

Application of the personal aeroallergen sampler to assess personal exposures to Japanese cedar and cypress pollens

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We have recently developed the Personal Aeroallergen Sampler (PAAS), a passive sampler for aeroallergens. In the present study, the applicability of the PAAS for personal exposure assessments of cedar and cypress pollens was investigated by comparing with existing reference samplers. To investigate the usability of the PAAS as a personal sampler for the airborne pollens, it was compared with the Institute of Occupational Medicine (IOM) sampler, a traditionally used active personal sampler. Overall, the result showed a good correlation between the two methods, that is, $R^2 = 0.8082$, suggesting the usability of the PAAS for the personal pollen samplings. The ratio of the pollen numbers collected by the PAAS to the IOM sampler was approximately 30%, which was consistent with our previous study investigating ambient dust particles. Meanwhile, the comparability of the PAAS to the Durham sampler, the most widely used stationary pollen trap, was also assured. Furthermore, we exemplified the seasonal peak of the personal pollen exposures was not necessarily reflected by the outdoor concentrations, indicating insufficiency of the stationary outdoor monitoring to represent the personal pollen exposures. The PAAS, a simple passive method, could be used in future field studies to elucidate the detailed mechanisms of allergic airway diseases such as cedar pollinosis.

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Introduction

In Japan, cedar pollen is one of major aeroallergens. For instance, exposure to airborne cedar pollens induces cedar pollinosis, similar allergic rhinitis and conjunctivitis symptoms, in allergic individuals (Ishizaki et al., 1987; Kitamura et al., 2005). At present, approximately 13 million people are thought to suffer from cedar pollinosis and the annual economic losses are estimated to be 286 billion yen. Meanwhile, the detailed mechanisms of cedar pollinosis are not fully understood partly because various environmental factors including air pollutants such as diesel exhaust particles (Muranaka et al., 1986), parasite infections (Kobayashi et al., 1999) and so on are suspected to intricately affect the disease mechanisms. Therefore, the interactions among the exposures to cedar pollens and those to the adjuvant environmental factors have to be better characterized to clarify the details of the disease mechanisms. To reveal the

abovementioned complex interactions, systematic assessments of personal exposures to airborne cedar pollens in conjunction with those to the adjuvant environmental factors are essential.

To date, various types of the pollen samplers have been developed to measure airborne pollens (Mullins and Emberlin, 1997). For instance, the Durham sampler (Durham, 1946), the IS Rotary pollen trap, the Burkard sampler based on the Hirst method (Hirst, 1952) and the real-time monitor using autofluorescence characteristics (Suzuki et al., 2005) have been developed and used to measure airborne cedar pollens. Among them, the Durham sampler is the most widely used in Japan. In this sampler, the pollens are collected on a glycerinated glass slide horizontally loaded between two parallel discs. As the cedar pollens are relatively large in size, that is, approximately 30 μm in diameter (Ministry of the Environment, 2006), they are collected on the slide by gravitational deposition. Meanwhile, the IS Rotary pollen trap is similar to the Durham sampler but the glass slide is inclined at an angle of 45° relative to the parallel discs to continuously face windward by means of a vertical tail fin equipped underneath the mount of the trap. The underlying structure of the IS Rotary pollen trap can increase the collection efficiency approximately five times compared to that of the Durham sampler. Thus, various types of the

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pollen samplers have been attempted to measure airborne cedar pollens in Japan. Nevertheless, these devices cannot be used as personal samplers as these are relatively large in size and designed as stationary samplers.

To accurately measure the personal exposures to aeroallergens including airborne cedar pollens in the vicinity of the human subject, we have recently developed the Personal Aeroallergen Sampler (PAAS), a passive sampler for airborne coarse particles (Yamamoto et al., 2006) (Figure 1). As shown in Figure 2, the human subject should wear the PAAS around his or her neck to collect aeroallergens adjacent to the breathing area. Briefly, the PAAS consists of two planetary rings in different sizes in which a substrate holder can rotate in all directions (Figure 1). The underlying structure resembling a gimbal enables the particle collection surface continuously directed upward regardless of inclination of the sampler. As the cedar pollens are relatively large in size, their behaviors in air are dominated by gravity. Consequently, the pollens are collected on the horizontally maintained collection substrate by gravitational deposition. Although the active samplers are occasionally impractical for the personal air samplings owing to noise, weight, dimension, limited lifetime of battery and so on, the PAAS is convenient as it is quiet, light and easy to handle.

