Original Article

Indoor Allergen Levels and Household Distributions in Nine Cities Across China

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Abstract

Objective Chinese allergic subjects have high levels of sensitization to house dust mite (HDM) and other indoor allergens. This study quantifies common indoor allergen levels in Chinese households.

Methods Dust samples were collected from nine cities. Major allergens Der p 1 and Der f 1 from *Dermatophagoides pteronyssinus and D. farinae*, and specific antigens of *Blomia tropicalis*, *Tyrophagus putrescentiae, Acarus siro*, and cockroach species *Blattella germanica* and *Periplaneta americana* were measured by ELISA.

Results HDM allergens were found in dust samples from bedding in 95% of the Chinese households. The median levels varied from <0.006 to 9.2 μg/g of dust, depending on the city. The percentages of households having HDM allergen levels associated with the risk of developing allergy sensitization and asthma were 65% and 25%, respectively. Specific antigens of the storage mite and cockroach were only found in samples from the southern and tropical regions of China. Levels of mite allergens were generally higher in samples from bedding compared to samples from the living room, even for storage mites, whereas levels of cockroach antigens were higher in the living room samples.

Conclusion HDM allergens are present in bedding dust samples from most Chinese households. Cities in southern and central China have relatively high levels of HDM major allergens compared to cities in northern and western China. Antigens of storage mites and cockroaches are not as common as HDM allergens.

Key words: *Dermatophagoides pteronyssinus*; *D. farinae*; House dust mite; Indoor allergen; Storage mite

Biomed Environ Sci, 2015; 28(10): 709-717 doi: 10.3967/bes2015.101 ISSN: 0895-3988

www.besjournal.com (full text) CN: 11-2816/Q

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INTRODUCTION

In developed countries, allergic respiratory disease has increased steadily over the past few decades. With the rapid economic development and industrialization of China, the prevalence of respiratory allergic disease has similarly increased over the past few years[1-5]. A multicenter study assessing the prevalence of skin prick test (SPT) sensitizations among more than 6000 patients with asthma and/or rhinitis showed high prevalence of sensitization to *Dermatophagoides farinae* (Der f), *D. pteronyssinus* (Der p), *Blomia tropicalis* (Blo t), and cockroach species *Periplaneta americana* (Per a), and *Blattella germanica* (Bla g)[6].

In addition to the strong genetic influence present on atopic sensitization and disease development, environmental allergen levels are a major risk factor for the development of allergic rhinitis and asthma. A causal relationship between levels of indoor allergens and asthma has been reported for dust mite allergens, and dust mite allergen concentrations of 2 and 10 μg/g of dust were proposed as exposure thresholds for the development of allergic sensitization and asthma, respectively[7-8]. Although it has been recently recognized that the proposed threshold concentrations are not absolute, due to the contributions and synergistic effects of other environmental factors, indoor allergen exposure still plays a prominent and critical role in the etiology and manifestations of allergic diseases.

Apart from house dust mites (HDM, *Pyroglyphidae*), storage mites belonging to the Glycyphagidae family (*Blomia*) and the Acaridae family (*Tyrophagus* and *Acarus*) have also been shown to be important allergenic sources, especially in regions with humid housing conditions[9-10]. Moreover, previous studies of children with asthma have reported an association between cockroach allergen exposure and cockroach sensitization[11-12].

Nationwide studies of indoor allergen levels in Chinese households have not yet been conducted. Previous studies in China have focused on specific geographic areas and have included few indoor allergens[13-18]. The objective of this study was to determine and compare the indoor allergen levels of dust samples from households across China. We also compared allergen levels in dust samples collected from bedding and the living room, as well as seasonal changes in indoor allergen levels.

MATERIAL AND METHODS

Study Population

Dust samples were collected from households from nine cities in mainland China: Guangzhou, Haikou, Wuzhou, Beijing, Shenyang, Nanjing, Xining, Chongqing, and Wuhan (data from Guangzhou and parts from Haikou have been published elsewhere[13-14] and are included for the sake of broadening the comparisons). Information about temperature and humidity were obtained from each city's Meteorological Administration. Other general information was obtained from http://www.wikipedia.org. A total of 546 households were visited, and 1319 dust samples were collected: 1024 from bedding and 295 from living rooms.

