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Visualization of Artificially Deposited Submicron-sized Aerosol Particles on the Surfaces of Leaves and Needles in Trees

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ABSTRACT

To understand the effect of aerosols on the growth and physiological conditions of trees in forests, it is important to know the state of aerosols that are deposited on the surface of the leaves or needles. In this study, we developed methods of visualization of submicron-sized aerosols that were artificially deposited from the gas-phase or liquid phase onto tree leaves or needles in trees. Firstly, we used field-emission scanning electron microscopy (FE-SEM) to observe black carbon (BC) particles that were artificially sprayed onto the leaves or needles. The distribution of BC particles deposited on the leaves and needles were distinguished based on the size and morphological features of the particles. The distribution and agglomerates size of BC particles differed between two spraying methods of BC particles employed. Secondly, we tried to visualize gold (Au) particles that were artificially sprayed onto the leaves using energy dispersive X-ray spectrometry (EDX) coupled to FE-SEM. We detected the Au particles based on the characteristic X-ray spectrum, which was secondarily generated from the Au particles. In contrast to the case of BC particles, the Au particles did not form agglomerates and were uniformly distributed on the leaf surfaces. The present results show that our methods provide useful information of adsorption and/or behavior of fine particles at the submicron level on the surface of the leaves.

Key words: Forest tree, Black carbon (BC) particle, Gold (Au) particle, Field-emission scanning electron microscopy (FE-SEM), Energy dispersive X-ray spectrometry (EDX)

1. INTRODUCTION

Rapid industrialization in East Asia has caused increases in the emission amounts of various air pollutants. These air pollutants have transferred long distances from the source, and have caused transboundary air pollution to neighboring countries. One of pollutants is aerosol. The aerosol particles that are deposited on the surface of the leaves of plants may influence not only the surface condition but also the growth and physiological process of leaves (Farmer, 1993). Black carbon (BC) is a kind of the aerosols. BC has mainly been emitted from four sources such as combustion of solid fuels, use of diesel engine, open forest burning, and industrial processes (Bond *et al.,* 2004). East Asia, South America, Western Africa, and South Asia are major source areas of BC (Bond *et al.,* 2004). However, we have still no enough information about the effect of aerosol on the growth of forest tree, in particular, about the effects of submicron-sized particles. For accurate evaluation of the effect of aerosol on the growth and physiological conditions in trees, it is important to understand the mechanism of adsorption of aerosol on the surface of the leaves. Burkhardt *et al.* (1995) observed the deposition of particles on the leaves by scanning electron microscopy (SEM) and fluorescence microscopy. In addition, the particulate matters or dust on the leaf surface were observed with SEM (Neinhuis and Barthlott, 1998; Coe and Lindberg, 1987; Krishnamurthy and Rajachidambaram, 1986; Fortman and Johnson, 1984). However, these studies observed larger size or aggregated particles and did not observe very initial condition of deposited particles at submicron level. Thus, the precise deposition and localization of aerosol particles at submicron level were still unclear.

The main objective of the present study was to establish the visualization method of submicron-sized BC and gold (Au) particles that were artificially deposited onto the surface of the leaves or needles of two tree species. We visualized the BC and Au particles by field-emission scanning electron microscopy (FE-SEM). However, it is necessary to identify the BC particles by its shape and size only because BC can not be detected by energy dispersive X-ray spectrometry (EDX). Therefore, for the establishment of the more secure method to visualize the deposition and localization of aerosol particles at the submicron level on the leaves, we conducted drop treatment and exposure to Au nanoparticles-based suspension, as a model, on the surface of the leaves and analyzed the characteristic X-ray which were secondarily generated from the Au particles by EDX.

2. MATERIALS AND METHODS

2. 1 Plant and Control Materials

We used leaves of two-year-old seedlings of *Castanopsis sieboldii* and needles of one-year-old seedlings of *Cryptomeria japonica* that were growing in campus of Tokyo University of Agriculture and Technology in Fuchu, Tokyo. We also used the polyethylene sheets (Unipack B-4, Seisannipponsha Ltd., Japan) which have low density and 0.04 mm of thickness as control substrates.