In our previous study (Yamamoto et al., 2006), the particle size distributions collected by the PAAS were compared with those by a reference active sampler, that is, the Institute of Occupational Medicine (IOM) personal sampler for which the particle size-selective collection performance was extensively investigated (e.g., Mark and Vincent, 1986). By comparing the results obtained by these two methods, the particle size-dependent collection performance of the newly developed passive sampler, that is, the PAAS, could be characterized. In our former study, we obtained good correlations between the two methods by measuring ambient dust particles and hence suggested the usability of the PAAS. Nevertheless, we have not tested the applicability for actual aeroallergens yet. Therefore, the goal of the present study is to ensure the applicability of the PAAS for the purpose of personal exposure assessments of actual aeroallergens. In particular, cedar and cypress pollens were selected as target aeroallergens because of abovementioned importance. Furthermore, to assure the comparability of the PAAS to the most commonly used pollen sampler in Japan, that is, the Durham sampler, the numbers of the pollens collected outdoors by the PAAS were compared with those routinely measured by the Durham sampler of the Tokyo Metropolitan Government (TMG).

Methods

Description of the PAAS

The PAAS made of stainless steel consists of two planetary rings in different sizes in which a substrate holder can rotate in all directions (Figure 1). The underlying structure

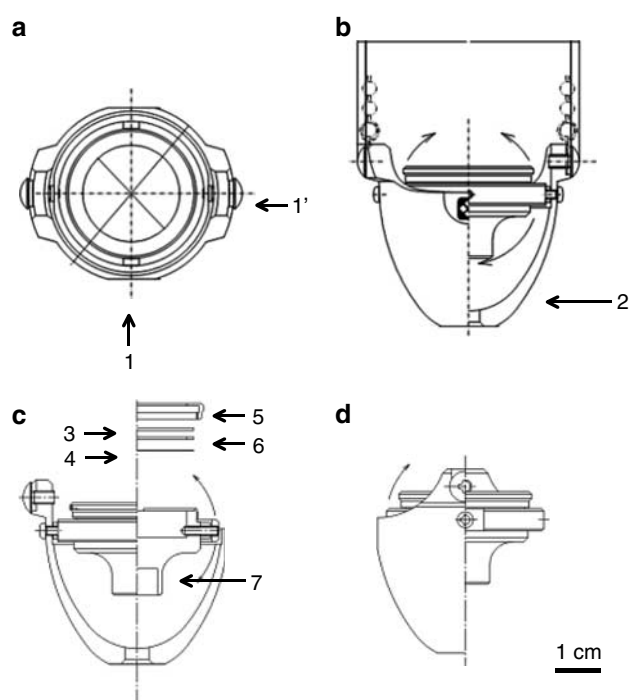


Figure 1. Schematic diagrams of the PAAS: (a) overhead view, (b) front view, (c) inside view and (d) side view. 1 and 1' — axes of the gimbal; 2 — protective outer shell; 3 — protective stainless mesh; 4 — collection substrate; 5 — substrate holder cap; 6 — Teflon ring; 7 — substrate holder body.



Figure 2. PAAS in use.

resembling a gimbal enables the particle collection surface continuously directed upward regardless of inclination of the sampler. Consequently, airborne cedar pollens can be collected on the horizontally maintained collection surface. As the PAAS is light and small, it is convenient for the personal samplings. The weight and dimension of the PAAS

are approximately 50 g and 50 mm (W) × 40 mm (D) × 40 mm (H), respectively. The mixed cellulose ester (MCE) membrane filters (25 mm diameter, 1.0 μ m pore size; Toyo Roshi Kaisha, Ltd, Tokyo, Japan) were used as collection substrates and the filters were immersed in mineral oil light white (MP Biomedicals, LLC, OH, USA) before loading on the substrate holder to prevent particle reentrainment from the filter substrates.

