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# Side-by-side comparison of automatic pollen counters for use in pollen information systems

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**Background:** Recent effort to build an unmanned pollen monitoring network in Japan has led to new developments in automatic pollen counters. In-the-field performance tests of these automatic counters have not been reported.

**Objective:** To characterize recently developed automatic pollen counters, with a view of using their data in pollen information systems.

**Methods:** We performed side-by-side comparisons between 2 recently developed automatic pollen counters and 2 reference samplers at 2 sites during the 2005 pollen season.

**Results:** Both automatic counters were found to have similar overall performance in terms of their correlations with the reference samplers. The linear correlation coefficient between the hourly values of the counters and one of the reference samplers was larger than 0.8 at both sites for both counters. Although these results are encouraging, our analysis also points to weaknesses of the investigated automatic counters in the areas of pollen discrimination, minimum measurable concentration, and calibration. Both counters were found to be affected by large concentrations of particulate matter, although the conditions and extent to which the particulate matter disrupted the measurements differ for the 2 sensors. The effect of particulate matter is particularly noticeable at the start and end of the pollen season, that is, when pollen concentration is low relative to particulate matter concentration. Further, it was found that one of the automatic counters could not differentiate snow particles from pollen grains.

**Conclusions:** The tested automatic pollen counters had good overall performances, but weaknesses in the areas of pollen discrimination, minimum measurable concentration, and calibration still have to be addressed for these counters to find widespread use in the allergy community.

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## INTRODUCTION

Monitoring of airborne pollen has recently attracted much attention because of its potential contribution to both allergen avoidance measures by providing individuals with allergy with pollen alerts<sup>1</sup> and the evaluation of cross-pollination between genetically modified crops and their wild relatives.<sup>2,3</sup> Automatic pollen monitoring has only been introduced recently in Japan in an attempt to provide the general public with alerts for Japanese cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*) pollen. The cedar and cypress tree species are major sources of airborne pollen, carrying potent allergens that have been reported to be the main cause of pollinosis in Japan.<sup>4</sup> Today, it is estimated that more than 1 in 10 Japanese citizens has pollinosis. Further, the cedar and cypress pollen share a common antigen (70% of Japanese patients with cedar pollen allergy also developed cypress pollinosis) and have their season shifted in time but overlapping so that the pollen season of the combined cedar and cypress pollen is unusually long. The cedar pollen season

starts in February and ends in the beginning of April, whereas the cypress pollen season starts in March and can last until the beginning of May, thus making a pollen season of approximately 12 weeks, twice as long as the typical 6-week ragweed pollen season. Thus, cedar and cypress allergy patients are exposed to pollen for a long time, making the development of a pollen alert system desirable. Current pollen alerts are generated by information systems that use the data collected by a network of automatic pollen counters as one of their model inputs to compute pollen forecasts.<sup>5–7</sup> Besides the application in allergy prevention, the spread of transgenes through pollen of genetically modified crops needs to be monitored to evaluate the impact of genetically modified crops, which could lead to disruption of natural habitats.<sup>2,3</sup> The environment evaluation of genetically modified crops requires detailed data on pollen dispersal that can only be collected with an automatic network of pollen counters.

Automatic pollen counters that are widely used in Japan include the KH3000 (Yamato, Yokosuka, Japan),<sup>8</sup> Kowa (Hamamatsu, Japan),<sup>9</sup> and NTT (Tokyo, Japan)<sup>10</sup> counters. Recently, a new pollen counter developed by Shinyei Corporation (Kobe, Japan) was introduced on the market and has triggered much interest because of its new pollen discrimination principle and its low cost. The design of the new automatic counter is based on the design of the standard particle counter in which a defined volume of air is circulated through a fine pipe that is intersected by a laser beam. When