2. 2 BC Particles Treatment

Aqueous suspensions of BC derived from carbon nanopowder with 30 nm of primary particle size were prepared. The BC particles that were produced from the suspensions with two types of aerosol generators, based on electrostatic and ultrasonic-forces were deposited on to surface of the leaves and polyethylene sheets. In addition, the leaves without the exposure were used as control materials. In the system of aerosol generator using electrostatic force, we used an electrospray system according to Lenggoro *et al*. (2002), which was equipped with a stainless steel capillary tube of 0.1 mm inner diameter (Naim *et al*., 2010). In the system of aerosol generator using ultrasonic force, an ultrasonic nebulizer operated at 1.7 MHz was used (Wang *et al*., 2008). We employed two methods because we assume that there are both the dry deposition and wet deposition of BC particles on the leaves or needles under natural condition. The size distribution of BC particles can be controlled by size of droplets and concentration of suspensions. The diameter of particles was designed to be approximately 100 nm. Duration of exposure was 2, 5 and 120 minutes for aerosol generator using electrostatic force and 0.5, 1 and 70 minutes for that using ultrasonic force. Distance between the outlet nozzle of aerosol generator and a tested material was 10 cm.

After exposure to BC, the leaves and polyethylene sheets were cut into the square (about 5 mm × 5 mm) and the needles were cut into a length of about 5 mm with steel blades (FA-10 or FHS-5, Feather, Japan). They were air-dried for at least three days in a desiccator (LH1-001-01, AS ONE, Japan). They were mounted onto specimen stubs for FE-SEM and then coated with platinum or platinum-palladium with a sputter coater (JFC1100 or JFC1500, JEOL, Japan) or a vacuum evaporator. The deposition of particles on the leaves and polyethylene sheets was observed under field-emission scanning electron microscope (S-4800, Hitachi, Japan or JSM-6301F, JEOL, Japan) at an accelerating voltage of from 0.5 to 5 kV.

2. 3 Au Particles Treatment

The commercially available suspensions including Au particles (diameter: 50 nm) (EMGC50, BBInternational, UK) were dropped with a Pasteur pipette on the surface of the air-dried leaves. In addition, the fresh leaves were exposed to Au particles which were generated from aerosol generator using electrostatic force. Duration of exposure was 10 minutes and distance between the aerosol generator and the leaves was 8 cm. In addition, the fresh leaves without the exposure were used as control materials. There are some differences of condition of exposure treatment between BC and Au because of slightly different purposes.

After drop treatment or exposure, the leaves were cut into the square (approximately 5 mm × 5 mm) with steel blades. After air-dried for at least three days in a desiccator, they were mounted onto specimen stubs for FE-SEM and then coated with carbon with a carbon coater (TB500, Quorum Technologies, Canada). The deposition of particles on the leaves was observed under field-emission scanning electron microscope (S-4800, Hitachi, Japan) at an accelerating voltage of from 1 kV to 15 kV.

For the detection of Au, the samples were analyzed by EDX (Genesis-XM2, EDAX, US) at an accelerating voltage of 10 kV or 15 kV, working distance of 15 mm, takeoff angle of 30°, and illumination current of about 2 nA.

3. RESULTS AND DISCUSSION

3. 1 BC Particles on the Polyethylene Sheets

Agglomerates of fine particles were deposited on the polyethylene sheets that were sprayed with suspensions of BC (Fig. 1a). The diameter of individual particles