Sampling Procedures

To evaluate the collection characteristics of the pollens by the PAAS, an active sampler was collocated. The IOM sampler (SKC Inc., PA, USA) connected to a portable pump (MP-Ö500; Sibata Scientific Technology Ltd, Tokyo, Japan) was used as a reference active sampler. The MCE filter was loaded in a filter holder of the IOM sampler. A sampling flow rate was adjusted at 21 min⁻¹ as instructed by the manufacturer. The IOM sampler was selected as a reference because it is the most commonly used personal inhalable sampler that conforms well to the inhalable particulate matter sampling criterion given by the American Conference of Governmental Industrial Hygienists (ACGIH, 2003).

Although the human subject should wear the PAAS around his or her neck (Figure 2), both the IOM sampler and the PAAS were attached to a handbag in this study to reduce burden of the subject to carry the pump of the IOM sampler. It should be stated the PAAS performance were likely to be affected by location of the sampler attached. Meanwhile, as a major objective of this study is to characterize the sampling performance of the PAAS relative to that of the IOM sampler, the performance evaluation of the PAAS is possible as long as the two samplers are collocated in the vicinity. The direct comparison of the PAAS with the IOM sampler can provide the absolute PAAS performance as the sampling performances of the IOM sampler have been well characterized by numerous studies (e.g., Mark and Vincent, 1986).

In this study, 28 personal and 22 stationary outdoor samplings for duration of 2 days were undertaken from February to April 2006. Specifically, the outdoor stationary samplings were performed outside the sixth floor room of the building in Bunkyo-ku located at the center of Tokyo from February to March 2006. In April 2006, the outdoor samplings were taken outside the fourth floor room of the building in Isehara-shi in Kanagawa Prefecture located in the suburban part of the Tokyo metropolitan area. The personal samplings were carried out during commuting in trains, staying outdoors and in the offices or houses and so on in the Tokyo metropolitan area. The human subject kept the time activity logs throughout the personal samplings. All samplings were taken from 0900 to 2100 of the day after next.

Microscopic Method

Glycerol (120 ml) (Assay (GC), 99.0%; Wako Pure Chemical Industries, Ltd, Osaka, Japan), 20 g gelatin (Wako Pure

Chemical Industries, Ltd, Osaka, Japan), 1.6 mg gentian violet R (Wako Pure Chemical Industries, Ltd, Osaka, Japan) dissolved in 4 ml ethanol (99.5%; Wako Pure Chemical Industries, Ltd, Osaka, Japan), 1 ml phenol standard solution (1 mg ml⁻¹ water solution), 70 ml ultra-pure water (Milli-Q Gradient A10 System; Millipore Corp., MA, USA) were mixed and stirred overnight to prepare gentian violet glycerin jelly. The jelly was refrigerated until use. The sampled filter was loaded onto a micro slide glass (1-mm grid interval; Matsunami Glass Industry, Ltd, Osaka, Japan) with mineral oil to make the MCE filter transparent. Hereafter, a cover glass (24 mm × 24 mm, 0.12 mm to 0.17 mm thickness; Matsunami Glass Industry, Ltd, Osaka, Japan) on which gentian violet glycerin jelly was melted by heat was placed on the slide glass. An optical microscope at ×100 magnification (×10 for both objective and ocular lenses; Eclipse E200; Nikon Corp., Tokyo, Japan) was used to quantitate the pollens on effective collection areas of the substrates of the PAAS and the IOM sampler, that is, the areas having diameters of 22 mm (= 380 mm²) and 19 mm (= 280 mm²), respectively. In this study, cedar and cypress pollens, which are both allergenic and crossreactive, were collectively counted as the major objective of this research is to investigate the collection performance of airborne allergenic pollens in general. Moreover, the combined counts of cedar and cypress pollens can increase the statistical power to characterize the performance of the sampler.

Pollen Data of the Tokyo Metropolitan Government

The numbers of the pollens collected outdoors by the PAAS were compared with those routinely measured by the Durham sampler of the Tokyo Metropolitan Government (TMG, 2006). In this study, the results obtained by the TMG monitoring station located in Kita-ku were compared with those measured outdoors by the PAAS installed at our sampling site in Bunkyo-ku. The TMG monitoring station in Kita-ku is located approximately 9 km north-northwest of our sampling site. At the TMG monitoring stations, the pollens were collected for 24 h from 0900 to 2100 of the following day. According to the TMG, the pollens collected by the Durham sampler were observed by an optical microscope within a square area of 18 mm × 18 mm (= 320 mm²) of the slide. As the TMG data were available on a 24-h basis whereas our samplings were taken for 2 days, the 2-day averages were calculated for the TMG data to enable comparison with our sampling results by the PAAS outdoors.

