

A comparison of airborne and dust-borne allergens and toxins collected from home, office and outdoor environments both in New Haven, United States and Nanjing, China

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Abstract In this study, the airborne and dust-borne concentrations of endotoxin, (1,3)- β -D-glucan and five house dust allergens were measured in office, home, and outdoor environments both in New Haven, United States and Nanjing, China. Air samples were collected using a BioSampler at a flow rate of 12.5 l/min for 30 min. Dust samples were simultaneously collected using a surface sampler. Dust samples went through extraction and dilution before analysis, while air samples were analyzed directly. Limulus Amoebocyte Lysate (LAL) Pyrochrome and GlucateLL assays were used to quantify endotoxin and (1,3)- β -D-glucan concentration levels, respectively. Enzyme-linked sorbent assay was used to measure the dust mites, cat, dog, and cockroach allergens. The experimental results indicated that endotoxin, (1,3)- β -D-glucan and allergen concentrations vary greatly both with samples and

environments. In all tested environments, endotoxin concentration ranged from 0.8 to 83.7 ng/m³ for air, and 7.8 to 14.3 ng/mg for dust. (1,3)- β -D-glucan concentration ranged from 0.1 to 9.8 ng/m³ for air, and 6.6 to 110 ng/mg for dust. Cockroach allergens were detected only in New Haven office and outdoor environments, and other allergens ranged from 0.1 to 90 ng/mg for dust samples, and from 1.5 to 1,282 ng/m³ for air samples. In general, similar profiles of allergens and toxins were observed in New Haven and Nanjing environments. Linear regression analysis showed that there were better endotoxin and (1,3)- β -D-glucan linear correlations ($R^2 = 0.78, 0.87$, respectively) between the dust and air samples compared to those of the allergens Der f 1 and Der p 1 ($R^2 = 0.5, 0.7$, respectively). This research contributes to the development of robust biological exposure assessment and the elaboration of airborne and dust-borne biomass in the living environments.

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1 Introduction

Allergens and toxins present in our environments have been shown to be associated with many health problems including lung diseases, allergies, inflammation, and

Sick-Building-Syndrome (SBS) (Douwes et al. 2003). A number of those allergens and toxins can be in an airborne state and contribute to the negative health effects via inhalation.

Among the allergens, dust mite allergens (Der f 1 and Der p 1), dog allergens (Can f 1), cockroach allergens (Bla g 1) and cat allergens (Fel d 1) are often measured in health-related studies, such as investigation of childhood asthma (Leaderer et al. 2002; Matsui et al. 2003; Custis et al. 2003). When these allergens are inhaled, they have the ability to enter the blood system and further interact with mast cells, thus triggering inflammable reactions by releasing histamine (Proud et al. 1992). Home exposures to these allergens in the airborne state are an important environmental factor in allergic sensitization and in the development and exacerbation of asthma (Leaderer et al. 2002). The concentrations of such allergens can be determined using the enzyme-linked immunosorbent assay (ELISA; Indoor Biotechnologies, Charlottesville, VA) (Leaderer et al. 2002; Matsui et al. 2003; Custis et al. 2003).

Endotoxin has been shown to be associated with many health impairments, and inhaled endotoxin is recognized as a potent inducer of airway inflammation (Dales et al. 2006). It has been shown that exposure to endotoxin is associated with pulmonary symptoms, airway bronchoconstriction, recruitment of neutrophils to the airways, and increased release of proinflammatory cytokines including tumor necrosis factor alpha, interleukin-6, and macrophage inflammatory protein (Thorne 2000). Endotoxin was also shown to be associated with respiratory diseases in the first two years of life (Dales et al. 2006). Its concentration is usually measured using kinetic chromogenic LAL assay. A 'no observed effect level' (NOEL) for endotoxin was proposed as 20 ng/m³ (Douwes et al. 2003).

In addition to endotoxin, (1,3)- β -D-glucan can also elicit inflammation (Czop and Kay 1991) and cause an increase in the number of lymphocytes in the blood when inhaled (Rylander 2005). (1,3)- β -D-glucan is generally quantified using modified kinetic chromogenic LAL assay, e.g., Glucatell assay (Associates of Cape Cod, Inc., East Falmouth, MA). Personal glucan exposure has been shown to range from 2.0 to 36.4 ng/m³ in garbage collection and from 4 to 366 ng/m³ in the wood processing industry (Rylander et al. 1999). Despite observed health effects, the dose-response

relationship for these above-mentioned allergens and toxins in the airborne state has rarely been established (Douwes et al. 2003).

