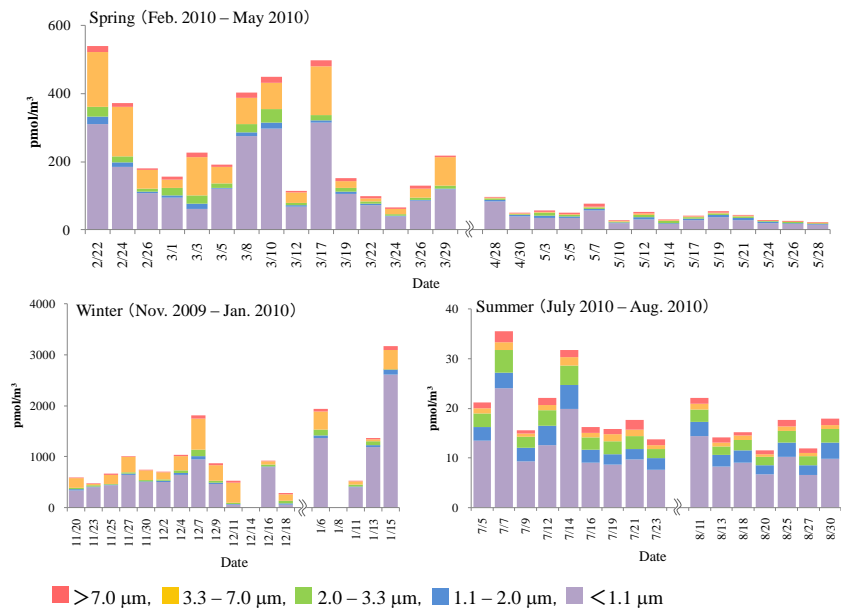
Figure 2. Concentrations of particle-associated PAHs in Beijing.
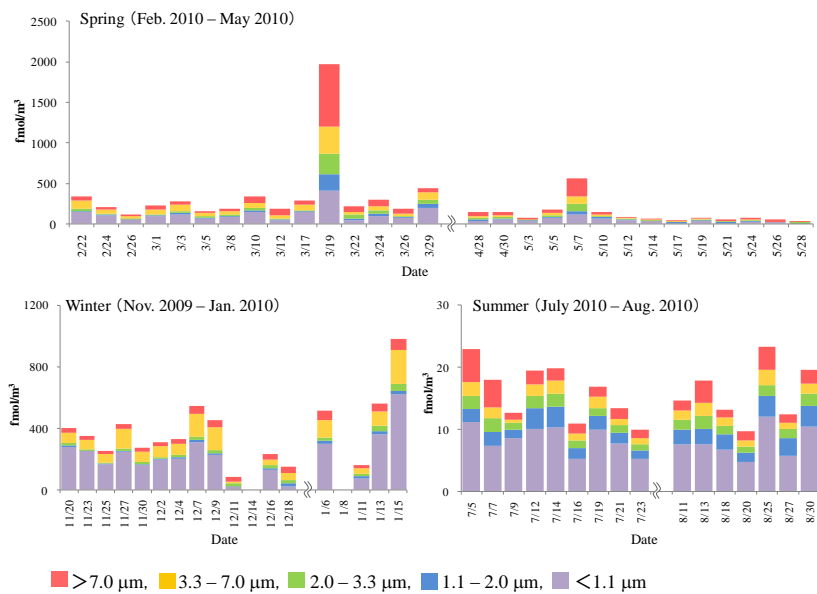
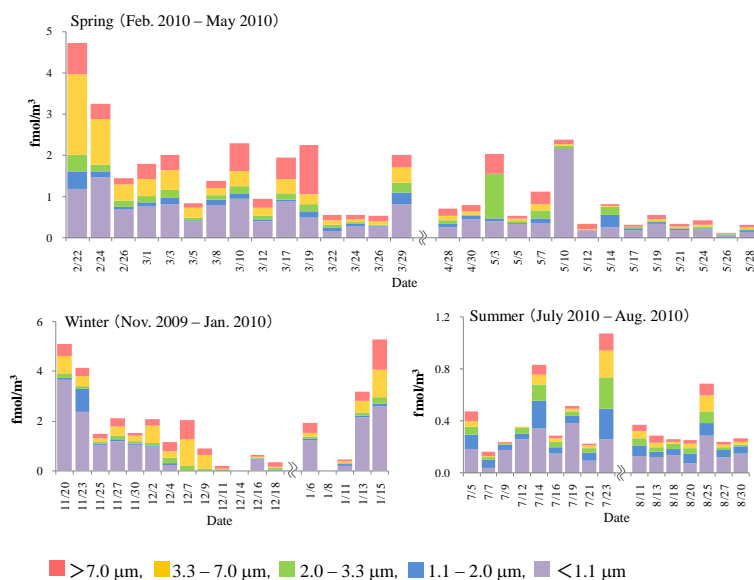Figure 3. Concentrations of 1-NP in Beijing.