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a particle passes through the laser beam, a scattered signal is detected, the intensity of which is related to particle size and optical index. The measured intensity of the scattered light can be related to particle size. In addition to the scattered intensity, the Shinyei counter includes a measure of the change in the polarization state of scattered light, which is known to be related to the particle shape and its internal structure. Pollen grains generate intensity and polarization signals that are different from those of nonpollen particles, so that pollen can be recognized from these 2 measures. The application of this pollen recognition principle may lead to some errors, because it was recognized that under certain circumstances (types of particulate matter) some overlapping between the scattered intensity and polarization values of pollen and particulate matter takes place. The KH3000 counter uses the spherical shape of pollen grains to discriminate them from other particles (the nearly perfect spherical shape of pollen is not found in particulate matter that lacks a biological origin such as sand and soot). To discriminate spherical particles from others, 2 laser beams and their respective detectors are used to measure the intensity of the scattered light from the same particle but measured at 2 different incident angles. If the particle is spherical and homogeneous, the 2 intensities will be the same. The existing automatic counters together with their main characteristics are listed in Table 1. Extensive comparisons against reference samplers, such as the gravimetric Durham sampler and the volumetric Burkard sampler (Burkard Manufacturing Co, Rickmansworth, England), remain scarce, resulting in poor characterization of actual counter performance in the field and, therefore, in ambiguity as to how the data should be used in a pollen information system. The new counter introduced by Shinyei and the lack of data on actual performance of the available counters prompted us to conduct our own side-by-side comparison.

MATERIALS AND METHODS

During the cedar and hinoki pollen season of 2005, we conducted extensive side-by-side comparisons between the KH3000 and Shinyei automatic pollen counters and the Durham and Burkard reference pollen samplers. Our measurement campaign was performed at 2 different sites, namely, on the roof of a 4-story building in the Chiba University Campus (140.133° east and 35.602° north) and on a 5-story building of the Akita Health Institute (140.898° east and 39.717° north). The measured items together with the measurement periods are given in Table 2. Although the

Burkard samplers used at both sites were of a different type, they were of a similar design except for the vacuum source, which uses a fan in the old version (7-day spore trap) and a mechanical pump in the new version (SporeWatch trap). The pollen counts on the Melinex tape used in the Burkard samplers and on glass slides used in the Durham samplers were performed under a microscope at a magnification of ×400 by trained staff. Both cedar and hinoki pollen grains were counted, because the automatic counters do not distinguish these pollen species, which are similar in size and shape. Durham daily counts were only available at the Chiba site and were determined by observing an area of 1 cm<sup>2</sup> (in this case, cedar and hinoki were counted separately). For the Burkard counts, the observed areas of the Chiba data and the Akita data were 0.5 × 5 mm<sup>2</sup> and 2 × 14 mm<sup>2</sup>, respectively. The KH3000 counters were operated with a sand gravimetric trap, which is thought to filter out yellow sand particles originating from deserts in China and Mongolia and sporadically blowing across Japan. The actual performance of the sand trap with regard to discrimination between pollen and sand has not been reported to our knowledge. The recently developed Shinyei pollen counter<sup>11</sup> exists in 2 versions: the original version, referred to as Shinyei, which has to be placed in a weather instrument shelter, and a modified version, referred to as NTT-Shinyei, which has a higher flow rate and is protected by an all-weather casing. The measurement results obtained with the original version of the NTT counter<sup>10</sup> are not shown because the NTT counter was updated to the NTT-Shinyei counter. The KP1000 Kowa counter<sup>9</sup> was found to be difficult to operate during a long period (we have had experience during 2 cedar pollen seasons) because of high running cost and repeated failures, so we decided not to include data from this counter in our study.

RESULTS

Figure 1 shows the variation in time of the daily deposition count and the average daily concentration for the Chiba site. The deposition count was determined by using a Durham sampler and counting separately cedar and hinoki pollen grains. The average daily concentration was obtained by averaging hourly concentrations measured by the automatic pollen counters. These counters cannot distinguish cedar from hinoki pollen grains and thus should be compared with observations of the total count of cedar and hinoki pollen grains. As shown in Figure 1, the hinoki pollen contribution to deposition counts was significant from the middle of March and predominant at the end of March. For the Akita

Table 1. Main Characteristics of the Most Widely Used Automatic Counters and the Burkard Reference Sampler

| Counter | Measurement principle       | Pollen discrimination  | Flow rate, L/min                  |
|---------|-----------------------------|------------------------|-----------------------------------|
| Burkard | Impactor and microscopy     | Microscope observation | 10                                |
| KH3000  | Scattering from 2 beams     | Spherical shape        | 4.1                               |
| Kowa    | Scattering and fluorescence | Size and fluorescence  | 4.0                               |
| Shinyei | Scattering and polarization | Size and shape         | 0.9 (original), 2.2 (NTT-Shinyei) |
| NTT     | Scattering                  | Size                   | 30                                |

Table 2. List of Instruments and Their Period of Operation at Both Measurement Sites

| Site (period of operation) | Durham | Burkard                                 | KH3000               | Shinyei              |
|----------------------------|--------|-----------------------------------------|----------------------|----------------------|
| Chiba (1/24/05–4/24/05)    | C      | 3/25–3/30 (SporeWatch, 1-hour sampling) | C (with sand filter) | C (NTT-Shinyei)      |
| Akita (3/28/05–4/25/05)    | NA     | C (7-day sampler, 2-hour sampling)      | C (with sand filter) | C (Shinyei original) |

Abbreviations: C, complete dataset; NA, dataset not available.