When assessing exposure to the airborne allergens and toxins, air sample needs to be taken. Among the sampling techniques, liquid impingement is widely used. Duchaine and co-workers have compared the use of AGI impinger against filter when using *Limulus* amoebocyte lysate (LAL) assay to quantify airborne endotoxin in swine barns and sawmills (Duchaine et al. 2001). And they indicated that impinger sampling was an acceptable sampling method for endotoxin sampling. A relatively new liquid sampler, the BioSampler, was designed to collect inhalable airborne particles while minimizing the problems associated with the AGI-30 impinger, e.g., particle bounce, reaerosolization as well as the integrity of biological cells (SKC, Inc., Eighty-Four, PA, USA). The collection efficiency of the BioSampler is close to 90% when sampling particles of 0.5 μ m and larger (SKC, Inc.). Microbial species and biological toxins often attach onto larger biologically inert particles, which correspondingly ensures their efficient collection when using the BioSampler. In addition, use of BioSampler allows direct transfer of aerosols into hydrosols that can be readily analyzed by microbiological techniques without further sample processing. As such, this sampling strategy eliminates the extraction step that is necessary when a filter is used.

Compared to the bio-toxic materials in the dust, airborne allergens and toxins are not well quantified and often neglected in many health endpoint investigations (Leaderer et al. 2002; Matsui et al. 2003; Custis et al. 2003). Fewer studies have quantified both airborne and dust-borne allergens, endotoxin, and glucan concentration at the same time for exposure assessments. In this study, we have chosen three environments: home, outdoor and office, where no apparent adverse health effects or impairments were observed or reported. Air and dust samples were collected from these environments using a BioSampler and a High Volume Small Surface Sampler (HVS3) (CS-3, Inc., Sandpoint, ID), respectively. Use of aerosol-to-hydrosol air sampling via the BioSampler simplified the sampling process compared to the filtering techniques. Biological contents of the samples, including 5 types of allergens (Der p 1, Der f 1, Fel d 1, Can f 1, Bla g 1), endotoxin and glucan were analyzed using ELISA, LAL, and Glucatell assay,

respectively. The experiments were independently conducted both in New Haven, United States and Nanjing, China.

This study was mainly designed to show that both airborne and dust-borne biological materials such as allergens, endotoxins and (1,3)- β -D-glucan should be considered for improving biological exposure assessment, and at the same time illustrate the relationship between the dust-borne and air-borne allergens and toxins through different environments in different geological locations (New Haven, US and Nanjing, China).

2 Materials and methods

2.1 Sample collection

In this study, the experiments were independently conducted both in New Haven, CT, United States and in Nanjing, Jiangsu, China. In the Nanjing experiment, only Der p 1, Der f 1, endotoxin and glucan concentrations were measured. For Nanjing and New Haven indoor environments (home and office), the experiments were conducted under similar indoor conditions: hard concrete floor, no apparent mold or water damage, and open windows. In the New Haven office environment, there is a central air conditioning system, while the Nanjing office environment does not have one. In addition, in the New Haven experiments, there were cats and dogs around the sampling areas, while in Nanjing, there were none. The outdoor samplings were conducted outside of one-story residence apartments and outside of a 6-story office building in New Haven and Nanjing experiments, respectively.

Air samples were collected from three air environments (home, outdoor, and office) using a Bio-Sampler operated at a flowrate of 12.5 l/min for 30 min. Deionized (DI) water of 20 ml (a standard volume) was used as the collection liquid. After 30 min sampling, the collected air samples were transferred into 50-ml tubes and stored at 4°C for subsequent biological analyses in the same day. For air samples, no sample processing and dilution were performed for all analyses including ELISA, endotoxin and glucan tests. The sampler was operated at a height about 1 m from the floor.

Dust samples were simultaneously collected using a commercially available surface sampler HVS3

(CS-3, Inc.) from the same environments. The surface samples were collected for 5 min from approximately 2 m² floor area or outside ground, which makes the samples equivalent to the mixture of several dust samples collected in a small area. The collected dust samples from each environment were well mixed, and a portion, about 10 mg for ELISA tests, and 2 mg for endotoxin and glucan tests, was measured, dissolved into 2 ml DI water, and subsequently went through the sample processing procedures. Three replicates from the samples collected were prepared for each of the environments investigated. During the dust collection process, it is possible that some of the dust would be re-suspended, especially in indoor environments. However, the re-suspension would be expected when walking or activities are taking place.

2.2 ELISA method

All procedures were carried out using pyrogen-free reagents and glassware. The dust was sieved using No. 50 mesh screen, 300 μ m diameter (Luczynska et al. 1998). For each dust sample, about 10 mg after sieving was deposited into a 2.0 ml conical screw cap microtube (Quality Scientific Plastics, Petaluma, CA) with 2 ml PBS-T containing 0.05% Tween 20. The 2-ml microtubes were then shaken on a titer plate shaker (Lab-line Instruments, Inc., Melrose Park, IL) for 2 h at a speed of 1,100 rpm under room temperature. Following the extraction, 20 min centrifugation at 2,500 rpm was performed for ELISA tests using a Centrifuge 5804 R (Eppendorf AG, Hamburg, Germany). The supernatant was pipetted into another 2-ml microtube which was labeled with the sample ID and stored at -70°C for next-day analysis.