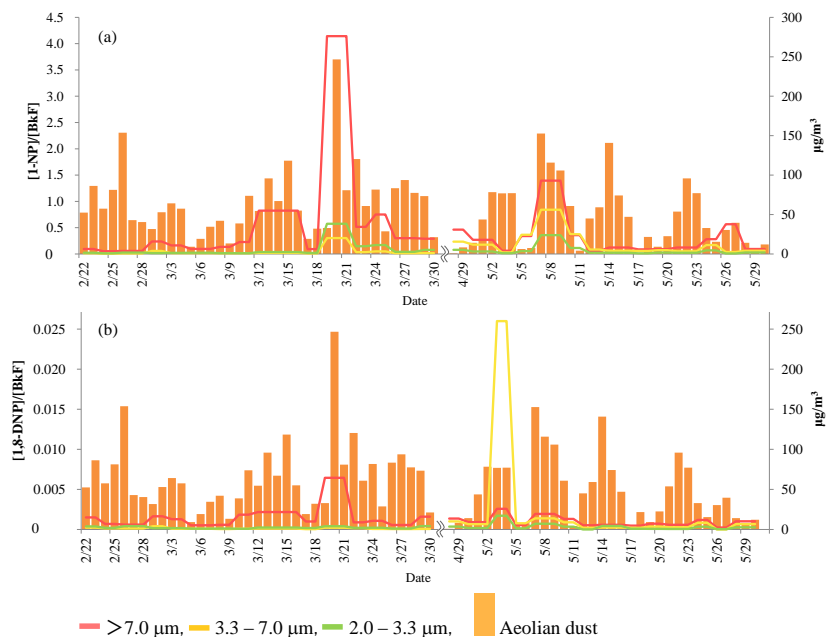Figure 4. Concentrations of 1,8-DNP in Beijing.

Figure 5. Daily variations in concentration ratios of 1-NP to BkF (a) and 1,8-DNP to BkF (b) in ambient airborne particles collected in Beijing during the spring of 2010. Bars indicate the concentration of aeolian dust.

The 1-NP/BkF ratio was significantly elevated during periods of heavy dust, particularly in the largest fraction of particle diameters (> 7.0μm), which mainly contains the dust particles carried to Beijing (Figure 5a) (Ohta et al. 2005). A similar increase in the ratio of 1,8-DNP/BkF was observed in the largest fraction during the heavy dust period (Figure 5b).

