



## Original article

# Assessment of airborne endotoxin in sandstorm dust and indoor environments using a novel passive sampling device in Al Zulfi city, Saudi Arabia – Establishing threshold exposure levels

Rajendran Vijayakumar<sup>a,\*</sup>, Faiz Abdulaziz Alfaiz<sup>a</sup>, Esam S. Al-Malki<sup>a</sup>, Tim Sandle<sup>b</sup>

<sup>a</sup> Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah 11952, Saudi Arabia

<sup>b</sup> Head of Microbiology, Risk Management and Sterility Assurance, Bio Products Laboratory, Elstree, United Kingdom



## ARTICLE INFO

## Article history:

Received 31 October 2020

Revised 3 December 2020

Accepted 6 December 2020

Available online 11 December 2020

## Keywords:

Airborne endotoxin

Bacterial endotoxin

Dust storms

Electrostatic dust cloth

Indoor

LAL test

Microorganisms

## ABSTRACT

The impact of sandstorm dust events affects local air quality and public health. These issues are becoming of greater concern in Saudi Arabia. There is a significant lack of research on airborne endotoxin exposure and analysis in the Middle East countries and no coherent body of research exists focusing on sandstorm dust in worldwide. In this study, we used a novel design of an aluminum foil plate (AFP) electrostatic dust cloth (EDC) for the passive air sampling of sandstorm dust. A total of 38 sandstorm dust samples were collected during sandstorm episodes occurring between January and April 2020 in both indoor (7 days,  $n = 20$ ) and outdoor environments (24 h,  $n = 18$ ). After exposure, and following an extraction procedure, bacterial endotoxin levels were measured using the *Limulus* Amoebocyte Lysate (LAL) gel clot method. The study highlights that the airborne endotoxin level observed was between 10 and 200 EU/m<sup>2</sup> in both indoor and outdoor environments, during a sandstorm event. Agricultural activities and farmhouses observed higher airborne endotoxin levels. In general, increased endotoxin levels were related to the severity of the sandstorms. Given that the observed values were high as per existing guidelines for respiratory health, we recommend the setting an occupational airborne exposure limit for bacterial endotoxin. This is the first report and further studies across various sandstorm-hit regions will need to be undertaken, together with various sampling methods, in order to assess for seasonal and geographic trends.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Sandstorms, also called dust storms, occur frequently and periodically under the strong winds which blow the dust across the dry deserts around the globe, especially in the Middle East countries. Sandstorm episodes in the Kingdom of Saudi Arabia are seasonal, with the majority of atmospheric transport happening within the February to May months (Griffin, 2007). According to the World Health Organization, dust storms contribute to poor air quality to the extent that sandstorms are attributed to the deaths of around

7 million people every year. The impact of dust events on local air quality and public health is becoming of greater concern in Saudi Arabia following the occurrence of more frequent and severe sandstorms in recent years (Ayoub Meo et al., 2013).

Dusts of sandstorms contain many microbial allergens including endotoxins. Endotoxins are lipopolysaccharides (LPS) naturally present within the outer membrane of Gram-negative bacteria and they are ubiquitous indoor bio-contaminants, with potent immune-stimulatory and proinflammatory properties that contribute to the development and severity of asthma and other respiratory symptoms (Shamsollahi et al., 2019; Thorne et al., 2005). Although the study of dust storm material has attracted many researchers worldwide (Behzad et al., 2018; Kim and Chung, 2010; Kwaasi et al., 1998; Vijayakumar et al., 2017), only limited studies have evaluated the effects of endotoxins on human health and such studies have been limited to endotoxin loads in house dust, collected from floors and beds by vacuuming, as a surrogate for exposure to airborne endotoxins (Dassonville et al., 2008; Gehring et al., 2004; Wickens et al., 2003). These studies have

\* Corresponding author at: Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah 11952, Saudi Arabia.

E-mail address: [v.kumar@mu.edu.sa](mailto:v.kumar@mu.edu.sa) (R. Vijayakumar).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

drawn an association between exposure to endotoxin in dust and a role in aggravating asthma in adults and children, and airflow obstruction (Lai et al., 2012; Rizzo et al., 1997). The concentrations of indoor airborne endotoxins are affected by factors within living environments other than house generated dust and endotoxin that have infiltrated from the outdoor air. Hence analyzing endotoxins from the dust generated from sandstorm is highly significant because allergic responses such as asthma, allergic rhinitis, hypersensitivity pneumonitis are the most common medical problems reported in Saudi Arabia and these conditions have a relationship with exposure to endotoxin and dust (Mendy et al., 2020; Pascoe et al., 2020) and the conditions are predominantly associated with inhalation exposure to sandstorm dust particles (Ayoub Meo et al., 2013). Even though researchers understand the health hazard by exposure of sandstorm dust, there have been no studies conducted, until this one, into the endotoxin levels recorded in dust storm samples.

Additionally, endotoxin level thresholds and associated respiratory ailments are reported in many workplaces, especially those set up for the purposes of laboratory animal handling, waste management, and fiberglass manufacturing (Freitas et al., 2016; Newton et al., 2017). Based on this, the Health Council of the Netherlands has suggested that an occupational health limit for airborne endotoxin be set for the general public (Ministerie van Volksgezondheid, 2010). However, there are currently no exposure limits for endotoxin in the Saudi Arabia and other Middle East countries, which also have a different environmental context. Thus, it is an important need to analyze to endotoxin levels in the environmental samples in frequent sandstorm hit regions. One reason for the paucity of data is due to insufficient knowledge in relation to sandstorm dust sample collection and analysis for endotoxin. Hence, an easy method of sample collection and analysis is also required.

Based on the above information, this study outlines the development of a passive air-sampling device to collect airborne endotoxin from sandstorm dust and presents the analysis of sampled for endotoxin levels collected from various indoor and outdoor environments. In addition, the paper recommends some airborne endotoxin levels for sandstorm dust and presents some precautionary measures to reduce exposure.

## 2. Materials and methods

### 2.1. Study location

The study location was Al Zulfi city, located in Riyadh province in the Central desert region of Saudi Arabia. The city it lies in the northern-central region of the Najd and to the south of the Samnan Valley. The city is surrounded by sand dunes to its north and west, which are known locally as the Al-Thoyr sands (Fig. 1); thus, there is a high possibility of frequent sandstorm episodes. Sandstorm dust samples were collected between January and April 2020 using a novel passive airborne endotoxin sampling device.

### 2.2. Sampling device

A novel passive sampling device was developed using aluminum foil plate (AFP) container. Briefly, a single electrostatic dust cloth (EDC) (Swiffer, 26.5 × 20.3 cm) prepared and placed into a round shape aluminum container (inner diameter 15.3 cm, outer diameter 17.3). The outer lid opening area exposed to the environment was (0.023 m<sup>2</sup>) (Fig. 2). The device was double wrapped with aluminum foil and subjected to 8 h at 200 °C for depyrogenation (Sandle, 2011). After depyrogenation, the AFP-EDC device placed in clean zip-lock polypropylene bag with label.