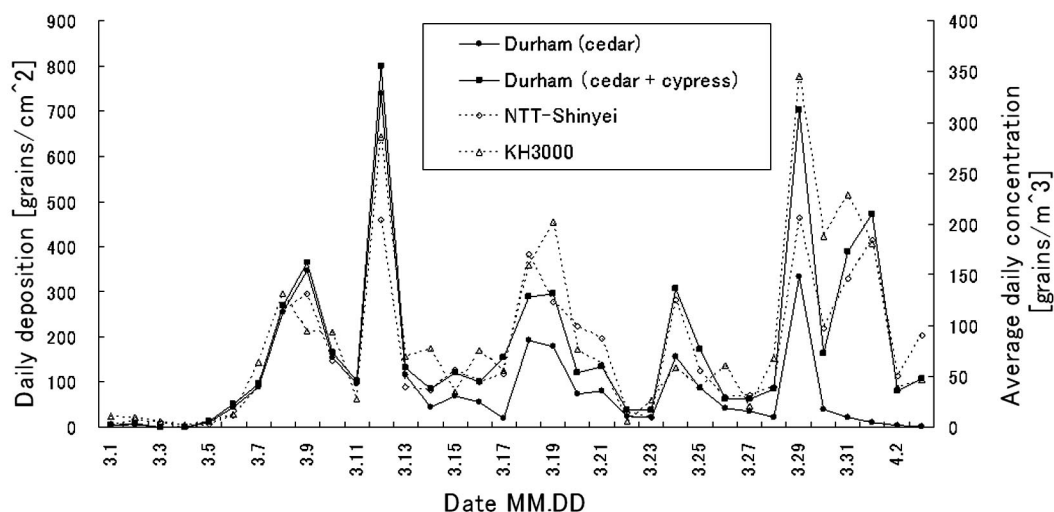


Figure 1. Comparison between daily deposition and daily average concentration of pollen at the Chiba site for the complete pollen season. The daily deposition counts for the cedar and hinoki total and for the cedar only are shown to illustrate the contribution of hinoki to the total count of pollen. Average daily concentrations were computed from hourly concentration values measured by automatic counters.

site, hinoki pollen contribution is known to be negligible during the entire pollen season because of the quasi-absence of the hinoki species in this area. From Figure 1 it is clear that the variations in daily deposition counts determined by the Durham reference sampler are well reproduced by the variations in the daily average concentrations of the automatic counters. Note that the relation between deposition and concentration depends on local factors, such as irregular topography and micrometeorological conditions, and is therefore varying over space and time. In our comparison, we assume the average over a day of these factors to be constant. The linear correlation coefficients between the Durham counts and the concentrations of both automatic counters were higher than 0.9, strongly suggesting that the automatic counters correctly approximate daily pollen variations.

Figure 2 shows the pollen concentration time series as measured with the automatic counters and the reference sampler at both sites. Timing of the pollen bursts are well reproduced by the 2 automatic counters. This is further evidenced by the high values of the linear correlation coefficients between the counters and the reference sampler. For the Chiba data, both a Shinyei and a NTT-Shinyei counter were used and the correlations were computed on hourly concentrations. For the Akita data, a Shinyei module was used and the correlations were computed on 2-hour average concentrations. At the Chiba site, the linear correlation coefficients

were 0.89, 0.92, and 0.90 for the KH3000, Shinyei, and NTT-Shinyei counters, respectively. At the Akita site, the linear correlation coefficients were 0.83 and 0.81 for the KH3000 and Shinyei counters, respectively. It also appears from Figure 2 that the intensity of the burst is not always well determined by the automatic counters. All counters underestimated pollen concentrations, pointing to calibration problems in the automatic counters. Small variations in pollen concentrations ( $<100$  grains/ $m^3$ ) as measured by automatic counters did not compare well with those determined with the reference sampler. In the Akita time series of Figure 2b, we found a high correlation between a sleet episode recorded by the Japan Meteorology Agency on March 29 to 30 and high counts of the KH3000. During this sleet episode, no pollen was observed in the Burkard reference concentration series. This strongly suggests that the KH3000 counted snow particles as pollen grains. This phenomenon was not observed with the Shinyei counter.