Dust Collection and Extraction

All study participants had been living in their homes for at least 1 year before the dust collection was performed, and they were all asked not to replace their bedclothes for 2 weeks prior to the dust collection. Dust was sampled by a trained technician using a handheld vacuum cleaner equipped with a filter trap (ALK, Hørsholm, Denmark) aimed at getting more than 1.0 gram of dust per sample. Bedding dust was collected from sheets, pillowcases, pillows, quilts, and mattresses. Living room dust samples were collected from sofas, carpets, furniture, and cushions. Mites and/or other microbes in the dust were killed by storing the samples at -18 °C before extraction. Large particles were removed from the dust before extraction in 0.125 mol/L $NH_4(HCO_3)$ (1:15; dust to buffer w/v) for 2 h at room temperature via some gentle shaking. The extract solution was filtered through a 0.22 μm filter and was stored at -18 °C before analysis.

Allergen and Antigen Level Measurements

Allergens and antigens were measured in extracted dust samples via sandwich ELISA using monoclonal antibodies against house dust mite allergens Der p 1 or Der f 1 and polyclonal specific antibodies raised in rabbits against the extracts of *Blomia tropicalis*, *Acarus siro*, *Tyrophagus putrescentiae*, and cockroach species *Blattella germanica* and *Periplaneta americana* (1:1, w:w). All antibodies, allergens, and extracts were from ALK (Hørsholm, Denmark).

For HDM, the sample results are reported as μg Der p 1 per g of dust, and/or, μg Der f 1 per g of dust.

For the other species tested in this study, the results are reported as arbitrary units (AU) per g of dust. All ELISAs were sandwich assays with allergen/ antigen-specific catching antibodies as first layer, samples, controls, and standards as the second layer, followed by specific peroxidase labeled polyclonal antibodies in the third layer and detection by TMB (TMB substrate reagent, BD Biosciences, USA).

The measured allergen levels of Der p 1 and Der f 1 were calibrated using commercially available 'universal' multi-allergen standards (INDOOR Biotechnologies, USA) containing eight purified allergens, including Der p 1 and Der f 1. The calibration procedure has previously been described by Filep et al.[36]. The previously published allergen levels in dust samples from Guangzhou and Haikou[13-14] were recalculated accordingly. The lower limit of quantification (LOQ) was 0.0060 µg/g for Der p 1 and 0.0054 µg/g for Der f 1. LOQ was 0.3 AU/g for *B. tropicalis*, 4.8 AU/g for *A. siro* and *T. putrescentiae*, and 1.2 AU/g for cockroach.

Statistical Analysis

Concerning the statistical treatment, the samples below LOQ were assigned a value of half the limit. The allergen levels were expressed as medians and interquartile ranges (IQRs). The Mann-Whitney test was used to compare allergen levels between two groups using Graph Pad Prism, version 5.04, with 95% confidence intervals. *P*<0.05 were considered to be significant.

RESULTS

The geographical locations of each of these cities represented in this study are shown in Figure 1. Table 1 shows the numbers of households, dust sample collection periods, outdoor temperature, relative humidity, city location, climate, and population.

Levels and Distributions of Indoor Allergens in Bedding Samples

The allergen levels of the bedding dust samples from each city are shown as median levels with IQR in Table 2. Der p 1 and Der f 1 were found in almost all the investigated cities, although they varied in their relative amounts, depending on geographic region. The total concentration of the HDM group 1 allergens (Der p 1+Der f 1, median value) varied from below LOQ to 9.2 µg/g of dust across all cities, as shown in Figure 1. Six cities (i.e., Wuhan, Guangzhou, Nanjing, Wuzhou, Haikou, and Chongqing) had HDM allergen levels above 2 µg/g of dust. All these cities are located in the subtropical region of China. In northern and western China, HDM allergen levels are generally 50 times lower than those in the subtropical region. The Der f 1 allergen was found in almost all the bedding samples from Beijing and Shenyang but only in 16% of the samples from Xining. The Der p 1 allergen levels for these three cities were also generally low, as less than 50% of the samples showed Der p 1 levels above LOQ.

Figure 2 shows that 95% of the dust samples collected from bedding contained detectable amounts of Group 1 HDM allergens, 65% of the samples had levels at or greater than 2.0 µg/g of dust, and 25% had levels at or greater than 10.0 µg/g of dust. The percentages of samples from Wuhan, Guangzhou, and Nanjing with HDM allergen levels at or above the 10 µg threshold were 45%, 38%, and 37%, respectively.