### 2.3. Sampling locations– Outdoor samples

Sampling devices were prepared and planned for collection proactively with assistance from the Al Zulfi metrological prediction report. During sandstorm episodes the AFP-EDC device was exposed horizontally with fixed by 3 M adhesive tape (3 M, USA), the sample was collected at a height of 1.5-meters above the ground level. The total duration of exposure was a minimum of 24 h. Outdoor samples were collected from the residential area and educational institute campus at the College of Science Al-Zulfi, Majmaah University. In this study, we refer to sand and dust storms using the definitions provided by the World Meteorological Organization (WMO). The classifications for dust storms are: denoted + (strong), 2+ (severe) and 3+ (extreme) (Querol et al., 2019).

### 2.4. Indoor samples

Airborne endotoxin from indoor samples were collected at similar heights of 1.5 m using customized AFP-EDC device. The sampling duration was 7 days and samples were collected from the homes, bedrooms, kids' playrooms and working offices, student laboratories at the College of Science Al-Zulfi. Another set of samples were taken from indoor environment of horse stable at Al-Zulfi, Saudi Arabia, which is considered as animal handling environment and hence a potential worst-case environment.

### 2.5. Endotoxin analysis

Endotoxin extraction from the EDC device was undertaken using a method described by Thorne et al. (2005). However, a modification was required to the Thorne method; briefly, after sampling dust loaded into the EDC, the dust was measured and the weight difference recorded. After weighing, each single EDC was cut into 6 pieces and soaked in 40 ml of endotoxin-free water with 0.05% Tween 20. The content was agitated (125 agitations/minute) for 1 h at 37 °C. From the extract, 2 ml was extracted and centrifuged at 1000g for 1 h. The supernatant was collected and stored at –20 °C until the endotoxin analysis was performed.

The detection of endotoxin levels in dust samples was performed using the gel-clot *Limulus* Amoebocyte lysate (LAL) assay method, in which endotoxin detected by forming the clotting reaction between LPS and LAL (Endosafe, Charles River, USA). All samples were equilibrated to room temperature and diluted with endotoxin-free water (EndoSafe, Charles River, USA). Firstly, the endotoxin test was standardized by performing the 'inhibition / enhancement test' and adjusting the pH of the extracts (the concern with inhibition is that endotoxin recovery could be less than expected; and with enhancement, that the level of endotoxin quoted is artificially high). All the samples were processed at a pH range between 6.0 and 8.0, as required by the assay. The testing methodology was followed as per the method outlined by the United States Pharmacopoeial Convention chapter <85> "Bacterial Endotoxin Test" (USP, 2012) and CEN standard EN 14031: 2003 "Workplace atmospheres. Determination of airborne endotoxins".

The detectable endotoxin limit was calculated based on formula (Eq.1) for the Maximum Valid Dilution (MVD). Where:

$$MVD = \frac{\text{Endotoxin limit}(\frac{EU}{mL})}{\lambda} \quad (1)$$

Samples were tested in the presence of both positive and negative controls. A quadruplicate test was performed for each dilution. The minimum sample dilution, prepared with water dilution was 1:16, and the maximum dilution performed was 1:12800. At first, a 10-fold dilution series was prepared, followed by a two-fold dilution series. The amount of endotoxin in a sample was expressed in

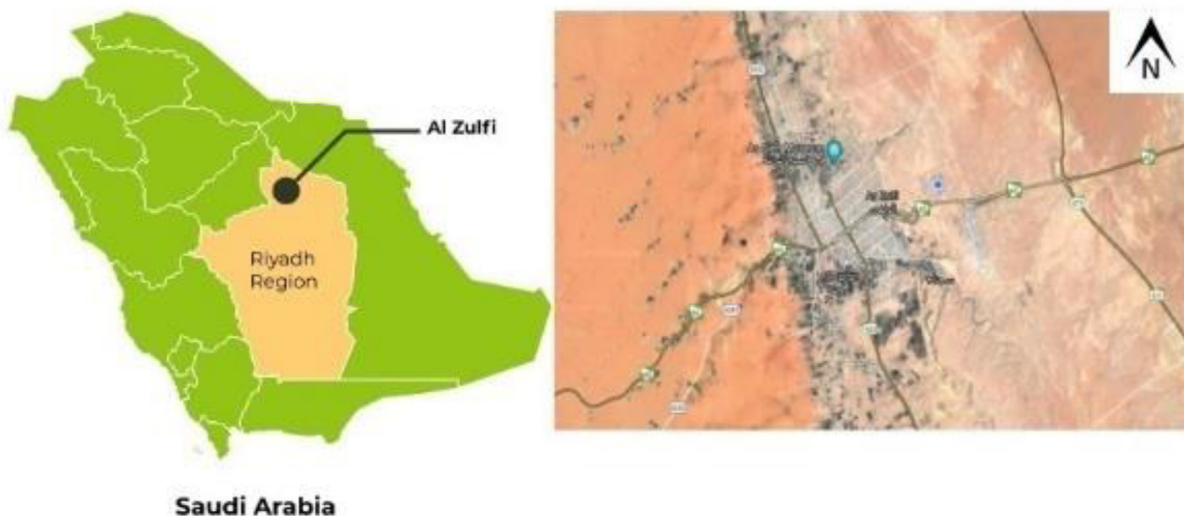


Fig. 1. Map of the study area Al-Zulfi city, Saudi Arabia.

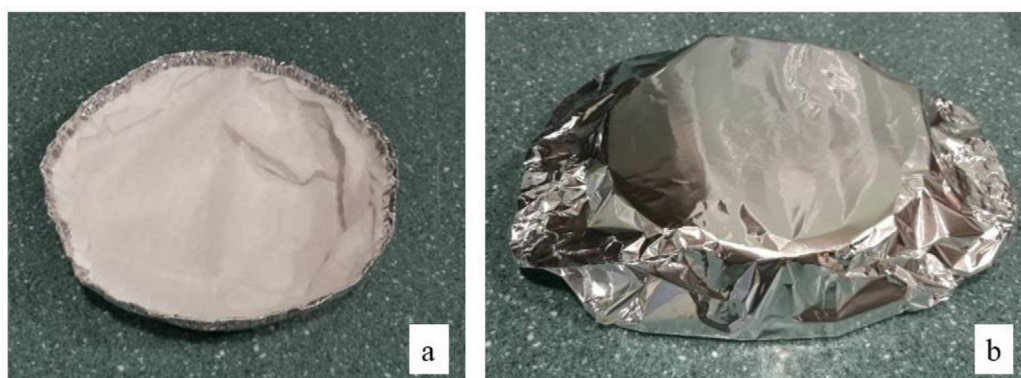


Fig. 2. A customized novel device of 'Aluminum foil plate (AFP) – electrostatic dust cloth (EDC) for passive air sampling of airborne endotoxin (a) EDC not wrapped with aluminum foil (b) Wrapped AFP-EDC device ready for depyrogenation.

endotoxin units EU/m<sup>2</sup>. The lysate sensitivity for the minimum endotoxin detection limit was 0.03125 EU/ml. A standard curve test was performed whenever a new lot of control standard endotoxin (CSE) and LAL reagent was received.