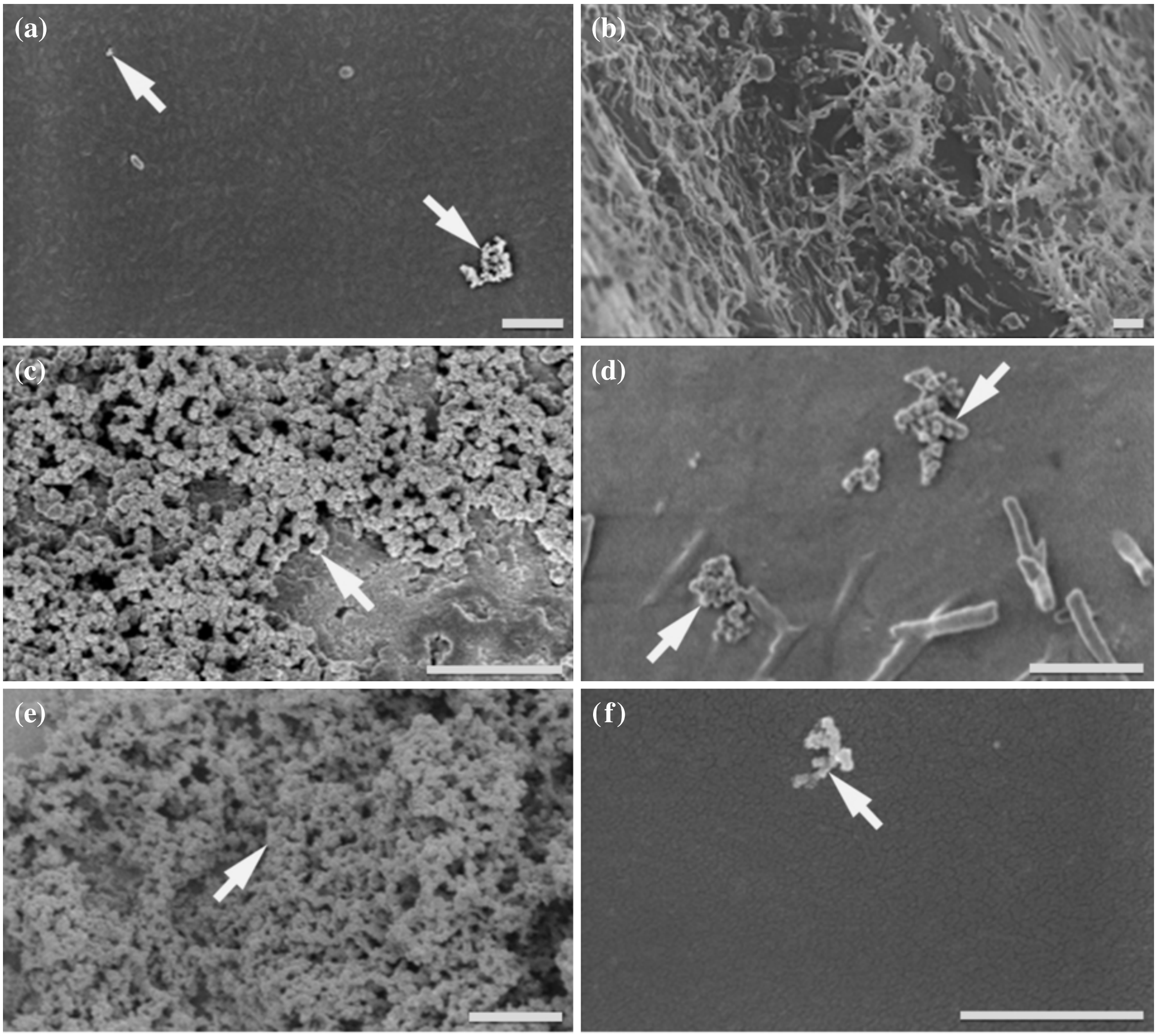

Fig. 1. FE-SEM images of the BC particles on polyethylene sheets (a) and the surface of the needles of *Cryptomeria japonica* (b, c and d) and the surface of the leaves of *Castanopsis sieboldii* (e and f). a: Agglomerates of BC particles that were sprayed by the method of aerosol generator by ultrasonic force. b: Particles with various size and shapes under natural conditions. c and e: Agglomerates of BC particles that were sprayed by the method of aerosol generator by electrostatic force. d and f: Agglomerates of BC particles that were sprayed by the method of aerosol generator by ultrasonic force. Accelerating voltage is at 2.5 kV (a), 3 kV (b), 1 kV (c, d and e), 5 kV (f). Arrows indicate the BC particles. Bars = 1 μm.

was approximately 100 nm. In contrast, there were no similar particles on the surface of fresh polyethylene sheets, which were not exposed to BC. Therefore, we are confident that the particles deposited on BC-treated polyethylene sheets were derived from suspensions of BC, which were artificially sprayed. The shape of deposited BC particles was caused by characteristics of BC because of similar shape and size of BC particles on the leaves or needles. The size and shapes of BC particles deposited on the polyethylene sheets in the present study were similar to those of BC particles that were previously detected by Li *et al.* (2003) in the atmosphere by transmission electron microscopy (TEM).