Dust allergen (Der p 1, Can f 1, Der f 1, Fel d 1, Bla g 1) analysis was conducted using ELISA kits (Indoor Biotechnologies, Charlottesville, VA). A standard allergen dilution series (200 to 0.5 ng/ml) was prepared in PBS-T containing 0.05% Tween 20 for each set of allergen tests. About 100 μ l volumes of standards with doubling dilutions (from the ELISA kit) in duplicate, sample extraction in triplicate, negative controls (PBS-T containing 0.05% Tween 20) were pipetted into pre-designated wells in a 96-well microplate (NUNC, Maxisorp Cert., Rochester, NY) and the manufacturer's instructions were followed for each step of the assay. The microplate was finally placed inside a spectrophotometer, SpectraMax (Molecular Devices, Inc.,

Sunnyvale, CA), and the optical density was measured using endpoint assay at 405 nm after 30 min color development. Sample concentrations were determined by 4-parameter standard curves (Sigmoid function) of optical density versus standard concentrations ($r^2 > 99\%$). The standard curve was generated by software based on the optical densities and concentrations of the standards of doubling dilutions. The allergen standards were provided with the analysis kits.

2.3 Endotoxin method

About 10 mg of sieved dust was deposited into a 2-ml tube containing 2 ml of DI water (0.05% Tween 20). PBS buffer was not used because of its interference with the assay. The 2-ml microtubes were then shaken on the titer plate shaker for 2 h at a speed of 1,100 rpm at room temperature. A final dust extraction was then carried out by vortexing the mixture for 1 min. To control matrix inhibition and enhancement, samples were diluted to 10^{-3} in DI water (0.05% Tween 20) prior to analysis.

Endotoxin content analysis was conducted using a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay (Associates of Cape Cod, Inc., East Falmouth, MA). A standard endotoxin dilution series of 0.005, 0.05, 0.5, and 5 EU/ml was prepared in lysate reagent water (LRW) for each set of test reagents. For endotoxin measurement, 40- μ l volumes standard in duplicate, sample extract dilutions (10^{-3}) in triplicate, negative controls (LRW and 0.05% Tween 20), and positive product controls (spiked value of 0.25 EU/ml) were pipetted into pre-designated wells in a 96-well microplate. The microplate was then incubated at 37°C for 15 min. During the incubation, Limulus Amebocyte Lysate agent was reconstituted using Glucashield buffer (Associates of Cape Cod, Inc., East Falmouth, MA) to remove interference from (1,3)- β -D-glucans. After incubation, 40 μ l of LAL agent were pipetted into each well. The plate was placed inside a spectrophotometer, SpectraMax, pre-mixed for 10 s, and the LAL assay was allowed to proceed for 60 min in the kinetic mode to measure the onset time for color development. Using log-log standard curves of onset time versus standard concentrations ($r^2 > 99\%$), sample concentrations were calculated. The standard curve was generated by software based on the optical densities and concentrations of endotoxin standards

(0.005, 0.05, 0.5, and 5 EU/ml) that are supplied by the assay manufacturer. The unit of endotoxin, EU, was converted to ng by dividing 10.

2.4 (1,3)- β -D-Glucan method

About 10 mg of sieved dust was deposited into a 2-ml (1,3)- β -D-glucan free tube containing 2 ml of extraction solution (0.5 N NaOH in DI) (Foto et al. 2004). (1,3)- β -D-glucan is present in both soluble (10%) and non-soluble forms, and use of NaOH helps unwind the triple-helix structure of glucan, thus making it soluble (Thorn and Rylander 1998). In addition, NaOH can also remove endotoxins (Lee et al. 1999), which avoids the overestimation of glucan content since endotoxin also reacts with LAL reagent. The 2-ml microtubes were then shaken on the titer plate shaker for 2 h at a speed of 1,100 rpm at room temperature. A final filter extraction was then carried out by vortexing the mixture for 1 min. To control for matrix inhibition and enhancement, samples were diluted to 10^{-3} in DI water prior to analysis. The diluted extraction solution from the dust was neutralized using Tris-HCl Buffer (Fisher Scientific, Pittsburgh, PA).

(1,3)- β -D-glucan analysis for the samples was conducted using the quantitative kinetic Glucatell lysate assay (Associates of Cape Cod, Inc., East Falmouth, MA). A standard (1, 3)- β -D-glucan series of 200, 100, 50, 25, and 5 pg/ml was prepared. A 25- μ l volume of (1, 3)- β -D-glucan standards in duplicate, sample extract dilutions in triplicate, negative controls (0.5 N NaOH neutralized by Tris-HCl), and positive product controls (spiked value of 25 pg/ml) were pipetted into pre-designated wells in a 96-well microplate. The microplate was then incubated at 37°C for 15 min. During the incubation, Glucatell lysate agent was reconstituted using 2.8-ml LRW and 2.8-ml Pyrosol buffer (Associates of Cape Cod, Inc., East Falmouth, MA). After incubation, 100 μ l was pipetted into each well of the 96-well plate. The plate was placed inside a spectrophotometer, SpectraMax, pre-mixed for 10 s, and the LAL assay was allowed to proceed for 80 min in the kinetic mode to measure the onset time for color development. Using log-log standard curves of onset time versus standard concentrations ($r^2 > 99\%$), sample concentrations were calculated. The standard curve was generated by software based on the optical

densities and the concentrations of glucan standards (200, 100, 50, 25, and 5 pg/ml) that were supplied by the assay manufacturer.