Figures 6–9 show atmospheric concentrations of airborne particles, gaseous NO_2, particle-bound PAHs, particle-bound 1-NP, and particle-bound 1,8-DNP in Wajima. The concentrations of the particle-bound 1-NP and 1,8-DNP during the heavy dust storm (March 15 – 22) were significantly higher than those during non- or low-dust periods, as was the case in Beijing. The 1-NP/BkF and the 1,8-DNP/BkF ratios were significantly elevated during periods of heavy dust, particularly in the fractions of particle diameters ranging from 2.0 – 3.3 and 3.3 – 7.0μm, which mainly contain the dust particles carried to Japan (Figure 10) (Ohta et al. 2003).
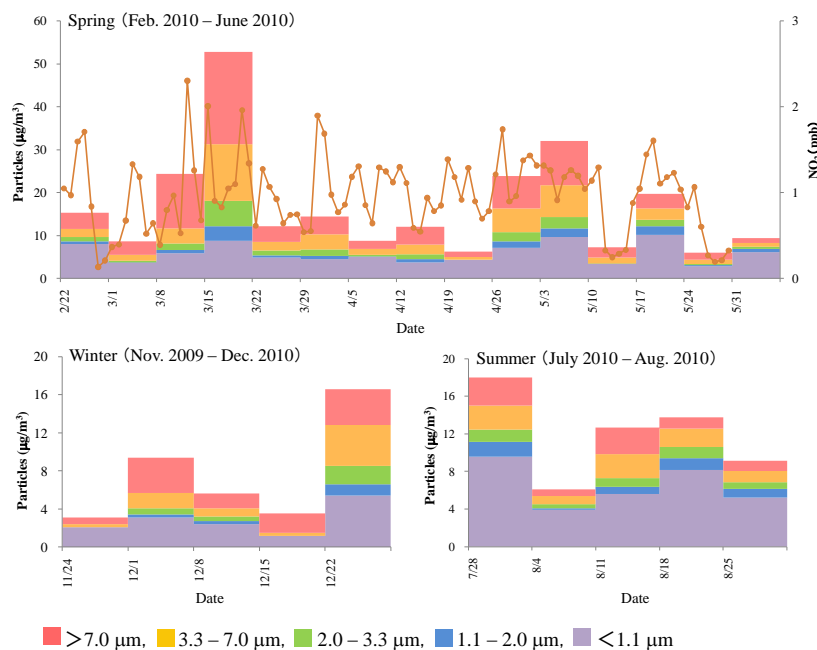

Figure 6. Concentrations of ambient airborne particles (bars) and NO_2 (circles) in Wajima.
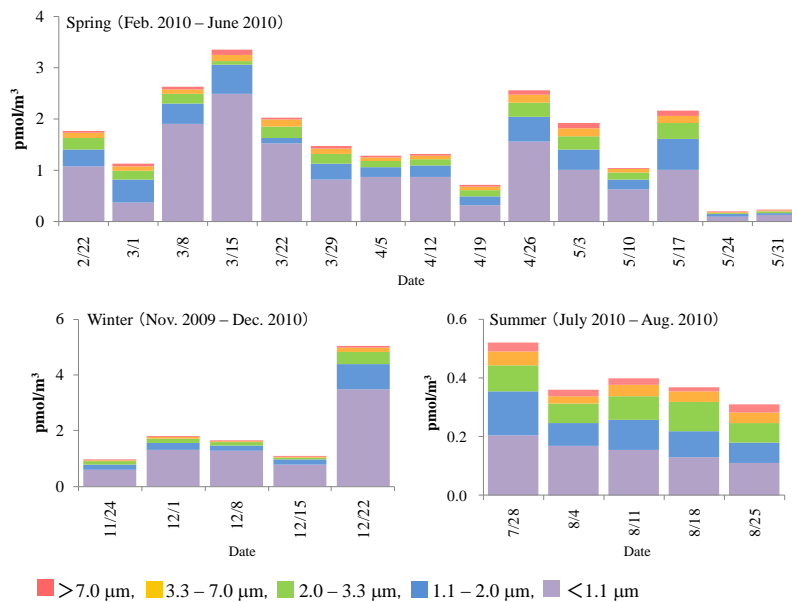
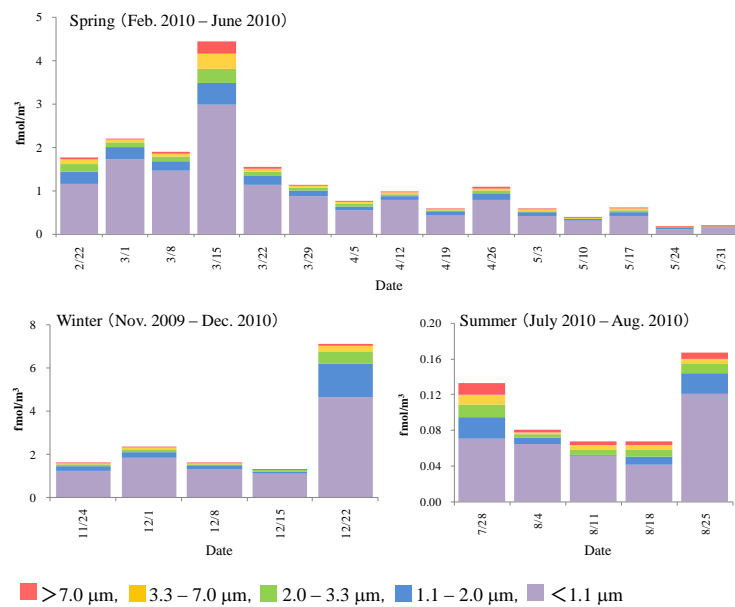Figure 7. Concentrations of particle-associated PAHs in Wajima.