### 2.6. Statistical analysis

The significance of the observed endotoxin levels of various tested samples were compared using Student's *t* test; here a *p* value less than 0.05 was considered as statistically significant.

### 3. Results

During the study period, 38 sandstorm dust sample sets were collected. These consisted of 20 samples from the indoor environments of homes, educational institutes such as a staff office room, student's laboratories, and a farmhouse and stable. Another 18 samples were collected from the outdoor environment including residential areas, outside the entry of the college gate, agricultural areas in desert region and the outside of horse stable.

#### 3.1. Airborne endotoxin levels in indoor samples

Regarding the indoor samples, the range of endotoxin levels observed in staff office was 2.5–12.5 EU/m<sup>2</sup>. However, in the indoor environment of the research laboratories, samples exposed near windows showed a slightly lower range of 2.5–10 EU/m<sup>2</sup>. In contrast, the samples collected from the homes showed higher levels, in the range of 5–50 EU/m<sup>2</sup>. For each sampling time the number of sandstorms and their severity were compared. This showed that the increased severity of sandstorm dust directly related to higher endotoxin levels. In the home, the site recording the highest levels was in bedrooms near to windows, where the collected samples showed a maximum of 50 EU/m<sup>2</sup>. Similarly, samples were collected from the farmhouses, which were in close proximity to agricultural farm areas, showing between 20 and 50 EU/m<sup>2</sup>. The highest levels of endotoxin observed was ≥200 EU/m<sup>2</sup>, from samples collected from a horse stable (Table 1).

#### 3.2. Airborne endotoxin levels in outdoor sandstorm dust samples

In the outdoor samples, all samples were exposed for 24 h. The endotoxin levels observed near the residential areas and the educational institute area were between 20 and 100 EU/m<sup>2</sup>. Some very

**Table 1**  
Airborne endotoxin levels in indoor samples (sampling duration 7 days).

Sample category (n)	Sampling location	Sandstorm episodes (number of occurrence)	Sandstorm dust severity <sup>#</sup>	Endotoxin levels <sup>§</sup> (≥EU/m <sup>2</sup> )	Range of Endotoxin levels (EU/m <sup>2</sup> )
Home (6)	Bedroom - near Window (Home 1)	1	+	50	5 to 50
	Bedroom - near Window (Home 2)	1	3+	25	
	Kids room - near window (Home 1)	1	3+	6	
	Bedroom - near Window (Home 3)	3	2+,3+	50	
	Kitchen - near window (Home 2)	1	+	5	
	Main hall- near window (Home 3)	1	2+	10	
	Educational institute -staff room (4)	Staff - office room	1	3+	
Cub board		1	2+	2.5	
Near window		1	2+	5	
Near window		1	+	5	
Educational institute - Laboratories (3)	Students research lab 1	1	+	2.5	2.5 to 10
	Students research lab 2	2	2+	5	
	Research lab 3	2	3+	10	
Farmhouse (4)	Bedroom - near window	2	+2+	25	20 to 50
	Room - near agricultural farms	2	+3+	50	
	Bedroom - near window	2	3+	20	
	Farmhouse	1	3+	50	
Horse stable (3)	Indoor - horse stable 1	1	2+	100	75 - 200
	Indoor - horse stable 2	2	+2+	200	
	Indoor - horse stable 3	1	+	75	

<sup>#</sup>Intensity of sandstorm dust are classified as per WMO, strong +, severe 2+, extreme 3+.

<sup>§</sup> Endotoxin levels are expressed in ≤ and ≥ as per gel-clot test method.

high values were observed in samples collected from dates and grass farming land. Samples collected from outside of a horse stable, which serves as a horse riding training area, showing more than 200 EU/m<sup>2</sup> (Table 2). The overall mean of endotoxin levels from the residential and educational institute area was 62 EU/m<sup>2</sup> and agricultural farm and horse stable recorded a mean value of 145 EU/m<sup>2</sup>. Outdoor samples were significantly higher in terms of endotoxin levels when compared to indoor samples (*p*-value 000183).

In this study, the severity of sandstorm was analyzed with endotoxin levels and it was found that storm severity is

directly proportional to increased level of airborne endotoxin (Figs. 3 and 4).

#### 4. Discussion

Sand and dust storm particles from Middle East-Central Asia contains both chemicals (including quartz, other silicate minerals, carbonates, oxides, sulphates, salts) and biological toxigenic compounds (pollen spores, bacteria, fungi and viruses) (Goudie, 2014). The biological agents are capable of surviving during the

**Table 2**  
Airborne endotoxin levels of outdoor samples collected (sampling duration 24 h) during sandstorm hit.

Sample category (n)	Sampling location	Sandstorm dust severity <sup>#</sup>	Endotoxin levels <sup>§</sup> (≥EU/m <sup>2</sup> )	Range of Endotoxin levels (EU/m <sup>2</sup> )
Residential area - Home (7)	Home 1 - Roof top	+	30	20 to 100
	Home 2 - Roof top	+	25-50	
	Home 3 - Roof top	3+	100	
	Home 4 - Roof top	3+	75	
	Home 5- Roof top	+	50	
	Home 6- Roof top	3+	100	
	Home 7- Roof top	2+	20	
Educational institute (4)	Near entrance	2+	100	75 to 100
	Staff - car parking area	2+	75	
	Students - car parking area	3+	100	
	Exit road	2+	75	
Agricultural activities (5)	Dates farming	3+	> 200	50 to > 200
	Grass farming	3+	> 200	
	Combined agriculture land	3+	150-200	
	Dates, lemon - mixed	2+	100	
	Farms - Agricultural	+	50	
Horse stable (2)	Outside stable - near horse training area (stable 1)	3+	> 200	> 200
	Outside stable - near horse training area (stable 2)	3+	> 200	

<sup>#</sup>Intensity of sandstorm dust are classified as per WMO, strong +, severe 2+, extreme 3+.

<sup>§</sup>Endotoxin levels are expressed in ≤ and ≥ as per gel-clot test method.



**Fig. 3.** Pictures illustrates severity of sandstorm hits in Al-Zulfi city; (a) no sandstorm with clear sky; (b) strong +; (c) extreme 3+.

long-range transport of dust; they have been shown to disperse globally and they can affect human health far from the point of origin (Behzad et al., 2018; Griffin, 2007; Kim and Chung, 2010; Vijayakumar et al., 2017). In particular, bacterial endotoxin is a potential biological compound within the sandstorm dust and many studies have reported that airborne endotoxin exposure in both indoor and outdoor workplaces has been linked to various

health hazards (Farokhi et al., 2018; Reed and Milton, 2001; Thorne et al., 2005). In the context of this study, although respiratory health issues have been increasing among the Saudi population in recent years ostensibly due to frequent sandstorm hits, there has not hitherto been a study conducted to analyse the airborne endotoxin in sandstorm dust, despite the causal relationship between endotoxin and respiratory illness. One obstacle to this