All bedding dust samples from the city of Haikou contained storage mite *B. tropicalis* antigens, with a median value of 27.9 AU/g of dust. In Wuzhou city, low levels of *B. tropicalis* antigens were found in 68%

Figure 1. Cities participating in the study and HDM allergen levels. HDM allergen levels (Der p 1+Der f 1, median value in µg allergen/ gram dust) are shown in brackets. Sample sizes collected from bedding in each city were as follows: Wuhan (*N*=100), Guangzhou (*N*=204), Nanjing (*N*=288), Wuzhou (*N*=28), Haikou (*N*=51), Chongqing (*N*=100), Shenyang (*N*=75), Beijing (*N*=82), and Xining (*N*=98). (*) below LOQ.

Table 1. City Characteristics

City	Population[*] (million)	Involved Households	Bedding Samples	Living Room Samples	Collection Time	[‡]Outdoor Temperature (°C)	[‡]Relative Humidity (%)	Climate[*]	Location[*] (Latitude/Longitude)
Nanjing	8.0	98	288		2006-2007	17.0	76.4	Humid subtropical influenced by monsoon	32^{0}03'N/118^{0}46'E
Guangzhou	12.7	107	204	200	2006-2007	24.0	55.0	Humid subtropical influenced by monsoon	23^{0}10'N/113^{0}23'E
Wuhan	9.8	50	50 50		Jan, Feb 2011 Jul, Aug 2011	5.0 28.9	76.5 79.0	Humid subtropical with abundant rainfall	30^{0}35'N/114^{0}17'E
Chongqing	16.7	50	50 50		Jul, Aug 2011 Jan, Feb 2012	28.9 9.3	74.5 81.5	Humid subtropical influenced by monsoon	29^{0}59'N/106^{0}54'E
Haikou	1.8	51	51	51	May, Jun 2009	29.1 24.7	83.4 84.0	Humid subtropical/tropical up to seasons	20^{0}02'N/110^{0}20'E
Xining	2.1	50	49 49		Jul, Aug 2010 Jan, Feb 2011	17.8 -4.4	63.5 44.5	Cold, semi-arid	36^{0}38'N/101^{0}48'E
Beijing	17.6	50	30 52		Jul, Aug 2010 Jan, Feb 2011	26.9 -2.3	76.0 38.5	Humid continental influenced by monsoon	39^{0}55'N/116^{0}24'E
Shenyang	7.9	50	25 50		Jul, Aug 2010 Jan, Feb 2011	24.5 -9.1	78.0 57.9	Temperate climate influenced by monsoon	41^{0}48'N/123^{0}25'E
Wuzhou	3.0	40	28	40	Nov, Dec 2008	16,9 *21.9*	70.5 *78.7*	Humid subtropical influenced by monsoon	23^{0}48'N/111^{0}32'E

Note. [‡]Data from local Meteorological Administration; average temperature and humidity during dust collection and/or annual average data. [*], Data from http://www.wikipedia.org.

of the bedding dust samples with a median value of 0.41 AU/g of dust (Table 2). The median value of *B. tropicalis* antigens in other cities is below LOQ. A storage mite *T. putrescentiae* antigen was found in bedding samples from Haikou, Chongqing, and Wuhan, with median concentrations of 24, 6.4, and 2.9 AU/g of dust, respectively (Table 2). The median levels of *A. siro* antigens were lower than LOQ in all the investigated cities. However, in Xining city, 22% of the samples were positive to *A. siro*. Cockroach antigens were found in 98% of the bedding samples from Haikou city, with a median level of 22 AU/g of dust. The prevalence of cockroach antigens in samples from Guangzhou city was 51%, with a median level of 1.3 AU/g of dust.

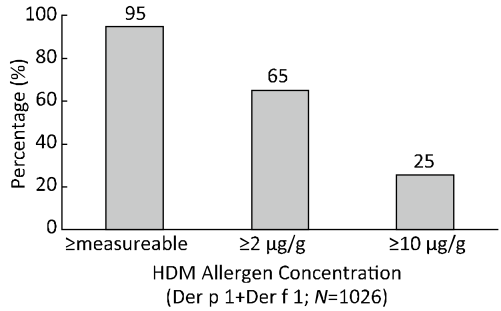

Figure 2. Percentages of the 546 Chinese households with house dust mite allergen concentrations larger than the given threshold values.