2.5 Statistical analysis

Linear regress analysis was applied to study the relationships between the dust-borne and airborne allergens and toxins, and their respective R^2 values were presented. Due to the high variation and some low concentrations, we transformed the concentration by first multiplying the data by 10, then taking its log.

3 Results and discussion

In this study, the concentrations of dust-borne and airborne allergens, endotoxin and glucans were investigated by combining air sampling and microbiological analysis techniques. Positive product control obtained 80–120% of the spiked value, which is in the acceptable manufacturer's assay range of 50–200% recovery (Associates of Cape Cod, Inc., East Falmouth, MA). Blank and negative controls were not detected within the detection limits of ELISA and LAL assays. All results were averages of three replicates from the same air and dust samples collected in the environments studied.

For home air samples of the New Haven experiment, the endotoxin concentration level was about 0.8 ng/m^3 , and glucan concentration level was about

9.9 ng/m^3 as shown in Fig. 1a. None of the allergens (Der p 1, Der f 1, Bla g 1, Can f 1, Fel d 1) were detected using our assay. For the Nanjing home experiment, airborne endotoxin concentration was about 90 ng/m^3 , and glucan concentration was very low, less than 1 ng/m^3 as shown in Fig. 2a. Der p 1 allergen concentration was about 120 ng/m^3 . Sohy et al. (2005) have reported that mean airborne endotoxin concentration was 0.18 ng/m^3 , lower than our values, for 100 dwellings in Strasbourg, France. For problematic indoor conditions, airborne glucan concentration was reported as high as 41.9 ng/m^3 on average (Rylander 1999), which is higher than our values. It seemed that airborne endotoxin, glucan and allergen concentrations vary greatly with samples and environments.

For our dust samples in the New Haven experiment, endotoxin concentration level was close to 8 ng/mg , and glucan concentration was about 90 ng/mg as shown in Fig. 1b. In the Nanjing experiment, dust-borne endotoxin concentration was measured as 15 ng/mg , and glucan concentration was measured as about 10 ng/mg . Sohy et al. (2005) reported that mean dust-borne glucan concentration was about 3.24 ng/mg , lower than our value, for a bedroom floor and 1.7 ng/mg , lower than our value, for a mattress sample (Custovic et al. 1999). The dust mite allergens Der p 1 and Der f 1 were detected and their concentrations were 0.1 and 2.8 ng/mg , respectively, for the New Haven experiment as shown in Fig. 1b. Three other allergens, dog (Can f 1), cat (Fel d 1), and

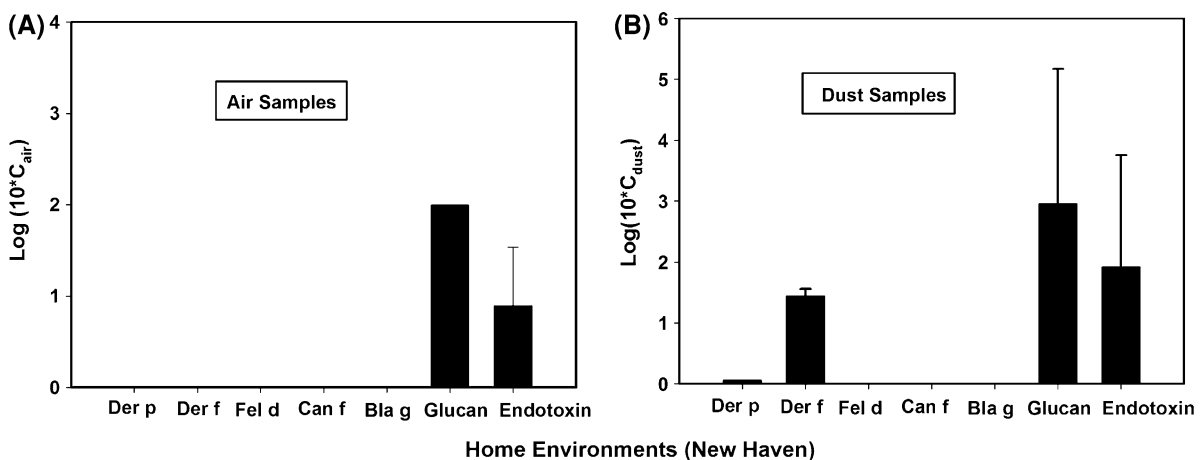


Fig. 1 Allergen, endotoxin and glucan concentrations in the home environment (New Haven, CT, USA). Error bars stand for standard deviation for three repeats. **a** Airborne, C_{air} (ng/m^3). **b** Dust-borne, C_{dust} (ng/mg)