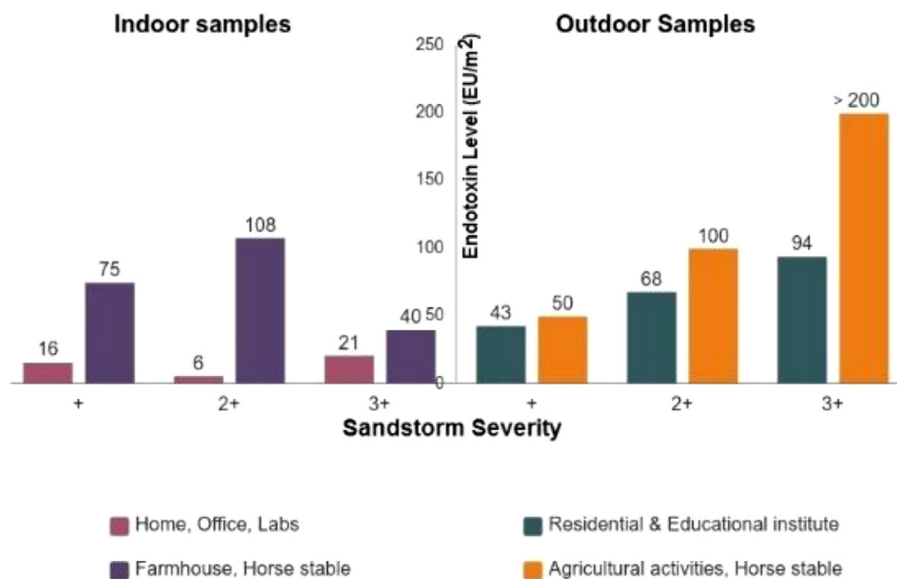


Fig. 4. Observed endotoxin levels compared with sandstorm severity for both indoor samples and outdoor sandstorm dust samples.

could be that dust sample collection, endotoxin extraction, and testing methodologies for assessing airborne endotoxin from sand dust has not yet standardised. Thus, one aim of the present study was designed to study the endotoxin levels of sandstorm settled dust in outdoor and indoor environment in Saudi Arabia by using novel AFP-EDC device.

#### 4.1. Novel design of passive air sampling device

Passive dust sample collection was conducted using electrostatic dust fall collector. This was first developed by Noss et al. (2008) for indoor airborne endotoxin exposure. In this study, we modified and developed a new device which contained a single electrostatic cloth (Swiffer, Procter and Gamble, USA) positioned in a round AFP. Previously, many researchers have attempted passive airborne dust samples collection methods using pizza box, Petri dishes etc., (Karlsson et al., 2002; Würtz et al., 2005). These devices have some demerits including cardboard boxes releasing particles, being difficult to depyrogenate, and possessing collection efficiency issues in relation to the smaller surface exposure area in Petri dishes. Our device has overcome these issues and has the advantage of being easy to use by the samplers; moreover, the device can be easily depyrogenated. It is also of a low-cost, it is straightforward to track samples using marker pen, and to fix the device onto the sampling surfaces. The airborne dust settles onto the AFP surface and is captured by the electrostatic properties of Swiffer cloth. This form of capture has been previously reported as an effective sample collection mechanism for airborne and surface settled endotoxin by various authors (Adams et al., 2015; Cox et al., 2017; Kilburg-Basnyat, 2015). The EDC test device provides similar types of results as using active air sampler to capture endotoxins, bacteria and fungi (Kilburg-Basnyat, 2015). Thus, the present study AFP-EDC device will provide a feasible, useful tool by which to assess exposure to airborne endotoxin in home and work environments.

#### 4.2. Indoor samples

Analyzing endotoxin levels in indoor environment is very important, given most people spend most of their time in an indoor environment. Occupational studies have linked indoor concentra-

tions of airborne endotoxin to adverse respiratory health effects in adults, including increased bronchial hyper responsiveness, lung function decline, and obstructive airway disease (Eduard et al., 2009). With our study airborne endotoxin samples collected from various indoor environments while sandstorm episodes. Firstly, 6 samples were collected from the home showing in the range of 5 to 50 EU/m<sup>2</sup> (mean 16.3 EU/m<sup>2</sup>) Similarly, samples were collected from the farmhouses, which are very near to agricultural farm areas, showing 20 to 50 EU/m<sup>2</sup>. The reason why such levels were detected was because dust could diffuse through gaps of windows and settle closer to areas around windows. Another variation is with the design of the home. As an example, authors from Japan reported that indoor endotoxin concentrations were significantly associated with the household characteristics in addition to outdoor endotoxin concentrations (Yoda et al., 2017).

There are other samples collected from the farmhouses, which are close to agricultural farm areas, showing 20 to 50 EU/m<sup>2</sup>. Each of the homes in this area are floored by carpets and this may be another contributory factor to the higher levels of airborne endotoxin recorded. This finding is similar to a report by Mazique et al. (2011) who found higher level of endotoxin in rooms with carpet flooring compared with homes with wooden flooring. A mean level of endotoxin of 33.44 EU/m<sup>2</sup> was recorded from the farmhouse samples. A further factor when farmhouses are compared to residential areas, farmhouses are not cleaned to the same extent, which could provide another reason for the higher levels of endotoxin. This has been evidenced by data that shows how frequent cleaning reduces indoor airborne endotoxin concentrations (Dassonville et al., 2008; Mazique et al., 2011). Such studies indicate that the variation of endotoxin levels in between houses and rooms is due to building characteristics or living environment, especially where the endotoxin level of settled dust is increased by lower level of cleaning and some cases by the presence of pet animals such as cat or dog.

In the workplace, the concentration of endotoxin responsible for triggering respiratory effects (including asthma) is often below the permissible exposure limits or occupational exposure limits (Brooks, 2013) thus, dust exposure at the workplace has to be controlled for the protection of the employees. In the present study, the range of endotoxin levels observed in a staff office was 2.5–12.5 EU/m<sup>2</sup>. However, in the indoor environment of student

research laboratories, samples that were exposed near windows showed lower ranges, at 2.5–10 EU/m<sup>2</sup>. On most of the days of sampling, student activity was very limited and the maximum occupancy was 20 students, which accounts for the lower level. With other indoor samples, the highest levels of endotoxin observed across all indoor samples was greater than 200 EU/m<sup>2</sup>, which was collected from horse stable. These results have parallels with very high concentrations of endotoxins reported (98,990 EU/m<sup>3</sup> and 83,640 EU/m<sup>3</sup>) inside swine and poultry buildings from other studies (Jonges et al., 2015; Lawniczek-Walczyk et al., 2013). Another study conducted in a horse stable reported that geometric means of personal exposure endotoxin were 608 EU/m<sup>3</sup> (Samadi et al., 2009). However, high values of airborne endotoxin vary according to the size of farms, sampling methods, extraction procedures and analysis.