Table 2. Indoor Allergen Levels in Bedding Dust Samples Collected in Nine Cities

City	Median Values per Gram of Dust (interquartile range) [% positive[4]]					
	Der p 1 μg/g	Der f 1 μg/g	Blo t AU/g	Tyr p AU/g	Aca s AU/g	Cockroach AU/g
Nanjing *N*=288	1.3 (5.0) [99%]	3.3 (5.7) [100%]	B[2] (NA[5]) [27%]	B[2] (NA[5]) [29%]	B[2] (NA[5]) [1.7%]	B[2] (NA[5]) [11%]
Guangzhou[1] *N*=204	0.33 (1.9) [100%]	5.4 (10) [100%]	B[2] (NA[5]) [27%]	N[3]	B[2] (NA[5]) [1.5%]	1.30 (6.5) [51%]
Wuhan *N*=100	1.6 (6.0) [99%]	5.2 (7.0) [100%]	B[2] (NA[5]) [33%]	2.9 (5.5) [51%]	N[3]	B[2] (NA[5]) [23%]
Chongqing *N*=100	1.1 (2.1) [100%]	0.54 (1.2) [99%]	B[2] (NA[5]) [37%]	6.4 (23) [60%]	N[3]	B[2] (NA[5]) [37%]
Haikou[1] *N*=51	6.9 (6.8) [100%]	B[2] (NA[5]) [35%]	28 (41) [100%]	24 (24) [100%]	B[2] (NA[5]) [0%]	22 (61) [98.0%]
Xining *N*=98	B[2] (NA[5]) [43%]	B[2] (NA[5]) [16%]	B[2] (NA[5]) [21%]	B[2] (NA[5]) [3.6%]	B[2] (NA[5]) [22%]	B[2] (NA[5]) [15%]
Beijing *N*=82	B[2] (NA[5]) [39%]	0.10 (0.65) [99%]	B[2] (NA[5]) [21%]	B[2] (NA[5]) [7.3%]	B[2] (NA[5]) [3.3%]	B[2] (NA[5]) [38%]
Shenyang *N*=75	0.0060 (0.016) [48%]	0.19 0.65) [95%]	B[2] (NA[5]) [16%]	B[2] (NA[5]) [13%]	B[2] (NA[5]) [8.0%]	B[2] (NA[5]) [27%]
Wuzhou *N*=28	1.9 (4.6) [100%]	1.7 (5.8) [100%]	0.4 (1.4) [68%]	N[3]	B[2] (NA[5]) [3.6%]	B[2] (NA[5]) [36%]

Note. [1], Data recalculated from the reference[12-13] due to calibration against the reference from INDOOR Biotechnologies; B[2]: Below LOQ: 0.0060 μg/g for Der p 1, 0.0054 μg/g for Der f 1, 0.3 AU/g for Blo t, 4.8 AU/g for Aca s and Tyr p, 1.2 AU/g for cockroach levels; N[3]: no tests, due to the limited sample amount; % positive[4]: Percent of samples with results>LOQ; NA[5]: Interquartile Range not relevant when median<LOQ.

Levels and Distributions of Indoor Allergens in Living Rooms of Three Cities

Dust samples were collected from living rooms in three cities: Guangzhou, Haikou, and Wuzhou. The results are shown in Table 3. The median levels of HDM allergens and storage mite antigens were significantly lower in the living room samples, except for *B. tropicalis* antigen levels in Wuzhou. Cockroach antigen levels in living room samples were statistically significantly higher (Guangzhou and Wuzhou) or similar (Haikou) compared to bedding samples collected from the three cities.

Seasonal Variations of HDM Allergen Levels in Household Samples

Seasonal variations of HDM allergen levels were investigated in dust samples from Guangzhou and Nanjing. The results from Guangzhou dust samples have been published elsewhere[14]. In Nanjing city (Figure 3), there was a clear tendency for HDM allergen levels to vary in bedding samples according to the season, with minimum levels in March through June and higher levels between July and December.