3.2 BC Particles on the Surface of the Leaves

Particles of various shapes and sizes were found on the surface of the needles of the seedlings of *Cryptomeria japonica* that were not exposed to BC (Fig. 1b). Agglomerates of fine particles of which diameter was approximately 100 nm were deposited on the surface of the needles after exposure to BC particles (Fig. 1c, d, e and f). The size and shapes of these particles were almost similar to those of original BC particles that were artificially sprayed to the surface of polyethylene sheets using aerosol generators (Fig. 1a, c, d, e and f). Therefore, we conclude that the agglomerates of particles on the leaves or needles were derived from the BC particles which were generated from suspensions using aerosol generator.

Distribution and localization of particles, such as size and density (number of agglomerates per unit area) of agglomerates of particles, differed between two exposure methods of BC particles (Fig. 1c, d, e and f). The aerosol generator by electrostatic force produced larger agglomerates of BC particles (Fig. 1c and e). In contrast, the aerosol generator by ultrasonic force produced smaller agglomerates of BC particles (Fig. 1d and f). In addition, the density of agglomerates on the leaves by aerosol generator by electrostatic force was higher than that by ultrasonic force. No significant differences in size and density of agglomerates between species were observed. Naim *et al.* (2010) suggested that different adhesion force induced different concentration of TiO_2 particles at submicron level on the surface of substrate. In this experiment, particles on the leaf surface were in dry condition from aerosol generator and were deposited by electrostatic force. In contrast, particles on the leaf surface were in wet condition from ultrasonic nebulizer and were deposited by difference of hydrophilic property on the leaf surface. Thus, difference in adhesion force between two types of exposure methods might induce such different distribution and localization of particles.

3.3 Au Particles on the Surface of the Leaves

We observed that the Au particles were deposited on the surface of the leaves of the seedlings of *Castanop-*