#### 4.3. Outdoor samples

The endotoxin levels in the outdoor sandstorm dust samples were remarkably interesting, especially in the context of there being no previous studies analyzing the endotoxin content of sandstorm dust. In contrast, there are many studies available in relation to endotoxin levels in outdoor rural, urban and school environments (Jacobs et al., 2014; Ortiz-Martínez et al., 2015; Yoda et al., 2017). In this study, samples in outdoor environments were exposed for 24 h (from the rooftops of residential homes) and the endotoxin levels observed near residential area and educational institute area ranged from 20 up to 100 EU/m<sup>2</sup>. This is comparatively higher than maximum ambient concentration observed from the polluted urban environment in Beijing, from a different study, which was recorded as 75EU/m<sup>3</sup> (Guan et al., 2014). Hence, our findings trigger concern that endotoxin in sandstorm dust is relatively comparable to the levels of endotoxin recovered from polluted urban areas. The second set of outdoor samples were collected from dates and grass farming land; these samples showed > 200 EU/m<sup>2</sup>. These levels are unsurprising because as soil and vegetation of agricultural land, dust carrying these particles already identified as potential sources of airborne endotoxin. Similar to indoor samples collected in the agricultural activities area, the outdoor samples collected from various farming activities including dates, grass, lemon and combined agricultural land showed geometric mean values of airborne endotoxin at 145.8 EU/m<sup>2</sup> for a 24 h exposure. Many reports suggest that agricultural activities could have a significant impact of increased endotoxin concentration in the air (Pavilonis et al., 2013; Spaan et al., 2006). Samples collected from outside of horse stable, which is horse riding training area showed more than 200 EU/m<sup>2</sup>, probably because these areas are covered with wet soil and active animal activities. This is higher than similar study done by Freitas et al. (2016) where it was reported that animals of housing rats, mice, guinea pigs, rabbits or hamsters kept in exposed workplaces had higher concentrations of endotoxin, with a median value of 34.2 EU/mg of dust.

Comparing the endotoxin levels in different environments, outdoor samples are significantly higher than indoor samples (*p*-value 0.000183). Our results do contrast with Yoda et al., (2017) as they found that indoor endotoxin concentrations were higher than outdoor concentrations. In addition, they discovered that the indoor endotoxin concentrations significantly correlated with outdoor concentrations in relation to multiple samples. However, the ambient outdoor environment was totally different to the present study.

While there have been no previous studies examining household dust, during the last decade, occupational exposure to endotoxin via bioaerosol inhalation has been documented in various environments worldwide. Furthermore, an associations with endotoxin levels in the air and ill-health effects have been documented

(Liebers et al., 2020). Hence, the measurement of exposure to endotoxin in different workplaces can contribute to understanding these complex and multifactorial scenarios. To fully interpret the totality of data, this requires a review of testing methods, geographical change, type of activities taking place etc. Looking at some of these studies, airborne endotoxin concentrations were between 10 and 300 EU/m<sup>3</sup> in a cheese production plant in France (Simon and Duquenne, 2014). Whereas, in a fish processing factory airborne endotoxin levels were found to range from 3 to 92 EU/m<sup>3</sup> (Dahlman-Höglund et al., 2016). Another interesting study was undertaken by Straumfors et al. (2015) in high dust generated environment of grain stores. This revealed a geometric mean for airborne endotoxin of 662 EU/m<sup>3</sup>. Further, a report by Basinas et al. (2017) collected 38 personal exposure measurements from dairy farmers and reported that dairy farm workers can be exposed to high and variable levels of inhalable dust and endotoxin (900 EU/m<sup>3</sup>) and consequently such workers may be at risk of respiratory disease. Similarly research into such diverse working environments as greenhouses with plants, sawmill industries, waste composting plants, poultry farming and metalworking industries, show comparable airborne endotoxin ranges (Dahlman-Höglund et al., 2016; Giofrè et al., 2018; Gutarowska et al., 2015; Straumfors et al., 2018; Thilings et al., 2015).

#### 4.4. Concerning seasonal variation

Hardly any seasonal variation was found in endotoxin concentrations. In this study, samples were collected from January to April, which is the end of winter in the central region of Saudi Arabia and the main time of sandstorm activity. In this study location, climates in the desert region are either cold winter or a hot summer, there is no equivalent season of autumn–winter and spring summer. Hence, we were not able to compare full seasonal variations. However, from a report by Park et al. (2000) the lowest levels of airborne endotoxin levels were observed in the winter. The researchers also they reported that no seasonal influence indoors based on floor dust samples was observed, based on tests conducted in North American homes. It follows that the effect of seasonal on dust endotoxin remains unclear, because there are various contradictory reports worldwide, some of which state that airborne endotoxin levels are at their highest in spring and autumn, while other reports indicate that levels are uppermost in the summer (Gehring et al., 2004; Hwang et al., 2016; Wickens et al., 2003).

#### 4.5. Health risk to people

Concerning occupational risk to the public, many authors have reported that inhalation exposure to dust, endotoxin, and microorganisms may place the exposed subjects at risk of developing respiratory complaints and other health problems. For example, Farokhi et al. (2018) reported that respiratory health effects due to endotoxin are not only an effect of high exposure levels, since respiratory effects are also apparent in exposure groups below 100 EU/m<sup>3</sup>. Therefore, both low exposure in the environment and long-term effects have to be considered since development of adverse health effects is generally not only based on the level of airborne endotoxin exposures, but also on the duration of exposure. The sandstorm dust duration varies between several hours to several days. Thus public must be aware about a heterogeneous mixture of bioaerosols include airborne endotoxin present in dust and preventive measure are recommended for workers who exposed outside dust storm since these people are at high risk to develop allergic diseases.

#### 4.6. Exposure limit

Concerning the exposure limits of airborne endotoxin, the implementation of a general health based threshold airborne endotoxin limit for outdoor sandstorm dust is not possible at the moment. Endotoxin levels are on the product of various environmental factors such as temperature, wind speed and severity of dust storm so identifying a threshold limit is technically challenging. In addition, dust contains other bioaerosols, minerals and particulate matters that are very heterogeneous and differ from area to area (Behzad et al., 2018). Furthermore, an exposure level would need to differ for indoor and outdoor environments. Our study results found that there is significant variation of sandstorm dust endotoxin between residential and agricultural environments (Farokhi et al., 2018; Liebers et al., 2020). However, setting endotoxin limits should become a subject of interest to governmental institutions in order to develop guidelines to protect the public health and safety of their inhabitants in areas prone to sandstorms. Specific to this study, because there are no guidelines that recommend maximum endotoxin exposure during dust storm, there is a need for such measures to be developed. By way of comparison, the Dutch Expert Committee on Occupational Safety of the Health Council recommends a health-based occupational exposure limit of 90 EU/m<sup>3</sup> (Castellan et al., 1987). In a different study, based on the occupational exposure limit, a tentative limit of 30 EU/m<sup>3</sup> was recommended for the general population living in the surroundings of livestock farms (Farokhi et al., 2018; Liebers et al., 2020). Setting a different level, the social economic council in the Netherland introduced a threshold limit value of 200 EU/m<sup>3</sup>. These variations in cut-off values show the need for further clinical based research. Comparing these reference values, the present study showed that median values airborne endotoxin levels in residential, educational institutes and agricultural areas were 75 and >200 EU/m<sup>2</sup>. Airborne endotoxin increases in values based on wind speed and intensity of sandstorm.