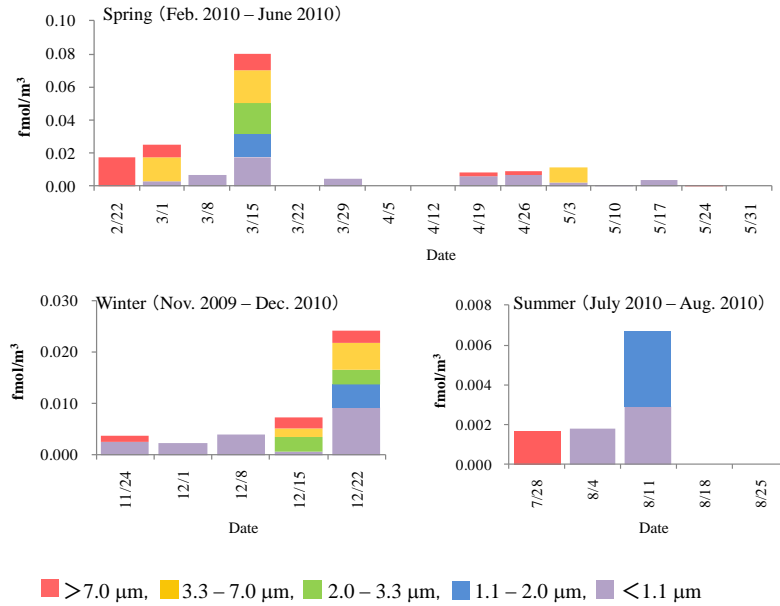Figure 8. Concentrations of 1-NP in Wajima.
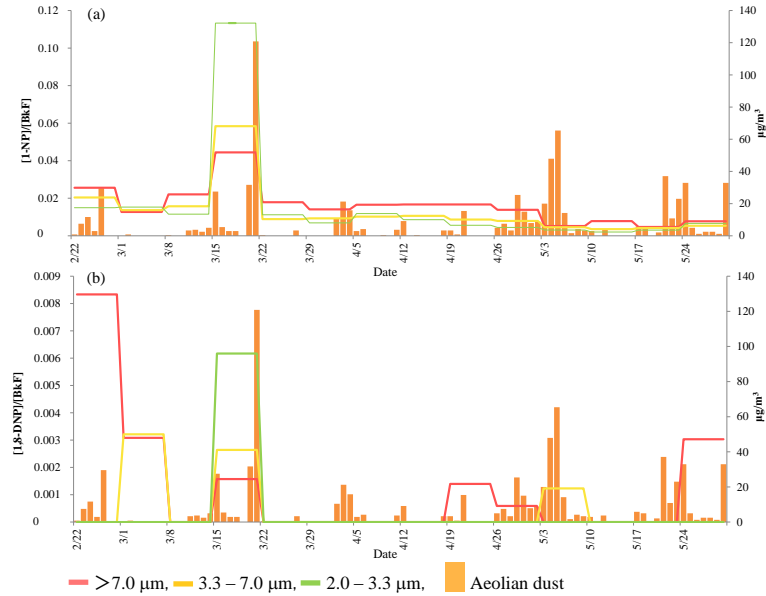
Figure 9. Concentrations of 1,8-DNP in Wajima.

Figure 10. Daily variations in concentration ratios of 1-NP to BkF (a) and 1,8-DNP to BkF (b) in ambient airborne particles collected in Wajima in spring of 2010. Bars indicate the concentration of aeolian dust.

Atmospheric PAHs can adsorb onto mineral dust particles when the dust plumes pass over polluted regions (Falkovich et al. 2004). Our laboratory-scale experiments have shown that the heterogeneous nitration of pyrene by gaseous NO_2 is promoted on mineral dust aerosols (Kameda et al. 2016). Thus, a considerable fraction of the aerosol-bound 1-NP and 1,8-DNP during the heavy dust episodes was likely formed on desert dust particles. It is worth noting that the mineral dust aerosols easily catalyse the heterogeneous nitration of PAH and may consequently lead to a significant increase in toxic NPAHs in the environment, which are believed to be predominantly produced from combustion sources.