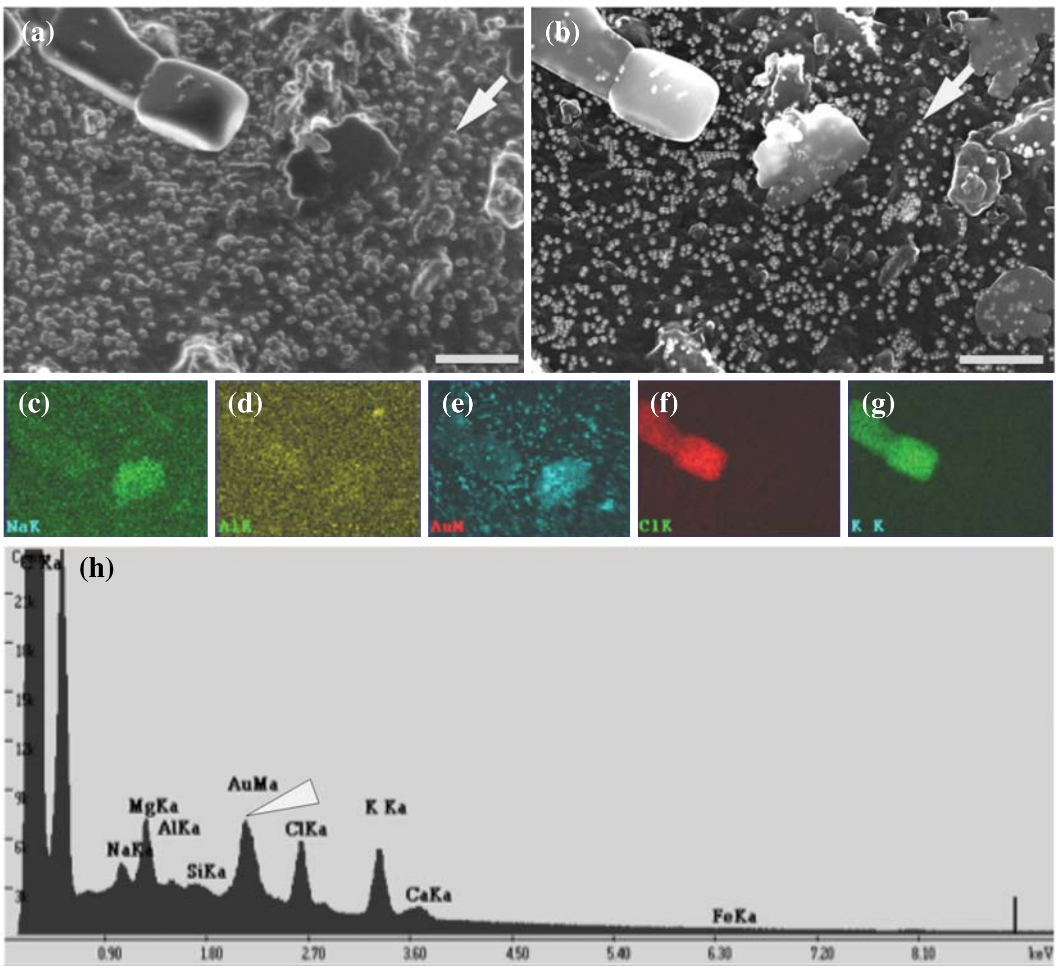

Fig. 2. FE-SEM and EDX images of the surface of the leaves of *Castanopsis sieboldii* after drop treatment of Au particles. a and b: Secondary electron images viewed at accelerating voltages of 1 kV (a) and 15 kV (b). c, d, e, f and g: Elemental mapping images of EDX (c: sodium, d: aluminum, e: gold, f: chlorine, g: potassium). These five figures were taken in the same area of leaf surface, indicating the intensity level of the characteristic X-ray of each of elements. h: EDX spectrum by area analysis at Fig. 2b. Arrows indicate the Au particles. Arrowhead indicates peak of spectrum of Au. Bars = 1 μm.

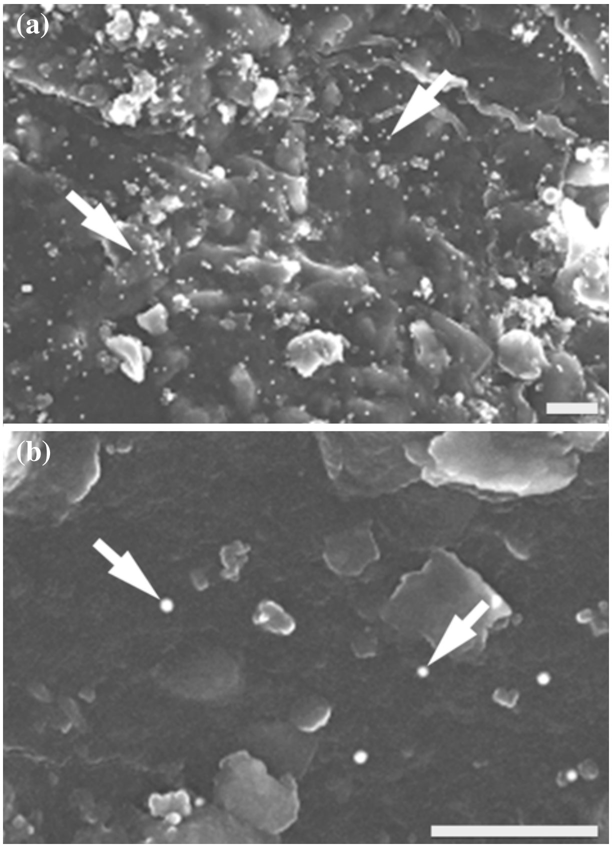

Fig. 3. Secondary electron images of the surface of the leaves of *Castanopsis sieboldii* after exposure to Au particles, taken at 5 kV. a: The Au particles were individually distributed without making agglomerates. b: Higher magnification than Fig. 3a. The Au particles, which were circular (diameter: 50 nm) and white, were deposited on the leaves. Arrows indicate Au particles. Bars = 1 μm.

sis sieboldii (Fig. 2a, b and e). The diameter of individual particles was approximately 50 nm. The EDX spectrum by area analysis showed clear peaks from particles corresponding to the element gold (Au) (Fig. 2h). Elemental mapping images of Au by the characteristic X-ray analysis of EDX corresponded approximately to the images of Au particles under FE-SEM (Fig. 2e). Needless to say, there are originally no or very little (undetectable) Au particles on the surface of the leaves or needles, which are grown under natural condition. Therefore, it is concluded that these particles on the surface of the leaves were derived from the suspensions of Au particles which were artificially conducted by the drop treatment.