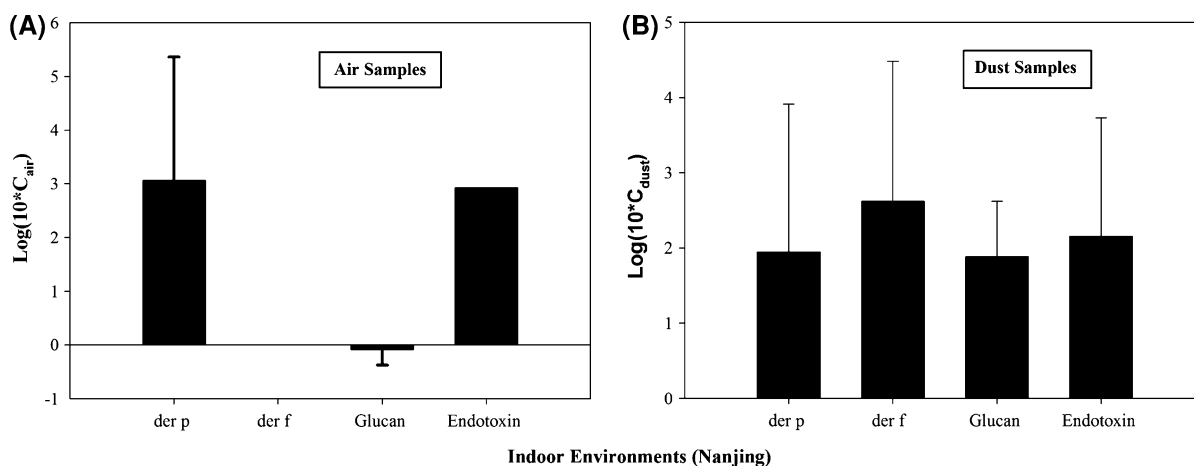


Fig. 2 Allergen, endotoxin and glucan concentrations in indoor environment (Nanjing, Jiangsu, China). *Error bars* stand for standard deviation for three repeats. **a** Airborne, C_{air} (ng/m³). **b** Dust-borne, C_{dust} (ng/mg)

cockroach (*Bla g 1*), were not detected in the dust based on the detection limits of ELISA tests. Custovic et al. (1999) have indicated that the airborne dust mite allergen concentration was usually low in the absence of vigorous disturbance. For the Nanjing experiment, dust-borne *Der p 1* and *Der f 1* concentrations were measured as 9 and 43 ng/mg, respectively, as shown in Fig. 2b. People spend approximately 80% of their time in home environments (Kreiss 1989), and the concentration levels of these allergens are an important indicator of the air quality in their environments. For indoor experiments, both the New Haven and Nanjing experiments have indicated that when comparing between endotoxin and glucan, higher concentrations in the dust

correspond to higher concentrations in the air or vice versa. But for allergens, no such trend was observed. The observed differences between the allergens and toxins were likely due to the mechanisms of their presence in both mediums. For the allergens investigated, the airborne levels were mainly due to the re-suspension from the ground, while for endotoxin and glucans, they naturally appear both in the dust and air.

For outdoor air, endotoxin concentration in our samples in the New Haven experiment was as high as 4.4 ng/m³, and glucan concentration was about 5.6 ng/m³ as shown in Fig. 3a. Cockroach allergen outdoors was detected at a level of 1,282 mU/m³ (unit of cockroach per volume of air). There is no

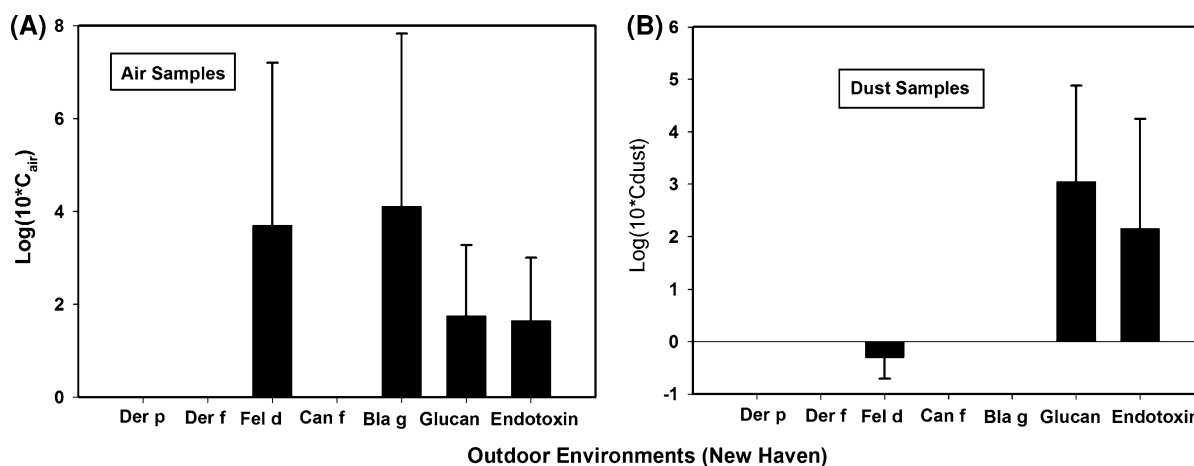


Fig. 3 Allergen, endotoxin and glucan concentrations in the outdoor environment (New Haven, CT, USA). *Error bars* stand for standard deviation for three repeats. **a** Airborne, C_{air} (ng/m³). **b** Dust-borne, C_{dust} (ng/mg)