#### 4.7. Recommendations and precautionary measures

In the current research findings, indoor environment endotoxin levels range 10 to 200 EU/m<sup>2</sup> and outdoor test limits showed a minimum of 200 EU/m<sup>2</sup>. Hence, this study recommends, cautiously, that wearing facemasks outdoors that filter dust pollutants and the avoidance of unnecessary exposure during sandstorms can be adopted in order to reduce the level of airborne endotoxin exposure. For indoor environment, improved hygiene practices such as frequent cleaning using a detergent and mopping the floor near windows may decrease indoor endotoxin levels.

#### 4.8. Limitations

There are some limitations in this study. First, the sampling duration differs between indoor and outdoor samples. Outdoor samples were only exposed for 24 h due to a blown dust cover on the EDC; however indoor samples, subject to less harsh conditions, were exposed for 7 days. A second limitation in this study is that since sandstorm dust weights could not be measured with accuracy, this means the endotoxin levels measured from the EDC cloth can only be expressed as EU/m<sup>2</sup>. This does not allow a direct comparison to be made with most other studies where data is expressed as EU/m<sup>3</sup> based on active air sampling (BS EN 14031, 2003). However, dust samples endotoxin levels can be expressed both EU/g and EU/m<sup>2</sup>, and this approach is generally accepted as per previous literatures (Noss et al., 2008). A third limitation is that this study could only analyze a minimum number of samples due to the occurrence of sandstorm episodes and difficulties in sample collection. Another important limitation is this study

measured endotoxin values using the gel-clot technique, which detects the presence of endotoxin through the clotting reaction between LPS and lysate and concentration determined semi-quantitatively by subsequent dilutions. This method is simplest and least expensive and it has been used many similar studies previously (Paba et al., 2013). However, further comparative studies using other endotoxin testing methods, such as kinetic chromogenic or turbidimetric methods, may yield more precise data.

#### 4.9. Future directions

Overall, the present study results suggest that an endotoxin limit needs to be established for sandstorm dust with a view to informing about public health risks. To establish a reliable limit, similar kinds of studies are needed consisting of a large series of samples. The collection of field data must accompany clinical studies to demonstrate the extent that the effect of airborne endotoxin in sandstorm dust induces an allergic reaction to local communities. Besides the above scenarios, we consider that desert dust alert and monitoring systems might be a very powerful tool for alerting the most sensible or exposed population to air pollution to take special measures to protect themselves from high desert dust exposure to airborne endotoxin levels.

### 5. Conclusion

In conclusion, this study developed a novel method: a simple passive air sampling device suitable for the collection of sandstorm dust for endotoxin analysis. Using this device, the indoor and outdoor airborne endotoxin content in sandstorm dust observed in residential areas recorded up to 100 EU/m<sup>2</sup>. More than 200 EU/m<sup>2</sup> were observed in agricultural farmhouses indoor and outdoor activities. These levels are up to three times higher in ambient air compared with the proposed threshold values, hence presenting a probable public health issue. It is of interest that levels of airborne endotoxin are directly proportional to the intensity of sandstorm dust. This is the very first report analyses the endotoxin level in sandstorm dust and similar kinds of studies needed, taking a larger series of samples, collected using passive and active sampling in other desert regions. Finally, this study suggests that measures need to be taken to reduce occupational exposure levels and airborne endotoxin studies should become the norm in order to alert the public health department through air quality monitoring programs in desert regions.

### Funding

This research work was funded by the Basic Science Research Unit, Deanship of Scientific Research at Majmaah University, Kingdom of Saudi Arabia (Project number 1439-8).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgement

The authors extend their appreciation to the Basic Science Research Unit, Deanship of Scientific Research at Majmaah University, Kingdom of Saudi Arabia for funding this research work (Project number 1439-8). Authors also would like to thank Mr. Bader Abdulrahman, Mr. Faizal Almutairi for their technical support for sample collection in horse stable and agriculture farms.