Results and discussion

An example of microscopic images of the pollens collected on the substrates of the IOM sampler and the PAAS is shown in Figure 3. Although the PAAS could selectively

collect coarse particles including cedar and cypress pollens, the IOM sampler could also collect finer dust particles, making visual investigation of the pollens difficult. As the PAAS could minimize collection of interfering finer dust particles onto the substrates, it made visual counting of the pollens efficient. This may be a part of advantages of the present passive sampling method.

In Figure 4, the numbers of the pollens collected by the IOM sampler and the PAAS are plotted for the personal and outdoor samplings. Furthermore, the pollen concentrations in air are indicated by the secondary x axis in Figure 4. To obtain the pollen concentrations in air, the numbers of the pollens collected by the IOM sampler were divided by the collected air volume, that is, 5.76 m^3 for 2 days, and the collection efficiency of the IOM sampler given by the following inhalable particulate matter sampling criterion (ACGIH, 2003):

$$\text{IF}(d_a) = 0.5(1 + \exp(-0.06d_a)), \quad (1)$$

where d_a is the aerodynamic diameter of the pollen in μm . We tentatively assumed $d_a = 33 \mu\text{m}$ for the cedar pollens and obtained $\text{IF}(d_a) = 57\%$. The present assumption of the aerodynamic diameter of the cedar pollens will be explained later. Overall, the results showed good correlations between the two methods, that is, the square of the correlation, R^2 , of 0.8082 for the personal samplings, suggesting the usability of the PAAS for the purpose of personal exposure assessment of airborne cedar and cypress pollens.

The ratios of the pollen numbers collected by the PAAS to the IOM sampler were almost equal for the personal and outdoor samplings, that is, approximately 30%. Meanwhile, our previous study investigating the collection efficiencies of ambient dust particles (Yamamoto et al., 2006) indicated the higher efficiencies in the outdoor samplings than in the personal samplings probably due to higher atmospheric turbulence outdoors. Although future research is necessary to elucidate the underlying mechanisms of this discrepancy, the observed coincidence in the collection efficiencies was likely due to higher occurrence of the personal pollen exposures in the open atmospheres than in the rooms. As the cedar and cypress pollens are exclusively of outdoor origins, the pollens were likely collected outdoors in the majority of instances. Consequently, the collection efficiencies of the pollens by the personal samplings are likely to conform to those characterized by the outdoor samplings.

In our previous study (Yamamoto et al., 2006), the ratios of the particle numbers collected outdoors by the PAAS to the IOM sampler were 6.6%, 15%, 42% and 116% for those with projected area diameters, d_{PA} , of 10 to $20 \mu\text{m}$, 20 to $30 \mu\text{m}$, 30 to $50 \mu\text{m}$ and 50 to $100 \mu\text{m}$, respectively. In terms of aerodynamic diameter, d_a , these ranges correspond to 7.2 to $14 \mu\text{m}$, 14 to $22 \mu\text{m}$, 22 to $36 \mu\text{m}$ and 36 to $72 \mu\text{m}$, respectively, by assuming the empirical d_{PA}/d_a ratio of 1.39 for ambient dust particles (Noll et al., 1988; Noll and Fang,

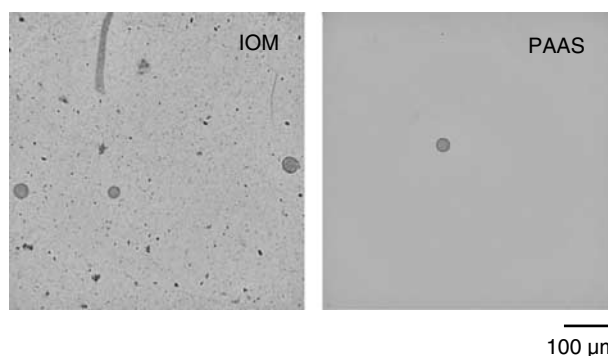


Figure 3. Microscopic images of the pollens collected on the substrates of the IOM sampler and the PAAS.

