INVESTIGATION OF INDOOR MICROBIAL POLLUTANTS IN HOMES OF ASTHMATIC SCHOOL-AGED CHILDREN

ASTIMLI OKUL ÇAĞI ÇOCUKLARIN EVLERİNDE İÇ ORTAM MİKROBİAL KİRLETİCİLERİN İNCELENMESİ

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ABSTRACT

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Some studies have reported various associations between indoor microbiological exposure and asthma in children. Although, little is known about the associations of potential determinants such as dwelling age, location of the house, smoke exposures, cleaning frequency, Etc., and aggravating asthma in children. This study examined indoor bacterial and fungal agents and potential determinants at home as a risk factor for asthma in children. Furthermore, the associations of biological agents with potential determinants were investigated. A case-control study was conducted among school children (aged 6–11 years) in the province of Ankara, Turkey. As case and control groups, 109 children with asthma and 130 age- and sex-matched healthy children were identified. The parents answered questions about the home characteristics and lifestyles of families. Dust samples were collected from children's living rooms and bedrooms, and endotoxin β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* spp. were measured in dust extracts. Associations between microbial markers, potential determinants, and risk factors for asthma were evaluated. Among the 109 children with asthma, the frequency of gender in the asthma group was approximately equal, and the asthma study children were, on average, eight years of age. The Childhood Asthma Control Test (ACT) score of 74.8% of the children

in the asthma group was more than 19. There were no statistically significant differences in age and gender in asthma and control groups. The endotoxin level was an inverse risk factor for the presence of asthma (OR = 0.324, 95% CI: 0.155–0.677). According to multivariate logistic regression, a high β-(1→3)-D-glucan level was a risk factor for the presence of asthma (OR = 3.162, 95% CI: 1.101–9.028). Furthermore, high β -(1→3)-D-glucan level was a risk factor for asthma exacerbation (OR = 2.563, 95% CI: 1.076–6.106) (P value= 0.034). Among the potential determinants that fitted multivariate modeling, dwelling age (>20), house floor (≤1), new furniture at home, smoke exposures at home, and houses without a separate kitchen are risk factors for the presence of asthma. All associations with *Aspergillus* and *Penicillium* were statistically nonsignificant in multivariate logistic modeling. According to multivariate logistic regression analyses dwelling age (>20), house floor (≤1) were determined as risk factors for uncontrolled asthma. Multivariate logistic regression analyses to determine the association between potential determinants and biological markers showed that having houseplants at home and the frequency of changing coverlets and bedsheets were the factors that were significantly associated with most of the biological markers.

Keywords: Asthma, endotoxin, β-(1→3)-D-glucan, *Aspergillus, Penicillium*, indoor air quality

ÖZET

ASTIMLI OKU ÇAĞI ÇOCUKLARIN EVLERİNDE İÇ ORTAM MİKROBİAL KİRLETİCİLERİN İNCELENMESİ

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Bazı araştırmalar, çocuklarda iç ortam mikrobiyolojik maruziyeti ile astım arasında çeşitli ilişkiler olduğunu bildirmiştir. Ayrıca, çevresel faktörler örneğin konut yaşı, evin konumu, sigara dumanına maruz kalma, temizlik sıklığı vb. ve çocuklarda astımı şiddetlendirme gibi potansiyel belirleyicilerin ilişkileri hakkında çok az şey bilinmektedir. Bu çalışmada, evdeki bakteriyel ve fungal kirleticilere maruz kalmak ve ayrıca çevresel faktörlerin çocukluk çağı astım ile ilişkileri incelemiştir. Ayrıca ev tozlarında ölçülen biyolojik kirleticilerin kaynakları ev özellikleri ve ailelerin yaşam tarzına göre belirlenmiştir. Türkiye'nin Ankara ilinde okul çağı çocukları (6-11 yaş) arasında bir vaka kontrol çalışması yapılmıştır. Vaka grubu için 109 astımlı çocuk ve kontrol grubu için 130 sağlıklı çocuk (astım olmayan) seçilmiştir. Ev koşulları, ailelerin yaşam tarzları ve çocukların sağlık durumları ile ilgili anket çocukların ebeveynleri tarafından yanıtlanmıştır. Çocukların en çok vakit geçirdikleri yerden yani oturma odası ve yatak odalarından toz örnekleri alınmıştır. Bu toz örnekleri ekstre edildikten sonra endotoksin, β-(1→3)-D-glukan, *Aspergillus* ve *Penicillium* spp. miktarları ölçülmüştür. Astım için biyolojik kirleticiler, potansiyel belirleyiciler ve risk faktörleri arasındaki ilişkiler değerlendirilmiştir.