DISCUSSION

In this study, we investigated mite and cockroach allergen and antigen levels in Chinese households

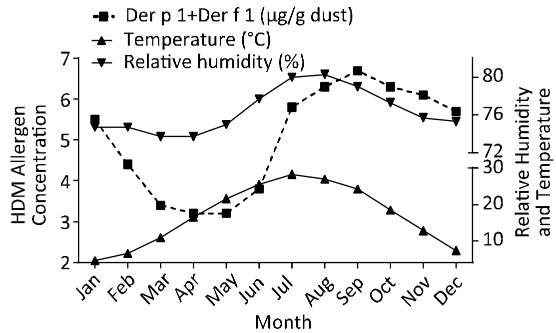

Figure 3. Yearly climate conditions and HDM allergen levels (Der p 1+Der f 1) in Nanjing city. Dust samples were collected three times from each household over about a three-month period between Dec 2008 and Feb 2010. Allergen levels for each month are the moving median values for the 3-month data. For example, the February allergen level was the median value of the data from January, February, and March. The temperature and humidity for each month was expressed according to the moving mean values for the 3-month data. Numbers of dust samples for each month were as follows: Jan: 30, Feb: 20, Mar: 16, Apr: 28, May: 50, Jun: 9, Jul: 22, Aug: 38, Sep: 14, Oct: 19, Nov: 21, and Dec: 21.

Table 3. Indoor Allergen Levels in Dust Samples Collected from Living Rooms in Three Cities

City	Allergen Levels: Median Values per Gram of Dust (IQR) [% positive⁴]				
	Der p 1⁶ μg/g	Der f 1⁶ μg/g	Blo t⁶ AU/g	Tyr p⁶ AU/g	Cockroach⁷ AU/g
Guangzhou¹ N=200	0.015 (0.092) [67%]	0.12 (1.2) [86%]	B² (NA⁵) [3.5%]	N³	8.3 (64) [88%]
Haikou¹ N=51	0.36 (2.5) [92%]	B² (NA⁵) [13%]	6.4 (15) [100%]	15 (19) [100%]	30 (114) [100%]
Wuzhou N=40	0.060 (0.14) [83%]	0.012 (0.047) [85%]	0.35 (0.58) [65%]	N³	27 (90) [85%]

Note. 1: Data recalculated from the reference[12-13] due to calibration against the reference from INDOOR Biotechnologies; B2: Below LOQ: 0.0054 μg/g for Der f 1 and 0.3 AU/g for Blo t; N3: No tests, due to the limited sample amount; % positive4: Percent of living room samples with results>LOQ; NA5: Interquartile Range not relevant when median < LOQ; 6: The median levels of HDM allergen and storage mite antigens are significantly lower (*P*<0.0001) in living room samples compared to bedding samples (shown in Table 2), except for Blo t in Wuzhou; 7: Cockroach antigen levels in living room samples are significantly higher (*P*<0.0001) in Guangzhou and Wuzhou and are similar to bedding samples in Haikou.

from nine different cities across China. In general, storage mite and cockroach antigens were found in dust samples from only a few cities, whereas HDM major allergens Der f 1 and Der p 1 were found in 95% of the dust samples in amounts ranging from <0.006 to more than 85 µg/g of dust. The numbers of households in China with levels of HDM allergens sufficient to lead to the sensitization and development of allergic diseases are similar to what has been described in the USA and Europe[19-20].

Allergen amounts in dust samples are not necessarily direct measures of individual exposure, but the immune system is likely to encounter allergens more frequently in an environment of high allergen concentration. Furthermore, studies have demonstrated that there exists a dose-response relationship between exposure to mite allergens and sensitization[7,21-23]. Cities in southern parts of China have up to 50-times more HDM major allergens compared to cities in northern and western parts of China, and this coincides with an HDM SPT sensitization prevalence from almost 80% in the southern parts to about 40% in northern China[6]. The relationship between allergen exposure levels and asthma is still not fully understood. Li et al. showed that asthma prevalence is strongly associated with sensitization to indoor allergens in Chinese patients[24]; however, Lau et al. did not find such an association among a German population[25].