## References

- Adams, R.I., Tian, Y., Taylor, J.W., Bruns, T.D., Hyvärinen, A., Tübel, M., 2015. Passive dust collectors for assessing airborne microbial material. *Microbiome* 3 (1), 46. <https://doi.org/10.1186/s40168-015-0112-7>.
- Ayoub Meo, S., Fahad A Al-Kheraiji, M., Fahad AlFaraj, Z., Abdulaziz Alwehaibi, N., Adnan Alderehim, A., 2013. Respiratory and general health complaints in subjects exposed to sandstorm at Riyadh, Saudi Arabia. *Pak. J. Med. Sci.* 29(2), 642–646.
- Basinas, I., Cronin, G., Hogan, V., Sigsgaard, T., Hayes, J., Coggins, A.M., 2017. Exposure to inhalable dust, endotoxin, and total volatile organic carbons on dairy farms using manual and automated feeding systems. *Ann. Work. Expo. Health.* 61 (3), 344–355. <https://doi.org/10.1093/annweh/wxw023>.
- Behzad, H., Mineta, K., Gojbori, T., 2018. Global ramifications of dust and sandstorm microbiota. *Genome. Biol. Evol.* 10 (8), 1970–1987. <https://doi.org/10.1093/gbe/evy134>.
- Brooks, S.M., 2013. Reactive airways dysfunction syndrome and considerations of irritant-induced Asthma. *J. Occup. Environ. Med.* 55 (9), 1118–1120. <https://doi.org/10.1097/JOM.0b013e318229a679>.
- BS EN 14031. Workplace exposure. Quantitative measurement of airborne endotoxins. Published, 17 April 2003.
- Castellan, R.M., Olenchock, S.A., Kinsley, K.B., Hankinson, J.L., 1987. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N. Eng. J. Med.* 317 (10), 605–610. <https://doi.org/10.1056/NEJM198709033171005>.
- Cox, J., Indugula, R., Vesper, S., Zhu, Z., Jandarov, R., Reponen, T., 2017. Comparison of indoor air sampling and dust collection methods for fungal exposure assessment using quantitative PCR. *Environ. Sci. Processes Impacts.* 19 (10), 1312–1319. <https://doi.org/10.1039/C7EM00257B>.
- Dahlman-Höglund, A., Lindgren, Å., Mattsby-Baltzer, I., 2016. Endotoxin in size-separated metal working fluid aerosol particles. *Ann. Occup. Hyg.* 60 (7), 836–844. <https://doi.org/10.1093/annhyg/mew036>.
- Dassonville, C., Demattei, C., Vacquier, B., Bex-Capelle, V., Seta, N., Momas, I., 2008. Indoor airborne endotoxin assessment in homes of Paris newborn babies. *Indoor Air* 18 (6), 480–487. <https://doi.org/10.1111/j.1600-0668.2008.00549.x>.
- Eduard, W., Pearce, N., Douwes, J., 2009. Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. *Chest* 136 (3), 716–725. <https://doi.org/10.1378/chest.08-2192>.
- Farokhi, A., Heederik, D., Smit, L.A.M., 2018. Respiratory health effects of exposure to low levels of airborne endotoxin – a systematic review. *Environ. Health.* 17 (1), 14. <https://doi.org/10.1186/s12940-018-0360-7>.
- Freitas, A.S., Simoneti, C.S., Ferraz, E., Bagatin, E., Brandão, I.T., Silva, C.L., Borges, M. C., Vianna, E.O., 2016. Exposure to high endotoxin concentration increases wheezing prevalence among laboratory animal workers: a cross-sectional study. *BMC Pulm. Med.* 16. <https://doi.org/10.1186/s12890-016-0233-1>.
- Gehring, U., Bischof, W., Borte, M., Herbarth, O., Wichmann, H.-E., Heinrich, J., LISA study group, 2004. Levels and predictors of endotoxin in mattress dust samples from East and West German homes. *Indoor Air* 14 (4), 284–292. <https://doi.org/10.1111/j.1600-0668.2004.00244.x>.
- Gioffrè, A., Marramao, A., Gesu, I.D., Samele, P., Paba, E., Marcelloni, A.M., Chiominto, A., Iavicoli, S., 2018. Exposure to airborne endotoxin in Italian greenhouses: environmental analyses. *Ind. Health.* 56 (2), 150–154. <https://doi.org/10.2486/indhealth.2017-0080>.
- Goudie, A.S., 2014. Desert dust and human health disorders. *Environ. Int.* 63, 101–113. <https://doi.org/10.1016/j.envint.2013.10.011>.
- Griffin, D.W., 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clin. Microbiol. Rev.* 20 (3), 459–477. <https://doi.org/10.1128/CMR.00039-06>.
- Guan, T., Yao, M., Wang, J., Fang, Y., Hu, S., Wang, Y., Dutta, A., Yang, J., Wu, Y., Hu, M., Zhu, T., 2014. Airborne endotoxin in fine particulate matter in Beijing. *Atmos. Environ.* 97, 35–42. <https://doi.org/10.1016/j.atmosenv.2014.08.005>.
- Gutarowska, B., Skóra, J., Stepień, Ł., Szponar, B., Otlewska, A., Pielech-Przybylska, K., 2015. Assessment of microbial contamination within working environments of different types of composting plants. *J. Air. Waste. Manag. Assoc.* (1995), 65(4), 466–478. <https://doi.org/10.1080/10962247.2014.960954>.
- Hwang, S.H., Park, D.J., Park, W.M., Park, D.U., Ahn, J.K., Yoon, C.S., 2016. Seasonal variation in airborne endotoxin levels in indoor environments with different micro-environmental factors in Seoul, South Korea. *Environ. Res.* 145, 101–108. <https://doi.org/10.1016/j.envres.2015.11.025>.
- Jacobs, J.H., Krop, E.J.M., Borrás-Santos, A., Zock, J.-P., Taubel, M., Hyvärinen, A., Pekkanen, J., Doekes, G., Heederik, D.J.J., HITEA schools study consortium, 2014. Endotoxin levels in settled airborne dust in European schools: The HITEA school study. *Indoor Air* 24 (2), 148–157. <https://doi.org/10.1111/ina.12064>.
- Jonges, M., van Leuken, J., Wouters, I., Koch, G., Meijer, A., Koopmans, M., 2015. Wind-mediated spread of low-pathogenic avian influenza virus into the environment during outbreaks at commercial poultry farms. *PLoS ONE* 10 (5). <https://doi.org/10.1371/journal.pone.0125401>.
- Karlsson, A.-S., Hedrén, M., Almqvist, C., Larsson, K., Renström, A., 2002. Evaluation of Petri dish sampling for assessment of cat allergen in airborne dust. *Allergy* 57 (2), 164–168. <https://doi.org/10.1034/j.1398-9995.2002.1s3297.x>.
- Kilburg-Basnyat, B. J., 2015. Validation of electrostatic dust collectors (EDCs) as effective passive samplers [Doctor of Philosophy, University of Iowa]. <https://doi.org/10.17077/jed.ctkvtfv1>.
- Kim, H.S., Chung, Y.S., 2010. On the sandstorms and associated airborne dustfall episodes observed at Cheongwon in Korea in 2005. *Air. Quality. Atmos. Health.* 3 (2), 83–94. <https://doi.org/10.1007/s11869-009-0054-y>.
- Kwaasi, A.A., Parhar, R.S., Al-Mohanna, F.A., Harfi, H.A., Collison, K.S., Al-Sedairy, S.T., 1998. Aeroallergens and viable microbes in sandstorm dust. Potential triggers of allergic and nonallergic respiratory ailments. *Allergy* 53 (3), 255–265. <https://doi.org/10.1111/j.1398-9995.1998.tb03885.x>.
- Lai, P., Fresco, J., Pinilla, M., Macias, A., Brown, R., Englert, J., Hofmann, O., Lederer, J., Hide, W., Christiani, D., Cernadas, M., Baron, R., 2012. Chronic endotoxin exposure produces airflow obstruction and lung dendritic cell expansion. *Am. J. Respir. Cell. Mol. Biol.* 47, 209–217. <https://doi.org/10.1165/rcmb.2011-0447OC>.
- Lawniczek-Walczuk, A., Górny, R.L., Golofit-Szymczak, M., Niesler, A., Wlazlo, A., 2013. Occupational exposure to airborne microorganisms, endotoxins and  $\beta$ -glucans in poultry houses at different stages of the production cycle. *Ann. Agric. Environ. Med.* 20 (2), 259–268.
- Liebers, V., Brüning, T., Raulf, M., 2020. Occupational endotoxin exposure and health effects. *Arch. Toxicol.* <https://doi.org/10.1007/s00204-020-02905-0>.
- Mazique, D., Diette, G., Breyse, P., Matsui, E., McCormack, M., Curtin-Brosnan, J., Williams, D., Peng, R., Hansel, N., 2011. Predictors of airborne endotoxin concentrations in inner city homes. *Environ. Res.* 111 (4), 614–617. <https://doi.org/10.1016/j.envres.2011.03.001>.
- Mendy, A., Metwali, N., Perry, S.S., Chrischilles, E.A., Wang, K., Thorne, P.S., 2020. Household endotoxin reduction in the Louisa Environmental Intervention Project for rural childhood asthma. *Indoor Air* 30 (1), 88–97. <https://doi.org/10.1111/ina.12610>.
- Ministerie van Volksgezondheid, W. en S., 2010, July 15). Endotoxins—Health-based recommended occupational exposure limit—Advisory report—The Health Council of the Netherlands [Publicatie]. Ministerie van Volksgezondheid, Welzijn en Sport. <https://doi.org/10/07/15/endotoxins-health-based-recommended-occupational-exposure-limit>.
- Newton, A.N., Davis, M., Koehler, K., Shreffler, W., Ahluwalia, S., Metwali, N., Thorne, P.S., Paigen, B.J., Matsui, E.C., 2017. Atopy as a modifier of the relationships between endotoxin exposure and symptoms among laboratory animal workers. *Ann. Work. Expo. Health.* 61 (8), 1024–1028. <https://doi.org/10.1093/annweh/wxx061>.
- Noss, I., Wouters, I.M., Visser, M., Heederik, D.J.J., Thorne, P.S., Brunekreef, B., Doekes, G., 2008. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl. Environ. Microbiol.* 74 (18), 5621–5627. <https://doi.org/10.1128/AEM.00619-08>.
- Ortiz-Martínez, M.G., Rodríguez-Cotto, R.I., Ortiz-Rivera, M.A., Pluguez-Turull, C.W., Jiménez-Vélez, B.D., 2015. Linking Endotoxins, African Dust PM10 and Asthma in an Urban and Rural Environment of Puerto Rico. *Mediators Inflamm.* 2015. <https://doi.org/10.1155/2015/784212>.
- Paba, E., Tranfo, G., Corsetti, F., Marcelloni, A.M., Iavicoli, S., 2013. Indoor Exposure to airborne endotoxin: a review of the literature on sampling and analysis methods. *Ind. Health.* 51 (3), 237–255. <https://doi.org/10.2486/indhealth.MS1325>.
- Park, J.H., Spiegelman, D.L., Burge, H.A., Gold, D.R., Chew, G.L., Milton, D.K., 2000. Longitudinal study of dust and airborne endotoxin in the home. *Environ. Health Perspect.* 108 (11), 1023–1028.
- Pascoe, C.D., Jha, A., Basu, S., Mahood, T., Lee, A., Hinshaw, S., Falsafi, R., Hancock, R.E. W., Mookherjee, N., Halayko, A.J., 2020. The importance of reporting house dust mite endotoxin abundance: Impact on the lung transcriptome. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 318 (6), L1229–L1236. <https://doi.org/10.1152/ajplung.00103.2020>.
- Pavilonis, B.T., Anthony, T.R., O’Shaughnessy, P.T., Humann, M.J., Merchant, J.A., Moore, G., Thorne, P.S., Weisel, C.P., Sanderson, W.T., 2013. Indoor and outdoor particulate matter and endotoxin concentrations in an intensely agricultural county. *J. Expo. Sci. Environ. Epidemiol.* 23 (3), 299–305. <https://doi.org/10.1038/jes.2012.123>.
- Querol, X., Tobias, A., Pérez, N., Karanasiou, A., Amato, F., Stafoggia, M., Pérez García-Pando, C., Ginoux, P., Forastiere, F., Gumy, S., Mudu, P., Alastuey, A., 2019. Monitoring the impact of desert dust outbreaks for air quality for health studies. *Environ. Int.* 130. <https://doi.org/10.1016/j.envint.2019.05.061>.
- Reed, C.E., Milton, D.K., 2001. Endotoxin-stimulated innate immunity: a contributing factor for asthma. *J. Allergy. Clin. Immunol.* 108 (2), 157–166. <https://doi.org/10.1067/mai.2001.116862>.
- Rizzo, M.C., Naspitz, C.K., Fernández-Caldas, E., Lockey, R.F., Mimiça, I., Solé, D., 1997. Endotoxin exposure and symptoms in asthmatic children. *Pediatr. Allergy. Immunol.* 8 (3), 121–126. <https://doi.org/10.1111/j.1399-3038.1997.tb00164.x>.
- Samadi, S., Wouters, I.M., Houben, R., Jamshidifard, A.-R., Van Eerdenburg, F., Heederik, D.J.J., 2009. Exposure to inhalable dust, endotoxins, beta(1->3)-glucans, and airborne microorganisms in horse stables. *Ann. Occup. Hyg.* 53 (6), 595–603. <https://doi.org/10.1093/annhyg/mep040>.
- Sandle, T., 2011. A practical approach to depyrogenation studies using bacterial endotoxin. *J. GXP. Compliance.* 15, 90–96.
- Shamsollahi, H.R., Ghoochani, M., Jaafari, J., Moosavi, A., Sillanpää, M., Alimohammadi, M., 2019. Environmental exposure to endotoxin and its health outcomes: A systematic review. *Ecotoxicol. Environ. Saf.* 174, 236–244. <https://doi.org/10.1016/j.ecoenv.2019.02.046>.
- Simon, X., Duquenne, P., 2014. Assessment of workers’ exposure to bioaerosols in a French cheese factory. *Ann. Occup. Hyg.* 58 (6), 677–692. <https://doi.org/10.1093/annhyg/meu027>.