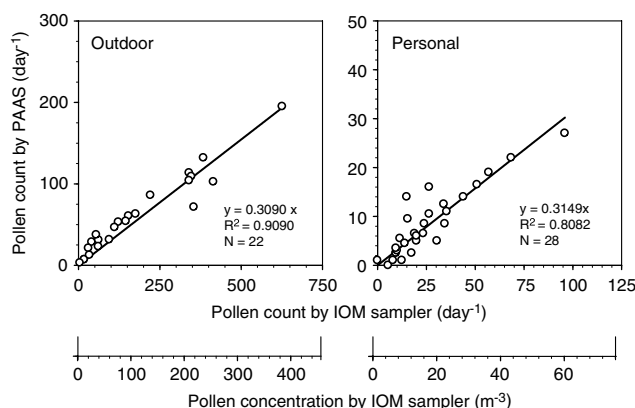


Figure 4. Numbers of the pollens collected by the IOM sampler and the PAAS by the personal and outdoor samplings. The pollen concentrations obtained by the IOM sampler are shown by the secondary x axis.

1989). Meanwhile, the aerodynamic diameters of the pollens were calculated by the following equation (Hinds, 1999):

$$d_a = d_{PA} \left(\rho_p / \rho_0 \right)^{1/2}, \quad (2)$$

where ρ_p is the effective pollen density and ρ_0 is the unit density ($= 1.0 \text{ g cm}^{-3}$). As the effective densities of the fresh pollens are generally in the range of 1.1 to 1.3 g cm^{-3} (Harrington and Metzger, 1963; Aylor, 2002; van Hout and Katz, 2004), we tentatively assigned the value of $\rho_p = 1.2 \text{ g cm}^{-3}$. Therefore, provided the projected area diameters of the cedar and cypress pollens are 30 and $20\text{--}30 \mu\text{m}$, respectively (Ministry of the Environment, 2006), their aerodynamic diameters correspond to 33 and $22\text{--}33 \mu\text{m}$, respectively, by assuming spherical shapes. As described above, the collection efficiency of ambient dust particles whose aerodynamic diameters were similar to those of the pollens, that is, $d_a = 22\text{--}36 \mu\text{m}$ ($d_{PA} = 20\text{--}30 \mu\text{m}$), was 42%. Thus, the collection efficiency of the pollens obtained by

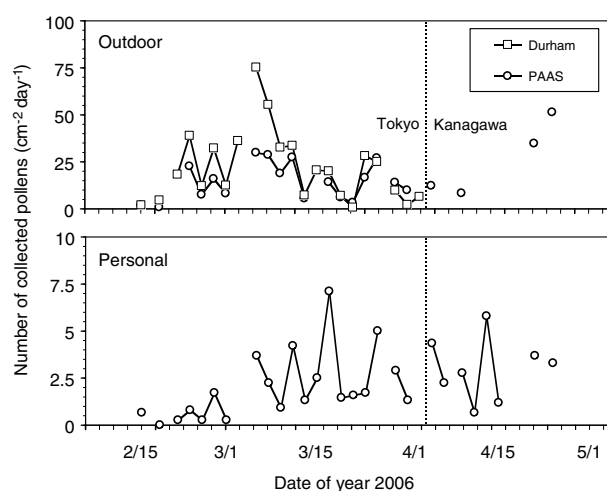


Figure 5. Numbers of the pollens collected by the Durham sampler at the TMG monitoring station and the PAAS.

this study, that is, 30%, was reasonably consistent with our former result obtained for ambient dust particles, that is, 42%.

Figure 5 exhibits the temporal trends of the numbers of the cedar and cypress pollens collected by the Durham sampler at the TMG monitoring station and by the personal and outdoor samplings by the PAAS. The numbers of the pollens collected by the Durham sampler were larger than those by the PAAS. The mean and maximum pollen counts obtained by the Durham sampler were 21.9 cm^{-2} and $75.3 \text{ cm}^{-2} \text{ day}^{-1}$, respectively, whereas those by the PAAS outdoors were 16.6 cm^{-2} and $51.3 \text{ cm}^{-2} \text{ day}^{-1}$, respectively. Meanwhile, the mean and maximum pollen counts obtained by the personal samplings by the PAAS were 2.3 cm^{-2} and $7.1 \text{ cm}^{-2} \text{ day}^{-1}$, respectively, which were considerably lower than the values obtained by the outdoor samplings. The personal concentrations became the maximum on 18 March 2006 whereas the outdoor concentrations reached the highest on 6 March 2006, excluding the data taken in Kanagawa. We speculate the observed time lag was due to invasion of the pollens into indoor environments with the course of the pollen season. As the airborne pollens of outdoor origins are expected to gradually invade and accumulate in the indoor environments as the pollen season advances for instance by air exchange between indoors and outdoors and/or carrying the pollens attached to the clothes to the indoor settings, we expect the observed time lag was due to increased pollen exposures indoors in the later period of the pollen season. The future research should further confirm the reasons of this phenomenon.