Particles in uniform size were individually deposited on the surface of the leaves of the seedlings of *Castanopsis sieboldii* without agglomerates after exposure to Au particles (Fig. 3a and b). These particles were similar to those of Au particles that were artificially conducted by drop treatment on the surface of the leaves. The EDX spectrum by area analysis showed clear peaks from particles corresponding to Au. Therefore, it is concluded that these particles on the surface of the leaves were derived from suspensions of Au particles which were artificially sprayed. FE-SEM and EDX methods with the characteristic X-ray analysis of Au particles provided us the accurate pattern of deposition of Au particles on the surfaces of leaves of seedlings at submicron level.

4. CONCLUSIONS

The leaves or needles in trees were exposed to BC nanoparticles. We visualized the adsorption of BC particles on the leaves or needles at submicron level by FE-SEM. We found agglomerates of BC particles on polyethylene sheets, leaves and needles. The Au particles were individually distributed without making agglomeration on the leaves. Therefore, the pattern of deposition and localization of aerosols on the leaves or needles at submicron level might be different depending on the type of particles and dispersion state of the starting suspension. We conclude that these methods are useful for observation of submicron-sized aerosols on the leaves and needles in the field.

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REFERENCES

Bond, T.C., Streets, D.G., Yarber, K.F., Nelson, S.F., Woo, J.-H., Klimont, Z. (2004) A technology-based global inventory of black and organic carbon emissions from combustion. Journal of Geophysical Research Atmospheres 109, D14203.

Burkhardt, J., Peters, K., Crossley, A. (1995) The presence of structural surface waxes on coniferous needles affects the pattern of dry deposition of fine particles. Journal

of Experimental Botany 46, 823-831.
Coe, J., Lindberg, S. (1987) The Morphology and Size Distribution of Atmospheric Particles Deposited. Japca-the International Journal of Air Pollution Control and Hazardous Waste Management 37, 237-243.
Farmer, A.M. (1993) The effect of dust on vegetation - a review. Environmental Pollution 79, 63-75.
Fortmann, R.C., Johnson, D.L. (1984) Effect of simulated rainfall on removal and In situ formation of particles on Taxus needles. Environmental Pollution Series B, Chemical and Physical 7, 297-316.
Krishnamurthy, K., Rajachidambaram, C. (1986) Factors associated with reduction in photosynthetic oxygen evolution in cement dust coated leaves. Photosynthetica 20, 164-168.
Lenggoro, I.W., Xia, B., Okuyama, K. (2002) Sizing of colloidal nanoparticles by electrospray and differential mobility analyzer methods. Langmuir 18, 4584-4591.
Li, J., Anderson, J.R., Buseck, P.R. (2003) TEM study of aerosol particles from clean and polluted marine boundary layers over the North Atlantic. Journal of Geophysical Research 108, ACH8.
Naim, M.N., Abu Bakar, N.F., Iijima, M., Kamiya, H., Lenggoro, I.W. (2010) Electrostatic deposition of aerosol particles generated from an aqueous nanopowder suspension on a chemically treated substrate. Japanese Journal of Applied Physics 49, 06GH17.
Neinhuis, C., Barthlott, W. (1998) Seasonal changes of leaf surface contamination in beech, oak, and ginkgo in relation to leaf micromorphology and wettability. New Phytologist 138, 91-98.
Wang, W.N., Purwanto, A., Lenggoro, I.W., Okuyama, K., Chang, H., Jang, H.D. (2008) Investigation on the correlations between droplet and particle size distribution in ultrasonic spray pyrolysis. Industrial & Engineering Chemistry Research 47, 1650-1659.

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