Astım grubundaki 109 astımlı çocuk arasında cinsiyet dağılımı yaklaşık olarak eşitti ve astımlı çocukların ortalama yaşı sekiz yaş olarak belirlenmiştir. Astım grubundaki çocukların %74,8'inin Çocukluk Çağı Astım Kontrol Testi (ACT) sonucu 19'un üzerinde belirlenmiştir. Ayrıca, astım ve kontrol gruplarında yaş ve cinsiyet açısından istatistiksel olarak anlamlı fark bulunmamıştır. Analizlere göre, bu çalışmada endotoksin seviyesi astım için ters bir risk faktörü olarak belirlenmiştir (OR = 0.324, %95 CI: 0.155-0.677). Multivariate lojistik regresyona göre, yüksek β-(1→3)-D-glukan konsantrasyonu astım için bir risk faktörü olarak bulunmuştur (OR = 3.162, %95 CI: 1.101–9.028). Ayrıca, yüksek β -(1→3)-D-glukan düzeyi astım atakları için bir risk faktörü olarak belirlenmiştir (OR = 2.563, %95 CI: 1.076-6.106) . Multivariate lojistik regresyon analizin sonucuna göre, evin yaşı (>20), evin bulunduğu kat (≤1), evde yeni mobilyalar, evde sigara dumanına maruz kalma ve ayrı bir mutfağı olmayan evler astım için risk faktörleri olarak bulunmuştur. Bu analizin sonucuna göre, *Aspergillus* ve *Penicillium* astım ile ilişkileri modellemede istatistiksel olarak anlamlı bulunmamıştır. Ayrıca, multıvariate analizine göre, evin yaşı (>20) ve evin bulunduğu kat (≤1), kontrolsüz astım için risk faktörleri olarak belirlenmiştir. Potansiyel belirleyiciler ve biyolojik belirteçler arasındaki ilişkiyi belirlemek için yapılan multivariate lojistik regresyon analizi, evde bitki ve çiçek bulunması ve nevresim ve çarşaf değiştirme sıklığının biyolojik kirleticilerin çoğuyla önemli ölçüde ilişkili olduğunu göstermiştir.

Anahtar Kelimeler: Astım, endotoksin, β-(1→3)-D-glukan, *Aspergillus*, *Penicillium*, iç ortam hava kalitesi

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LIST OF ABBREVIATIONS

ACT	Asthma Control Test
ANAOVA	Analysis of Variance
aOR	Adjusted odd ratio
BDG	β-(1→3)-D-glucan
BET	Bacterial Endotoxin Test
CFU	Colony forming unit
CI	Confidence interval
CO	Carbon monoxide
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CSE	Control Standard Endotoxin
DCBR	Dichloran Rose-Bengal Chloramphenicol
ECT	Exercise Challenge Test
EIA	Enzyme Immunoassay
EIB	Exercise-induced bronchoconstriction
EPS	Extracellular polysaccharide
ETS	Environmental tobacco smoke
EU	Endotoxin Unit
FEV_1	Forced Expiratory Volume
FVC	Forced Vital Capacity
GM	Geometric mean
GSD	Geometric standard deviation
HDM	House dust mite
HP	Hypersensitivity pneumonitis
IAQ	Indoor air quality
IQR	Interquartile Range
LAL	Limulus Amebocyte Lysate

LOD	Limit of Detection
LPS	Lipopolysaccharides
LRW	LAL Reagent Water
MVD	Maximum Valid Dilution
MVOC	Microbial Volatile Organic Compounds
NO_2	Nitrogen Dioxide
ODTS	Organic Dust Toxic Syndrome
OR	Odd ratio
PAH	Polycyclic Aromatic Hydrocarbon
PEF	Peak Expiratory Flow
PM	Particulate Matter
pNA	para-nitroanilide
RGW	Reagent Grade Water
SO_2	Sulfur dioxide
SPT	Skin Prick Test
VOC	Volatile organic compounds
WHO	World Health Organization
µg	Microgram

1. INTRODUCTION

Asthma is a significant and troubling issue for many children and parents. Asthma, like most illnesses, includes a wide range of symptoms as well as probable causes and consequences. Asthma is a complex disease that can occur depending on genetic characteristics, lifestyle, and environmental factors. In most cases, asthma appears in childhood and will likely last a lifetime. It is unknown why some people get asthma, and others do not, but it is likely related to a mix of environmental and genetic reasons. Due to the morbidity rate, severity, and economic effects of pediatric asthma, it is regarded as one of the leading causes of pediatric hospitalizations and a public health problem.