As demonstrated in Figure 5, the pollen counts obtained by the personal samplings by the PAAS were considerably lower than those measured by the outdoor samplings. Based on the present results, the ratio of the pollen numbers collected by the personal to the outdoor samplings by the

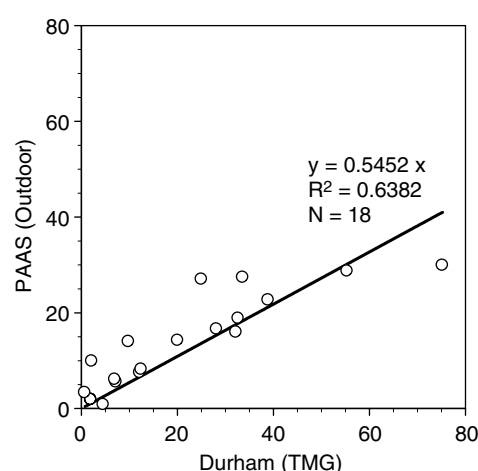


Figure 6. Numbers of the pollens ($\text{cm}^{-2} \text{ day}^{-1}$) collected by the Durham sampler at the TMG monitoring station and the PAAS outdoors.

PAAS was approximately 15%. As the personal samplings were taken outdoors in only a small portion of the time based on time activity logs, that is, approximately 9% including the time spent for commuting, the observed ratio was fairly consistent from the aspect of the time fractions spent indoors and outdoors. Nevertheless, it should be stated the ratio of the pollen counts collected by the personal to the outdoor samplings, that is, 15%, was slightly higher than the time fraction spent outdoors to the overall personal exposure sampling, that is, 9%. The number ratio of the pollens collected by the personal to the outdoor samplings should agree with the time fraction spent outdoors as the pollens are generally of outdoor origins. Nevertheless, the personal pollen exposures are likely increased indoors in the later period of the pollen season as explained previously. This might contribute to the excess of the ratio of the pollens collected by the personal to the outdoor samplings compared to the time fraction spent outdoors.

Thus, we indicated the personal pollen exposures were not necessarily reflected by the outdoor concentrations, highlighting the advantage of the PAAS over the stationary outdoor samplings for the personal exposure monitoring of aeroallergens having seasonal variation in atmospheric concentrations in particular.

Figure 6 demonstrates the relationship between the pollen numbers collected outdoors by the PAAS and the Durham sampler. The figure shows a good correlation between the two methods, that is, $R^2 = 0.6382$, even though these two samplers were located approximately 9 km far from each other. The numbers of the pollens collected by the PAAS were smaller than those by the Durham sampler, that is, approximately 55%, even though the particle collection mechanism is basically similar between the two methods, that is, gravitational settling. The smaller numbers by the PAAS are expected owing to the effects of the sampler's geometry.

Although the Durham sampler collects the pollens on an open-faced glass slide, the PAAS collects them on the substrate loaded in the hollow of the substrate holder, covered by a protective mesh and tightened by a stainless filter holder cap (Figure 1c). The underlying geometry of the PAAS might shade the particle deposition flux to the collection substrate, decreasing the particle collection efficiency compared to that by the Durham sampler.