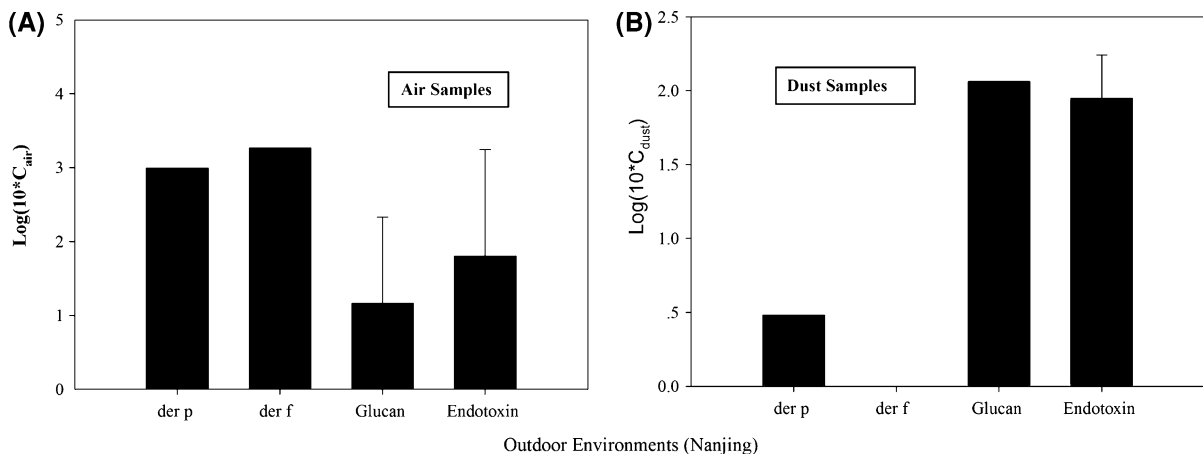


Fig. 4 Allergen, endotoxin and glucan concentrations in the outdoor environment (Nanjing, Jiangsu, China). *Error bars* stand for standard deviation for three repeats. **a** Airborne, C_{air} (ng/m³). **b** Dust-borne, C_{dust} (ng/mg)

established conversion between unit of cockroach and mass, e.g., nanogram. The concentration of airborne cat allergen was detected at 499 ng/m³ in the outdoor environment. Dog and dust mite allergens were below the detection limits of ELISA tests. For the Nanjing outdoor air experiment, glucan and endotoxin concentrations were measured at 1.5 and 6.3 ng/m³, respectively, as shown in Fig. 4a. Airborne Der p 1 and Der f 1 concentrations were measured as 98 and 184 ng/m³, respectively. Endotoxin is often used as a surrogate for environmental bacterial load, and glucan is used as a proxy for fungal species. Solomon et al. (2006) reported that the airborne endotoxin levels ranged from 0.6 to 8.3 EU/m³ (0.06 to 0.83 ng/m³) in flooded areas in New Orleans after Hurricane Katrina (Solomon et al. 2006). This endotoxin concentration is much lower compared to those obtained in our samples. In addition to actual differences in airborne endotoxin concentration, differences in analysis methods or possible sample inhibition could contribute to differences in endotoxin concentrations observed in different studies.

For outdoor dust samples in the New Haven experiments, as shown in Fig. 3b, endotoxin concentration was detected at 14.28 ng/mg, and glucan concentration was detected at 111 ng/mg. Cockroach and other allergens except cat allergen (Fel d 1) (0.05 ng/mg) were not detected in the outdoor dust samples. In the Nanjing experiment, glucan and endotoxin concentrations were measured at 11.6 and 8.9 ng/mg, respectively, as shown in Fig. 4b. Der p 1 concentration was

measured at 0.3 ng/mg. For outdoor experiments, as demonstrated in Figs. 3 and 4, similar trends as seen in the home experiments were also observed, except for glucan in the Nanjing experiments. No apparent trend was observed between airborne and dust-borne allergen concentrations in outdoor experiments.

As shown in Fig. 5a, office air samples in the New Haven experiments had higher Der f 1 concentration at about 110 ng/m³. Other allergens were not detected in the office air samples. Office air samples had 1.5 and 2.1 ng/m³ glucan and endotoxin concentration levels, respectively. One study reported that the average airborne glucan and endotoxin levels in office buildings ranged from 0.1 to 0.6 ng/m³, and 0.2 to 0.4 ng/m³, respectively (Rylander et al. 1992), which are lower than our values. For the Nanjing experiments, airborne glucan and endotoxin concentrations were measured at 1.2 and 19.4 ng/m³, respectively, as shown in Fig. 6a. Airborne Der p 1 concentration was estimated at 112.9 ng/m³.