The ratio of HDM major allergens Der f 1 and Der p 1 varied in the household dust samples from the different cities. However, this variation does not necessarily give rise to a differential molecular sensitization towards Der p 1 and Der f 1. Despite the low levels of Der f 1 in Haikou dust samples, the specific IgE prevalences against Der p and Der f are equally high among HDM asthmatic children in Haikou[13]. Allergic subjects from Guangzhou show very similar levels of specific IgE-reactivity towards both Der f 1 and Der p 1[26], despite relatively higher levels of Der f 1 in the dust samples[14]. In terms of sensitization, it is likely that the immune system cannot distinguish between exposure to Der f or Der f allergens due to their large degree of homology and identity[27-30], and there is no obvious linear relationship between allergen exposure levels and sensitization[28].

In subtropical and tropical regions, the storage mite *B. tropicalis* is known to be a major source of allergens[27,31]. In this study, specific *B. tropicalis* antigens were found in all dust samples collected in Haikou, and this supports the finding that *B. tropicalis,* together with HDM, is an important allergen source in Haikou[13]. The infestations of *T. putrescentiae* and *A. siro* have not been extensively investigated[25], but *T. putrescentiae* has been detected in Asian households at low levels[32-33]. The antigen prevalence of these mites is generally low in our study, except in samples from Haikou and Chongqing. However, the absence of any dose-response data makes it difficult to contextualize the detected levels with respect to sensitization and disease.

The relative importance of cockroach levels as allergen sources in China is not fully understood. Very few sera selected from across China show IgE-reactivity against cockroach allergens present on allergen micro-array chips[34]. Specific IgE against cockroach was found in 13% of the subjects who visited pediatric and respiratory clinics in Guangzhou, mainly at low IgE levels and with IgE cross-reactivity towards HDM allergens[35]. In this study, cockroach specific antigen levels in samples from Wuzhou and Haikou were up to twenty times higher compared to those in the Guangzhou dust samples. Further investigations are necessary to evaluate the importance of cockroach antigens in cities with high allergen levels, such as Haikou and Wuzhou.

In agreement with other studies, we found that HDM allergen levels in dust samples from bedding are significantly higher than those found in living room dust samples[14]. In addition, we found that antigens from *B. tropicalis* and *T. putrescentiae* showed significantly higher levels in bedding than in the living room in samples taken from the city of Haikou. Storage mites are associated with food items, but they are also detected in furniture, and the tropical climate of Haikou may favor the presence of storage mites in bedding. Only cockroach antigens showed higher prevalence and concentrations in living room samples compared to bedding samples; however, in Haikou there were no significant differences between living room and bedding samples.

The seasonal variation in HDM allergens from Nanjing dust samples is similar to what has previously been observed in Guangzhou[14]. The HDM allergen levels in dust peak during months with more than 75% relative humidity. In Nanjing, these conditions occur from July to December.

CONCLUSION

The percentages of Chinese households having

HDM allergen levels associated with a risk of developing sensitization and asthma were 65% and 25%, respectively. Cities in southern and central China have 50 times more HDM major allergens compared to cities in northern and western China. The distribution of Der p 1 and Der f 1 varies across China but is not necessarily reflected in differential molecular sensitization. Storage and cockroach specific antigens are mostly found in the tropical part of China. The presence of indoor HDM allergens, often at relatively high levels, in the majority of Chinese households might be an important factor contributing to the Chinese population's sensitization and development of allergic disease.

ACKNOWLEDGMENTS

We thank LIU Tian Tian and ZHONG Hai Feng for extensive work of dust extraction and allergen ELISA measurements, and we thank the staff at the involved clinics in for the dust collection. Last but not the least we thank the hundreds of households for providing their time and access to their houses.

CONFLICT OF INTEREST

ZHENG Yi Wu, LAI Xu Xin, GJESING Birgitte, and SPANGFORT D. Michael are all employed by ALK.

CONTRIBUTIONS

ZHENG Yi Wu designed the project, interpreted the data, and wrote the manuscript. LAI Xu Xin and GJESING Birgitte developed and validated the methods and commented on the manuscript. They were also involved in the project design. ZHANG Luo, ZHONG Nan Shan, and SPANGFORT D. Michael supervised the project and co-wrote the manuscript. ZHAO De Yu, ZHANG Chun Qing, CHEN Jian Jun, WEI Qing Yu, CHEN Shi, LIU En Mei, and NORBACK Dan were involved in the project design, collected dust samples, and commented on the manuscript.

Received: June 25, 2015;
Accepted: August 19 2015

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