Indeed, our former study (Yamamoto et al., 2006) indicated the numbers of ambient dust particles collected by the PAAS were smaller than the theoretical numbers calculated by the dry deposition model based on a smooth surrogate surface with a sharp leading edge (Kim et al., 2000). The particle collection efficiencies by the PAAS relative to the efficiencies by the dry deposition model were 57%, 39%, 32% and 27% for the particles with diameters of $d_a = 7.2\text{--}14$, $14\text{--}22$, $22\text{--}36$ and $36\text{--}72\text{ }\mu\text{m}$, respectively. Therefore, the cedar and cypress pollens known to have aerodynamic diameters of $33\text{ }\mu\text{m}$ and $22\text{--}33\text{ }\mu\text{m}$ calculated by Eq. (2), respectively, are expected to be collected at an efficiency of around 32%. Meanwhile, the abovementioned ratio of the pollen numbers collected by the PAAS to the Durham sampler was 55%, which was larger than the expected efficiency by our former result, that is, 32%. A possible cause of this discrepancy is the lower collection efficiencies by the Durham sampler than by the dry deposition model. Although the dry deposition model assumes the smooth surrogate surface with a sharp leading edge facing open atmosphere, the glass slide substrate is loaded between the two parallel discs in the Durham sampler. Consequently, the collection efficiencies by the Durham sampler are likely lower than those by the dry deposition model because of the samplers' geometries. In summary, it is expected that the collection efficiencies were the highest in the dry deposition model, higher in the Durham sampler and the lowest in the PAAS because of the difference of the samplers' geometries.

Although the present study demonstrated the applicability of the PAAS for the purpose of personal exposure assessments of airborne cedar and cypress pollens, the sampler can be used for other allergenic pollens, too. For instance, while ragweed pollen is one of the most serious allergens in the United States (Gergen et al., 1987), the pollens from many anemophilous plants such as oak, birch, corn, alder, pine and so on are also known aeroallergens. To assess personal exposures to these allergenic pollens, the methodology introduced in this study is directly applicable. For instance, as the diameters of ragweed pollens are generally around $20\text{ }\mu\text{m}$ (Harrington and Metzger, 1963), that is, $22\text{ }\mu\text{m}$ in aerodynamic diameter by Eq. (2), the collection efficiency, that is, the ratio of the pollen numbers collected by the PAAS to the IOM sampler, is expected in the range of 15% to 42%, which is comparable to those observed for cedar and cypress pollens by this study. Thus,

the PAAS is likely suitable for personal exposure assessments of other allergenic pollens, too.

As described above, the PAAS is light and small, that is, approximately 50 g in weight and $50\text{ mm (W)} \times 40\text{ mm (D)} \times 40\text{ mm (H)}$ in dimension. Therefore, the sampler can be used for child subjects although the existing active samplers are virtually impractical. As the allergen exposures in childhood are believed to be important for initial sensitization of the immune system and subsequent progression to allergic diseases (e.g., Holt et al., 1999; Platts-Mills et al., 2000; Sporik and Platts-Mills, 2001; Halken, 2003), accurate assessments of children's exposures to aeroallergens are essential to elucidate the mechanisms of the disease expressions in early life. Indeed, Ozasa et al. (2002) investigated the relationship between the airborne pollen concentrations and the prevalence of allergic pollinosis among schoolchildren by the ecological study. Besides such ecological studies, the PAAS should be used in the personal exposure assessment surveys involving child participants. The detailed information on early life exposures to airborne cedar pollens might provide the additional knowledge on the mechanisms of allergic cedar pollinosis.

Conclusions

In the present study, the applicability of the PAAS for the purpose of personal exposure assessments of cedar and cypress pollens was investigated by comparing with existing reference samplers. To investigate the usability of the PAAS for the purpose of the personal exposure samplings of cedar and cypress pollens, the PAAS was compared with the IOM sampler, a traditionally used personal sampler for airborne inhalable particles. Overall, the result showed a good correlation between the two methods for the personal samplings, that is, $R^2 = 0.8082$, suggesting the usability of the PAAS for the personal exposure assessment of airborne cedar and cypress pollens. The ratio of the pollen numbers collected by the PAAS to the IOM sampler was approximately 30%, which was quite consistent with our previous study investigating ambient dust particles. Meanwhile, to ensure the comparability of the PAAS to the most commonly used pollen sampler in Japan, the Durham sampler was used for comparison. The ratio of the pollen numbers collected by the PAAS to the Durham sampler was approximately 55%. The smaller numbers of the pollens collected by the PAAS than by the Durham sampler are likely due to the difference of the samplers' geometries. Furthermore, we indicated the seasonal peak of the personal pollen exposures was not necessarily reflected by the outdoor concentrations, indicating insufficiency of the stationary outdoor monitoring. Because of its simplicity of handling, the PAAS could be used in future exposure assessment studies to elucidate the detailed mechanisms of allergic airway diseases such as cedar pollinosis.

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