Office dust samples seemed to have detectable levels of all allergens studied in the New Haven experiment, except Der f 1, as observed in Fig. 5b. For the office dust samples, endotoxin concentration was detected at 7.8 ng/mg and glucan concentration was detected at 13.3 ng/mg. Der p 1 was detected at 0.5 ng/mg, cat allergen at 1.7 ng/mg, dog allergen at 0.4 ng/mg, and cockroach allergen at 1.1 ng/mg. These levels were measured in a setting where no apparent health impairments were observed or reported; thus these allergen concentrations measured here might suggest concentrations that are not yet causing effects in

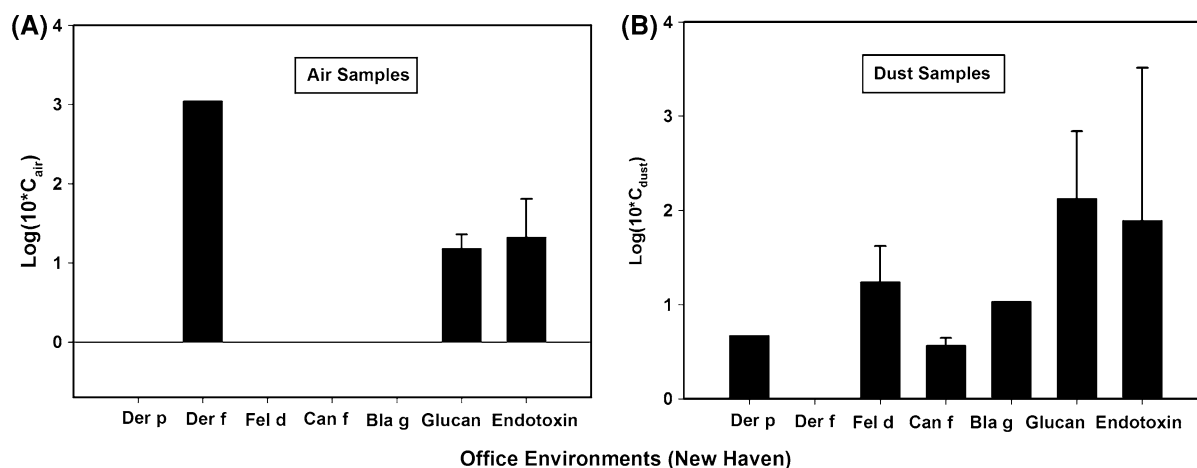


Fig. 5 Allergen, endotoxin and glucan concentrations in the office environment (New Haven, CT, USA). *Error bars* stand for standard deviation for three repeats. **a** Airborne, C_{air} (ng/m³). **b** Dust-borne, C_{dust} (ng/mg)

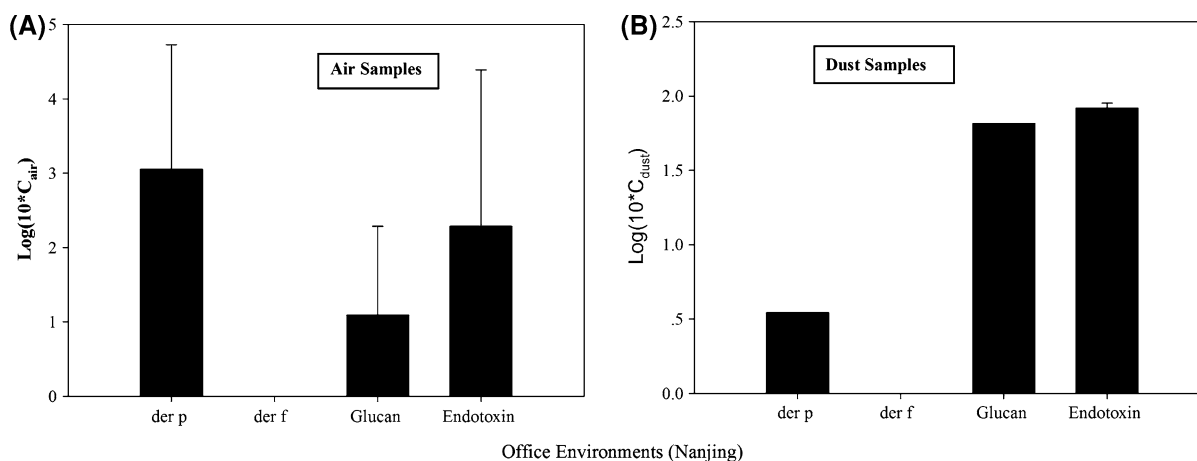


Fig. 6 Allergen, endotoxin and glucan concentrations in the office environment (Nanjing, Jiangsu, China). **a** Airborne, C_{air} (ng/m³). **b** Dust-borne, C_{dust} (ng/mg). *Error bars* stand for standard deviation for three repeats

healthy populations. However, additional data are certainly needed to ascertain this hypothesis. For the Nanjing experiments, glucan and endotoxin concentrations were measured at 6.6 and 8.3 ng/mg, respectively, as shown in Fig. 6b. Dust-borne Der p 1 concentration was estimated at 0.3 ng/mg. For office experiments, a similar trend when comparing endotoxin with glucan was also observed, except for glucan in the first set of experiments.