In Figure 3 and Figure 4, we examine in more detailed measurement errors in pollen concentration determined by the automatic counters for the Akita series. The concentrations measured by the automatic counters were first corrected for bias introduced by calibration errors using a linear calibration curve between the reference and the automatic counters, and then the residuals were computed and used to derive the absolute errors between the automatic counters and

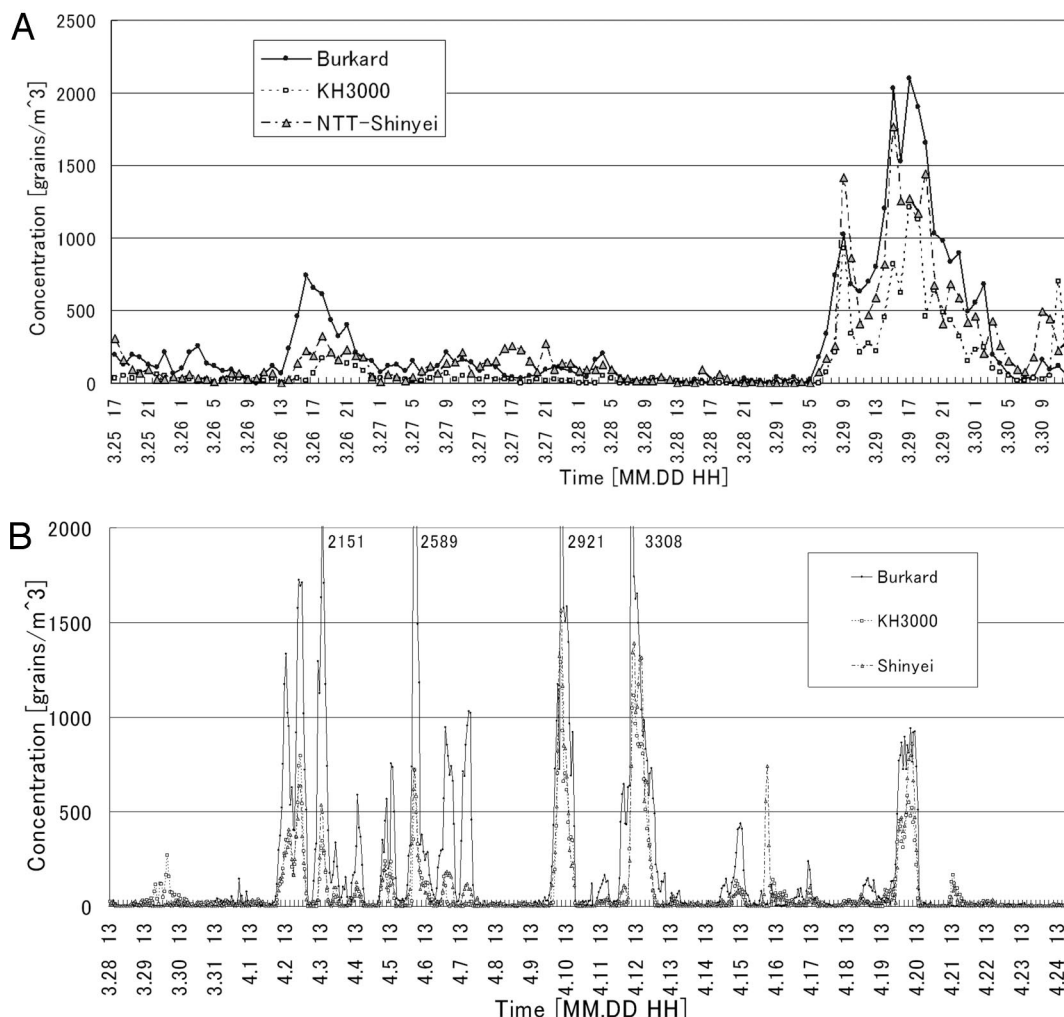


Figure 2. Comparison between pollen concentrations measured with a Burkard, KH3000, and Shinyei counter at the Chiba site (A) and the Akita site (B). In section B, the maximum value of the concentration axis was set to 2,000 grains/m<sup>3</sup> to make visible the variations for all counters (actual values for the Burkard counts that fall outside the plotted range are indicated on the right side of each peak). The 1-hour average and 2-hour average of pollen concentration are plotted for the Chiba site and the Akita site, respectively.