Children spend most of their time indoors, mostly in their homes and schools. Indoor air quality plays a crucial role in their health as children's immune systems are underdeveloped. Furthermore, the presence of germs, fungus, heavy metals, particulate matter, and other contaminants inside has an impact on growing children. Different types of indoor pollution can be found in home environments. Biological agents, or bioaerosols, are known as common indoor air pollutants. A variety of factors can cause biological pollution. The parameters that influence indoor air quality are home characteristics, family lifestyles, and outdoor air quality.

Exposure to bioaerosols has some health impacts, such as infections, respiratory diseases, asthma, allergies, and, in some cases, cancer. However, exposure to certain biological agents is beneficial for health, especially among children. Endotoxin, β-(1→3)-D-glucan, and fungi such as *Aspergillus* and *Penicillium* are common biological markers in indoor environments. Some studies investigated the association between these biological agents and respiratory diseases, especially among children. Although the results of these studies are conflicted, The contradictory findings may be due to geographical variations, differences in sampling methods and laboratory techniques, or different cultures.

In this study, biological agents were investigated in the houses in different regions of Ankara occupied by 6–11-year-old children, which was carried out through studying the investigation of indoor microbial pollutants in the homes of asthmatic school-aged children. This thesis was

supported by the Scientific and Technological Research Council of Turkey (TUBITAK) through the 117Y088 project.

1.1. AIM AND SCOPE

This thesis aims to determine the association of biological agents such as endotoxin, β-(1→3)-D-glucans, *Aspergillus*, and *Penicillium* with asthma in school children. Furthermore, the following aspects are intended to be determined at the end of the study:

- Determination of microbial agents (endotoxin, β-(1 → 3)-D-glucan, *Aspergillus*, and *Penicillium*) in house dust,
- Evaluation of the factors that cause changes in biological agent concentrations,
- The detection of biological agents in house dust and their relationship to the development of asthma in school-aged children,
- Determination of home characteristics and lifestyles of families that affect asthma morbidity
- Contribute to the improvement of air quality in indoor environments where children live.

The study's approach is carried out using five key tools, which are:

- Selection of the study population: The study population consisted of 6–11-aged children. The population includes 109 cases (with asthma) and 130 control children (without asthma). Patients in the case group with the diagnosis of asthma were followed by the Hacettepe University Pediatric Clinic. For selecting the control group, we got permission from the Ankara Provincial Directorate of National Education to reach children between 6–11 years of age who have not been diagnosed with asthma.
- Make a survey questionnaire: The survey was formed to determine the home's condition and its activities. In the survey, questions about the characteristics of the dwelling (location of the home, residential area, dampness at home, heating system, type of carpet, etc.), pet-keeping at home, and household activities (smoking, cooking methods, ventilation frequency, etc.) were found.
- House dust sampling: House dust samples were collected during home visits in the winter of 2019 as a part of a study in both case and control houses.

- Analyze biological agents: *Limulus* Amoebocyte Lysate (LAL) assay was used to detect endotoxin and β -(1 → 3)-D-glucan and culturable method for *Aspergillus* and *Penicillium*.
- Evaluation of the results: The results were evaluated with SPSS, Statgraphics, and GraphPad Prism.

1.2. INDOOR AIR QUALITY

Because individuals spend most of their time indoors, the air they breathe in these places has a significant impact on their health in both the short and long term. As a result, indoor air quality (IAQ) is a crucial factor to monitor to provide a healthy interior environment for the residence's occupants [1]. Nowadays, people spend about 70-90 percent of their time indoors, such as in their homes, occupational settings, schools, and other public indoors such as restaurants, cinemas, libraries, Etc. Children spend most of their time indoors, mostly in their homes and schools. Various studies on indoor air quality have found that concentrations of many indoor air pollutants are greater than those of outside air pollutants [2]. As a result, interior air quality has a significantly more prominent influence on human health than outside air quality.