In all experiments, endotoxin concentration measured in air samples was about 19 ng/m³ on average, higher than glucan concentration which was 3.3 ng/m³ on average. For dust samples, the average endotoxin concentration was 10.3 ng/mg, lower than glucan concentration which was 40 ng/mg. In general,

similar profiles of allergens and toxins were observed in the New Haven and Nanjing environments. Linear regression analysis showed that there were better endotoxin and (1,3)- β -D-glucan linear correlations ($R^2 = 0.78, 0.87$, respectively) between the dust and air samples compared to those of the allergens Der f 1 and Der p 1 ($R^2 = 0.5, 0.7$, respectively). Although the actual levels of allergens and toxins might fluctuate in different seasons both in New Haven, United States and Nanjing, China, the fluctuation would not affect the main conclusions here. The detection limits for airborne allergens or toxins depend on the detection limits of the relevant assays such as ELISA and the LAL method. The lowest standards used in this study vary with agents:

endotoxin (0.005 EU/ml), glucan (5 pg/ml) and allergens (0.5 ng/ml). However, the actual detection limits depend on the comparisons between the negative control and the samples. The concentrations of the samples were calculated based on the standard curve generated by the software. Therefore, the lowest calculated values (but significantly above that of the negative control) obtained for the samples can be treated as the detection limit.

Airborne and dust-borne allergens, endotoxin and glucan concentrations are often expressed in different units, e.g., ng/m³ for airborne and ng/mg for dust. Currently, many health investigations are focused on the relationship between the amount of allergens and endotoxins in the dust and health outcomes (Leaderer et al. 2002; Matsui et al. 2003; Custis et al. 2003). In contrast, although airborne allergens have direct effects on health via inhalation, the factors associated with exposure to such allergens are largely unknown or rarely studied. Dose-response relationship for exposure to these bioaerosols has not been established yet. A 'no observed effect level' (NOEL) for various health endpoints for endotoxin was proposed as 20 ng/m³ (Douwes et al. 2003). Also, a direct relationship between the dust and airborne endotoxin and glucan concentration levels has not been established either. Chew and co-workers have studied the relationship between dust and air sampling for culturable fungal exposure, and they have concluded that these two methods likely represent different types of exposure risk assessment (Chew et al. 2003). Custis and co-workers on the other hand have shown that airborne cat allergen (Fel d 1) is significantly correlated with samples from the floor (Custis et al. 2003). But in our study, we did not observe such a relationship ($R^2 = 0.5, 0.7$). To develop a quantitative relationship between these two allergen levels, a large number of samples would be needed in the environments in question. The effective percentage of the allergens in the dust that can effectively enter the human respiratory system also remains largely unknown. For airborne allergens, at least the amount of inhaled allergens can be calculated based on the allergen concentration in the air and the human breathing rate. Sohy et al. (2005) did not find any correlation between dust and airborne endotoxin levels in their study. They suggested that it is necessary to measure endotoxin in both dust and air to obtain a relevant exposure assessment.

The sampling time for the BioSampler can be further increased up to 8 h if a non-evaporating liquid is used (Lin et al. 1999), which would improve the level of detection of airborne allergens. In addition, this advantage allows the BioSampler to be customized into a standalone bioaerosol exposure assessment system as an aerosol-to-hydrosol sampling strategy (Lester and Ponce 2002). If a filter is used for sampling, a high collection efficiency is achieved, but the relatively low sampling flow rate and long sampling time can be a significant barrier for the application of this technique. In addition, an extraction step would be required for subsequent microbiological analysis. To avoid this step for quantifying airborne allergens, a gelatin filter might be an alternative since it can easily dissolve into the water. Different filter types and extraction procedures have been shown to considerably affect endotoxin exposure assessment (Douwes et al. 1995). Therefore, use of the aerosol-to-hydrosol sampling technique, e.g., the BioSampler, possibly presents a robust sampling method for toxin exposure assessment.

This study has demonstrated the feasibility of quantifying airborne allergens, endotoxin, and glucans by using available bioaerosol sampling and microbiological analysis techniques. It also measured general airborne concentration profiles of those common allergens as well as endotoxin and glucan in non-problematic living environments in different geological locations. Because of the presence of such material in the air, their analysis should not be neglected when evaluating health outcomes and effects. The use of only culturing techniques or total number of microbial species for exposure assessment does not explain the health endpoints sufficiently well since the non-culturable allergens, endotoxins, and glucans from those microbial species can also cause negative health effects. This study presented an example of combining air sampling with surface sampling as well as the use of molecular/bio-chemistry techniques in quantifying potential biological exposure. In future research, more samples in different environments would be needed to study the relationship between dust-borne and airborne allergens, endotoxin and glucans as well as their concentration ranges. Development and adaptation of a robust aerosol-to-hydrosol sampling technique could be one of the key steps toward adequate environmental biological exposure assessment and monitoring.

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