the reference sampler. Figure 3a-b shows detailed time variations in the corrected concentrations together with the reference concentration for a low-concentration region and a high-concentration region of the Akita series, evidencing poor (good) correlation between the automatic counters and the reference sampler for the low (high) concentration region. Figure 4 shows the absolute errors in concentration measurement between the automatic counters and the reference sampler as a function of the averaged concentration. The curves indicated as “observed” refer to errors estimated from differences between the reference sampler and the automatic counters, whereas the curves indicated as “statistical” refer to errors estimated from the theory of statistical fluctuations. When counting airborne pollen grains, unavoidable statistical fluctuations in the observations result from the random nature

of the observed process. These fluctuations are not related to any instrument error; that is, an estimate of the statistical fluctuations gives the lower limit to the measurement error for an ideal instrument. An estimate of the fluctuation error can be obtained from the SD of the observation distribution, which is known to follow a Poisson distribution in a counting experiment. The SD of a Poisson distribution is the square root of the mean of the counted events, which was used to compute the statistical errors of Figure 4. The difference in the statistical errors between the 2 automatic counters is explained by a difference in the sampled volumes (see the flow rate column of Table 1), that is, a difference in the number of counted pollen grains for the same concentration. For both automatic counters, the observed errors are found to be much larger than the statistical errors, pointing to the

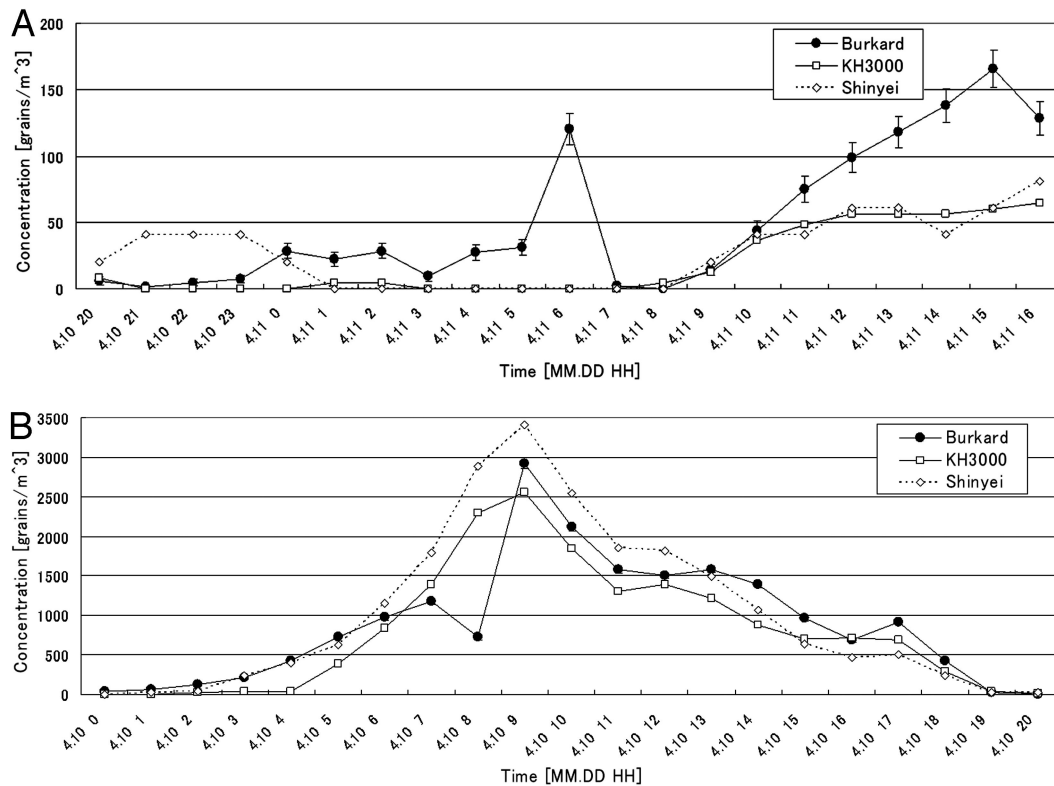


Figure 3. Detailed variations in pollen concentration with time at Akita as measured with a Burkard sampler and a KH3000 and Shinyei counter for a low-concentration region (A) and a high-concentration (B) region. The error bars of the Burkard series represent statistical uncertainty, which arises from statistical fluctuations in the pollen counts and were estimated as the square root of the counted pollen grains. (Note that the values of the error bars in section B are too small compared with the extent of the y-axis to be represented.)

existence of instrumental errors. For concentration in the 0 to 50 grains/m<sup>3</sup>, the error of the Shinyei counter is as large as the measured concentration, indicating that data collected with this counter should be used for concentrations larger than 50 grains/m<sup>3</sup>.