- Spaan, S., Wouters, I.M., Oosting, I., Doekes, G., Heederik, D., 2006. Exposure to inhalable dust and endotoxins in agricultural industries. *J. Environ. Monit.* 8 (1), 63–72. <https://doi.org/10.1039/B509838F>.
- Straumfors, A., Haldal, K.K., Wouters, I.M., Eduard, W., 2015. Work tasks as determinants of grain dust and microbial exposure in the Norwegian grain and compound feed industry. *Ann. Occup. Hyg.* 59 (6), 724–736. <https://doi.org/10.1093/annhyg/mev012>.
- Straumfors, A., Olsen, R., Daae, H.L., Afanou, A., McLean, D., Corbin, M., Mannetje, A.'t, Ulvestad, B., Bakke, B., Johnsen, H.L., Douwes, J., Eduard, W., 2018. Exposure to wood dust, microbial components, and Terpenes in the Norwegian Sawmill industry. *Ann. Work. Expo. Health.* 62 (6), 674–688. <https://doi.org/10.1093/annweh/wxy041>.
- Thilsing, T., Madsen, A.M., Basinas, I., Schlünssen, V., Tendal, K., Bælum, J., 2015. Dust, endotoxin, fungi, and bacteria exposure as determined by work task, season, and type of plant in a flower greenhouse. *Ann. Occup. Hyg.* 59 (2), 142–157. <https://doi.org/10.1093/annhyg/meu090>.
- Thorne, P.S., Kulhánková, K., Yin, M., Cohn, R., Arbes, S.J., Zeldin, D.C., 2005. Endotoxin Exposure Is a Risk Factor for Asthma: The National Survey of Endotoxin in United States Housing. *Am. J. Respir. Crit. Care. Med.* 172 (11), 1371–1377. <https://doi.org/10.1164/rccm.200505-7580C>.
- Vijayakumar, R., Aboody, M., Alturaiki, W., Alsagaby, S., Sandle, T., 2017. A study of airborne fungal allergens in sandstorm dust in Al-Zulfi, Central region of Saudi Arabia. *J. Environ. Occup. Health.* 6 (1), 27. <https://doi.org/10.5455/jjeos.20170120094512>.
- Wickens, K., Douwes, J., Siebers, R., Fitzharris, P., Wouters, I., Doekes, G., Mason, K., Hearfield, M., Cunningham, M., Crane, J., 2003. Determinants of endotoxin levels in carpets in New Zealand homes. *Indoor Air* 13 (2), 128–135. <https://doi.org/10.1034/j.1600-0668.2003.00187.x>.
- Würtz, H., Sigsgaard, T., Valbjørn, O., Doekes, G., Meyer, H.W., 2005. The dustfall collector—a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). *Indoor Air* 15 (Suppl 9), 33–40. <https://doi.org/10.1111/j.1600-0668.2005.00342.x>.
- Yoda, Y., Tamura, K., Shima, M., 2017. Airborne endotoxin concentrations in indoor and outdoor particulate matter and their predictors in an urban city. *Indoor Air* 27 (5), 955–964. <https://doi.org/10.1111/ina.12370>.