Indoor air pollution can be dangerous to newborns, children, the elderly, those with chronic obstructive pulmonary disease (COPD), and people with heart disease. As newborns and children's immune systems are underdeveloped and their metabolic rates are high, indoor pollution plays a crucial role in their health. Additionally, bacteria, fungi, heavy metals, particulate matter, and other pollutants indoors affects growing children.

Different types of indoor pollution can be found in home environments. Organic pollutants such as volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH), aldehydes, pesticides, and inorganic pollutants like sulfur dioxide (SO2), nitrogen dioxide (NO2), carbon dioxide (CO2), carbon monoxide (CO), and environmental tobacco smoke (ETS) are detected in indoor environments. Pollutants such as particulate matter, asbestos, manufactured mineral fibers, and radon are other indoor air pollutants. The most common indoor air pollutants are biological agents or bioaerosols such as bacteria, dust mites, allergens, fungi, β glucans, endotoxins, and mycotoxins [3].

A variety of factors can cause indoor air pollution. The parameters that influence indoor air quality are home characteristics, family lifestyles, and outdoor air quality. Furthermore, other

variables reduce indoor air quality or the accumulation of contaminants in the interior environment. However, the majority of contaminants in the home environment are caused by the individuals' lifestyle. For example, cleaning materials, cooking, and smoking at home may affect the daily concentrations of pollutants in the air, especially if ventilation systems are not available in the indoor setting [4]. Home characteristics such as dwelling age, location of the home, and building material are other parameters to determine IAQ. Some of the sources of indoor air pollution are furniture, heaters, and office equipment. Additionally, water damage at home, dampness and visible mold are potential variables that cause indoor air pollution. As a result, we can say that the occupants' behaviors and everyday activities can also impact the IAQ, either positively or adversely.

People are exposed to various indoor air contaminants depending on the features of the interior environment and occupants' lifestyles. The level of activity-related pollutants in the indoor environment fluctuates throughout time based on the density and duration of the activity. Persons who have been exposed to these environmental contaminants for an extended time are frequently the individuals who are most vulnerable to the health impacts of different indoor air pollutants [3]. Because pollutant concentrations can stay in the indoor environment for an extended period after certain indoor activities, health concerns may arise. Indoor air pollution has two sorts of health consequences. The first is acute health effects, which appear after a single or recurrent exposure. The second type is chronic health effects, which appear years after exposure or only after extended or repeated periods of exposure. Some acute health consequences involve headaches, eye, and nose irritation, dizziness, tiredness, and irritation in the throat. These acute symptoms are usually temporary and manageable. Asthma symptoms, hypersensitivity pneumonitis, and fever may also appear shortly after exposure to some indoor air contaminants. Chronic health impacts such as respiratory disorders, heart disease, and cancer, which can be debilitating and lethal, can manifest years after exposure to indoor air pollution [5]. Aside from these health issues, exposure to indoor air pollution can cause a wide range of health concerns that differ significantly from person to person.

Bioaerosols are one of the most significant indoor air pollution sources that can cause acute or chronic health concerns in people. The following sections offer general information about

bioaerosols and common biological agents in indoor environments and significant acute and chronic health effects from exposure to these agents.

1.3. BIOAEROSOLS

Microbial contamination is a significant source of indoor air pollution. Microbial contamination is caused by hundreds of bacterial and fungal species (particularly filamentous fungus) that grow in favorable situations [6]. Since bioaerosols are found almost everywhere in nature, eliminating them from any microenvironment is impossible. Airborne biological particles are microscopic and have dimensions ranging from 0.001 to 100 μm. Bioaerosols are biologically derived from plants or animals and include live organisms [7]. Thus, bioaerosols are composed of neither pathogenic nor non-pathogenic, living or dead microorganisms such as bacteria, fungi, endotoxins, high molecular weight allergens, β-(1→3)-D-glucans, viruses, Etc. [8]. Bacteria and fungus are the most regularly seen bioaerosols. These particles are also known as organic dust. Because of their tiny size and low weight, bioaerosols are transported from one habitat to another [9].

Bacteria and fungus are very varied and adaptable microorganisms that can grow in practically any environment. They may be found almost anywhere. They can also be seen as parasites or symbiotics in humans, animals, and plants. Fungi are eukaryotic, whereas bacteria are prokaryotic. Fungi are present in organic portions of plants, particularly soil, and are involved in decomposing cellulose in nature [10].