3.2. Mutagenicity of Airborne Samples and Contribution of the NPAHs

The direct-acting mutagenicity of the SOF of airborne particles collected from March 19–22 in Beijing towards the *Salmonella typhimurium* strain NM2009 was the highest among the dates studied (Figure 11a); this coincided with a period of elevated 1-NP and 1,8-DNP concentrations.

The mutagenicity was also high between March 15–22 in Wajima, during the onset of the heavy dust storm (Figure 11b). We previously showed that the direct-acting mutagenicity was strongly affected by primary emissions, particularly automotive emissions (Kameda et al. 2004). However, the finding that the high mutagenicity coincided with the high concentrations of dust suggests that secondarily formed NPAHs contributed to the ambient mutagenicity. The total contribution of 1-NP and 1,8-DNP to the ambient mutagenicity, based on the ambient concentrations obtained in this study and the specific direct-acting mutagenic activities previously reported (Oda et al. 1993), reached a maximum of 30% during the high-dust period in Wajima.

Thus, the strong mutagenic activities during the onset of the dust storm cannot solely be accounted for by the secondary formation of 1-NP and 1,8-DNP.
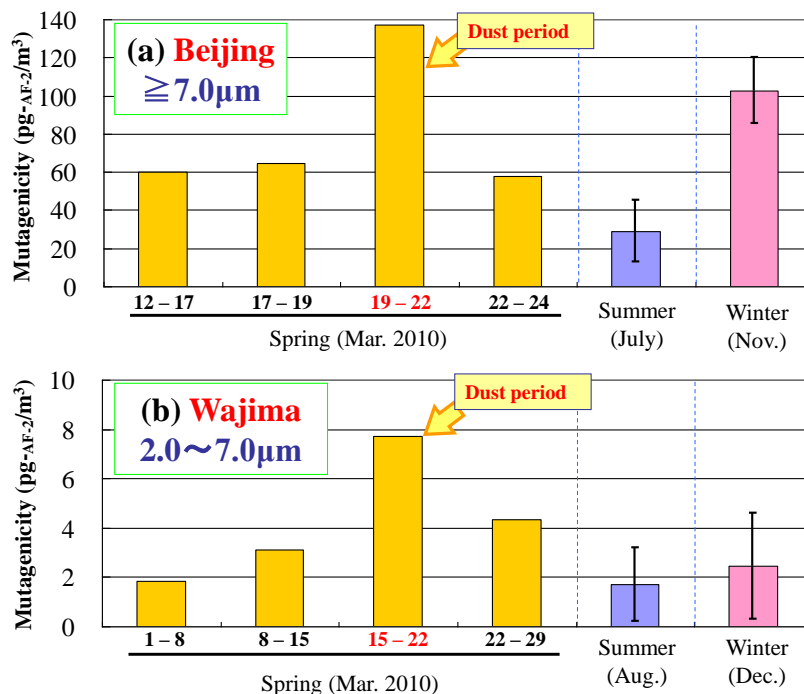
Figure 11. Direct mutagenicity (AF-2 equivalent) of soluble organic fractions of ambient airborne particles collected in Beijing (a) and Wajima (b).

Our results suggest that the other NPAHs formed by the heterogeneous reactions on dust particles contribute more to the direct-acting mutagenicity of the air when dust storms are observed.

Recently, Koike et al. (2014) examined the effects of several PAH derivatives, including 1-NP, on respiratory and immune cells and showed that they induced cytotoxicity and/or inflammatory responses. This indicates that the heterogeneous processes that form NPAHs may significantly affect respiratory health. Asthma can be induced by mineral dust aerosols as they damage airway epithelial cells and trigger pro-inflammatory responses that exacerbate the invasion and recognition of inhaled allergens (Honda et al. 2014). Thus, the catalytic nitration of PAHs on natural minerals needs to be considered as a significant environmental contributor to NPAH formation, as NPAHs are a class of major toxic compounds.

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