## DISCUSSION

A side-by-side comparison conducted at 2 different sites between reference samplers and automatic pollen counters revealed some weaknesses of the investigated counters in the areas of pollen discrimination, minimum measurable concentration, and calibration. These imperfections rooted in the design of the investigated automatic counters directly affect the accuracy of the measured pollen concentration and limit their applications. Apart from changes in counter design, which fall outside the scope of this report, we would like to point out a possible area of improvement. Because the 2 investigated automatic counters have been found to exhibit different characteristics in their ability to discriminate pollen from other particles, combining observations of both counters to filter out false peaks should lead to some improvements in the accuracy of these pollen counters when used simulta-

neously. Also some recommendations when setting up pollen counters in weather shelters (commonly used to protect instruments from sunshine and precipitation) may be useful. When operating a counter in weather shelter, the air should be sampled outside the shelter through a channel like the ones of the KH3000 or the NTT-Shinyei counters. Sampling inside the shelter could result in measurement errors, because pollen grains that are inevitably deposited inside the shelter are likely to be reemitted at a later time.

Pollen discrimination problems and calibration inaccuracies greatly hamper the use of automatic pollen counter data in a pollen forecasting system and should be addressed. The minimum measurable concentration of the investigated counters was found to be approximately 50 grains/m<sup>3</sup>, a concentration that may be too high to provide useful information to patients with allergy. The value of the minimum concentration to be monitored in a pollen information system has to be debated and agreed on by the allergy community. For the automatic pollen counters to be successfully integrated in a pollen monitoring network, further developments in the pointed areas have to be made.

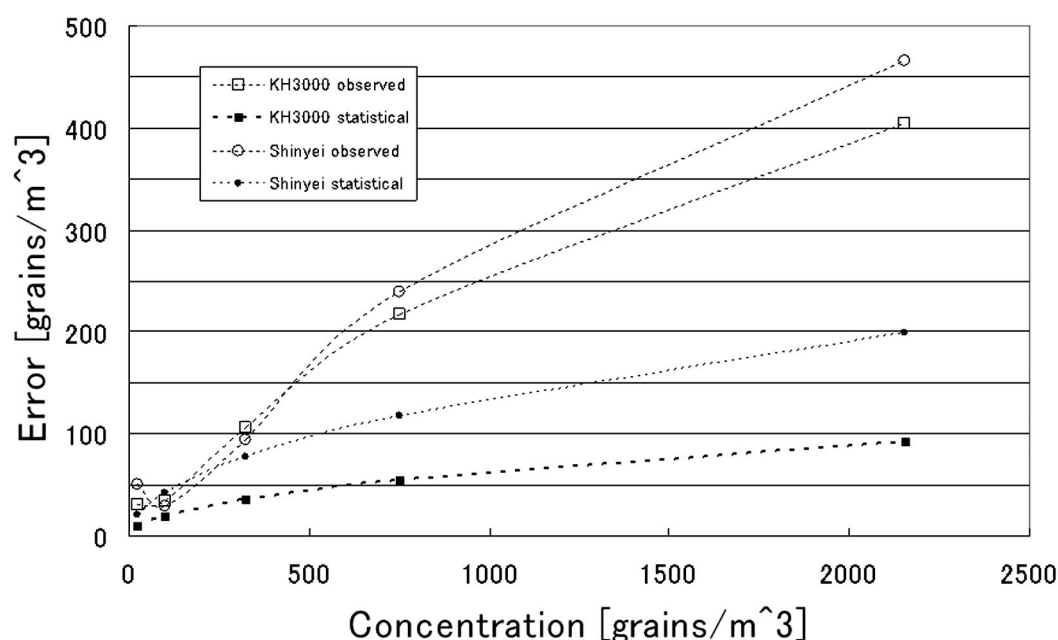


Figure 4. Error analysis of automatic pollen counters performed on the Akita dataset. Absolute errors are computed between the concentrations measured with the Burkard reference sampler and the automatic counters. The 2-hour average concentrations were used and errors computed in the following bins: 0 to 50, 50 to 150, 150 to 500, 500 to 1,000, and more than 1,000 grains/m<sup>3</sup>.

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