Both physical and biological factors determine the presence of bioaerosols. Physical parameters are the primary factors that influence the number of bioaerosols. In addition to temperature and relative humidity, diffusion, gravity, thermal and electrostatic forces are among the physical factors that impact the existence of bioaerosols [11].

Sources of bioaerosols in the indoor environment can be found indoors and outdoors. Bioaerosol levels and species may differ based on location, climate, season, temperature, the quantity of precipitation, building materials, dwelling age, and ventilation rate [12]. Although a low ventilation rate saves energy, it may raise the amount of bioaerosol in the interior environment and increase the risk of illnesses associated with it [10]. Foodstuffs, houseplants, household dust, pets, textile products, carpets, wall, and floor covering materials, and furniture surfaces are all sources of bioaerosols in the indoor environment [11][13]. Additionally,

common human behaviors such as coughing, talking, walking, and sneezing might produce bioaerosols [14]. Offices, schools, hospitals, and industrial sites are examples of crowded regions with a high bioaerosol concentration [10]. The sources of bioaerosols in workplaces are broad. Bioaerosols can be highly detectable in agricultural and food processing industries, waste composting, the livestock sector, and so on [15].

Recently, exposure to bioaerosols in indoor environments such as homes, workplaces, and schools has received much interest due to the potential effects on human health. As a result, the presence of bioaerosols has been linked to a variety of human illnesses, including pneumonia, influenza, measles, gastrointestinal sickness, and respiratory diseases such as asthma and allergy [16]. Nevertheless, under some conditions, exposure to specific biological markers is helpful to health in terms of creating a robust immune system and keeping children from allergies and asthma morbidities [17]. Even though the relevance of biological markers and their influence on human health has been acknowledged, it is still tricky to correctly explain their function in the onset or development of many symptoms and diseases.

1.3.1. Bacteria

There is no region on Earth where bacteria do not exist. They can be found in the air, soil, fresh and saltwater, on plants and animals, inside organisms, and even in glaciers [18]. Bacteria are unicellular, tiny organisms without a fundamental nucleus. Bacteria are classified into three types based on their shape: rod-shaped (bacilli), spherical (cocci), and helical (spirilla). Bacteria can also be categorized as either gram-positive or gram-negative [19]. Bacteria may grow on a wide range of natural substrates. Furthermore, environmental conditions such as the presence of oxygen, humidity, temperature, and pH are critical for bacterial growth [12]. Bacterial toxins are often classed as either exotoxins or endotoxins. Exotoxins are discharged into the environment instantly, while endotoxins are not secreted until the immune system eliminates the bacterium.

Bacteria can be found virtually anywhere. Many bacteria may be found in plants, animals, most foods and drinks, soil, water, and air. Based on the ambient circumstances, bacteria in the indoor air can be detected in concentrations ranging from $10–10^4$ CFU/m^3. The air contains saprophytes and parasites and pathogenic and non-pathogenic bacteria. The majority of airborne microorganisms are not harmful. Most of the pathogenic bacteria in the indoor environment are

caused by soil, water, plants, animals, and the outdoor environment. Bacteria are transmitted from the outside environment to the indoors, commonly by wind and dust. The majority of airborne bacteria are mesophilic bacteria, and the optimal temperature range for them to grow is 20–35^{o}C. Gram-positive cocci and Bacillus species are the most typically detected.

1.3.2. Fungi

They may be found all around the world, although they are more frequent in humid areas. Although it is estimated that there are 1.5 million fungal species worldwide, only 69,000 have been identified to date [20]. Fungi spores in the air can produce mycotoxins and microbial volatile organic compounds (MVOC). Some of these mycotoxins and MVOCs are harmful. Mycotoxins are secondary biomolecules that are poisonous and are created by fungi or molds. Nevertheless, the same mold species can generate several types of mycotoxins, whereas the same mycotoxins can be formed by multiple mold species [21].

Fungi are known as multicellular eukaryotic organisms. They are also heterotrophs and play a significant part in nutrient cycling in the environment. Fungi are organisms with a larger surface area compared to bacteria, and they can reproduce both sexually (under special conditions) and asexually [22]. Fungi require oxygen in order to reproduce. Carbohydrates, on the other hand, play a vital role in their development. Most fungi grow at temperatures ranging from 18 to 32°C. In rare cases, sporulation can occur at temperatures below 0°C. Extreme temperatures, such as 71 degrees Celsius, are frequently fatal to fungus. *Aspergillus fumigatus* and *Aspergillus niger* can tolerate a wide range of temperature values [23].

Most common fungus species thrive on a variety of foods or other organic materials such as paper, textiles, wood, Etc. Bacterial and fungal growth in the indoor environment was most prevalent in air conditioning equipment, toilets, showerheads, water-damaged carpets, moist ceiling panels, and walls [24].

Fungi, as well as their hyphae and spores, are commonly transported in the air. As a result, there is a considerable risk of exposure to airborne fungus through breathing. Fungi are crucial for human health because they frequently induce allergies and asthma attacks. Fungal spores range from 3 to 200 μm but are most commonly found at 10 μm. They may be found freely and in large quantities in the air for extended periods. Fungi may enter the indoor environment from

the outside via heating, air conditioning, ventilation systems, and windows and doors and contaminate the building materials [25].

The most prevalent fungi that cause disease in indoor environments are *Penicillium* spp., *Aspergillus* spp., and *Alternaria* spp. [26]. More than 80 different species of fungus have been discovered as the source of respiratory allergies. The most well-known allergenic fungi include *Cladosporium, Alternaria, Aspergillus,* and *Fusarium*. These fungi also irritate the respiratory tract. Non-biological particles transport allergenic fungal compounds independently of fungal spores, allowing them to enter the lungs considerably deeper. Additionally, allergens, enzymatic proteins, toxins, and VOCs might be created by the fungus, causing a variety of human health effects such as toxic effects, irritations, infections, and allergies [27].

In this thesis, endotoxin, β-(1→3) -D-glucans, *Aspergillus*, and *Penicillium* were investigated in the indoor environment. These biological agents are discussed in the following sections.

1.3.3. Endotoxin

Endotoxins are lipopolysaccharides (LPS) with powerful pro-inflammatory qualities found in gram-negative bacteria [28]. Endotoxin is a non-allergenic and highly immunogenic substance found in the cell walls of gram-negative bacteria. It is widespread in many workplace settings, but it is also found in the general environment, notably in household dust [29]. LPS are big molecules made up of a lipid and a polysaccharide formed of an O-antigen, an outer core, and an inner core bonded together by a covalent connection [30]. Lipid "A" part of lipopolysaccharide is responsible for endotoxin activity in the molecule. As bacteria decompose and release into the environment, endotoxin is produced. The primary difference between endotoxin and exotoxin is that endotoxin is released by bacterial cells. Figure 1.1 shows the structure of lipopolysaccharide.

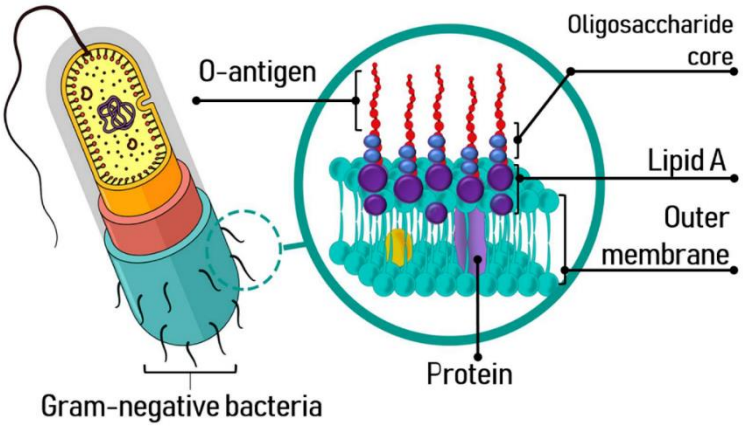


Figure 1. 1. Structure of lipopolysaccharide

People are continually exposed to endotoxin because it is simply attached to dust and easily inhalable [31]. Endotoxin response changes based on dosage, location, and route parameters. Endotoxin exposure has been linked to a reduction in lung diffusion capacity as well as a variety of symptoms and disorders, including fever, shivering, blood leukocytosis, neutrophilic airway inflammation, asthma symptoms, malaise, and bronchial obstruction [32].

Endotoxins have been identified as a significant contributor to occupational lung illness and organic dust toxic syndrome (ODTS) [33]. Indeed, abnormalities in pulmonary function caused by endotoxin exposure have been observed in a variety of occupational groups, including textile workers [34], dairy employees [35], livestock sector staff, and sewage treatment plant operators [36].

Based on the timing of exposure, endotoxin could either be a risk factor for asthma or have a protective effect against atopy [37]. Endotoxin is thought to be a reason for occupational asthma and has been linked to higher asthma severity in adults in the household environment. The effect of endotoxin exposure in the home on children with asthma is less apparent. Endotoxin in the house has been linked to the exacerbation of asthma symptoms in children, such as wheezing. On the other hand, other studies have shown no link between endotoxin and peak flow variability in children with asthma [37]. Moreover, some studies in the literature report adverse, protective, no associations between endotoxin exposure and expiratory diseases in children. The contradiction of results is not yet known.

1.3.4. β *glucans*

Glucans are known as glucose polymers that are abundantly spread throughout the environment. Glucans are found naturally in the cell walls of numerous microorganisms, such as most fungi, some bacteria, and plants (both higher and lower plants) [38]. Glucans are categorized generically based on the kind of intrachain linkage of the polymer, which can be α- or β- linked. The most common type of glucan discovered in fungus is β- linked glucans. β-(1→3) -D-glucan (BDG) is found in the fungal cell wall and is linked to proteins, lipids, and other carbohydrates [39].

β-(1→3)-D-glucans have potent immune-modulating properties. These actions are mainly attributable to β-(1→3) -D-glucans ' capacity to stimulate cellular and humoral mechanisms of the host immune system. Glucans have been used to treat high levels of cholesterol, diabetes, and cancer and improve the immune system [40]. Nevertheless, several investigations have reported that exposure to β-(1→3)-D-glucan has a contribution in bioaerosol-induced inflammatory and associated respiratory disorders [41]. As a result, the effects of inhaling β glucans appear to differ in proportion to some variables, such as the type of glucan and concomitant exposure [27]. Figure 1.2 shows the chemical structure of β-(1→3) -D-glucan.

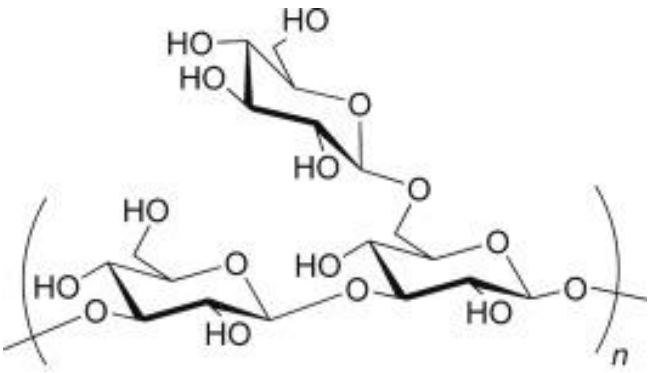


Figure 1. 2. Chemical structure of β-(1→3) -D-glucan

1.3.5. *Aspergillus*

Aspergillus is a genus comprising several hundred mold kinds found in a variety of climates across the world. Pier Antonio Micheli, an Italian priest, and scientist was the first to classify *Aspergillus* in 1729. *Aspergillus* species are saprophytic filamentous fungi typically seen in soil, degrading plants, seeds, and grains [42]. When environmental factors are appropriate, it is the

first fungal species to form colonies on a human-derived substrate. They are frequently noticed because of their capacity to withstand low humidity levels. According to studies, indoor environments contain more *Aspergillus* fungus than the outdoor environment.

Because *Aspergillus* spores are ubiquitous in the environment, humans are constantly and routinely exposed to them. As a result of this exposure, adverse health effects occur in their bodies. Invasive pulmonary aspergillosis, aspergilloma, and other hypersensitivity illnesses such as allergic asthma, hypersensitivity, pneumonitis, and allergic bronchopulmonary aspergillosis are examples of these diseases [43].

There is substantial worry about the possible health consequences of exposure to biological markers in the air. Molds pose a significant hazard to human health; their effects vary from mild allergies and severe asthma to widespread illnesses. Mold exposure in indoor environments is not often regarded as a distinct risk factor in the etiology of fungal illnesses unless certain variables are present that are required for specific infections [43].

Fungal allergens are thought to be less relevant in asthma etiology than dust in houses; yet, eradicating fungus from residential surroundings helps improve asthma. Adult asthma exacerbation has been linked to high concentrations of *Aspergillus* spp. and their allergens in the home [44][45]. According to some studies, the frequency of fungal colonies is higher in homes that offer healthcare for children with asthma, particularly in children's beds and rooms where children spend the majority of their time [46]. The macroscopic and microscopic image of *Aspergillus* spp. were depicted in figure 1.3.

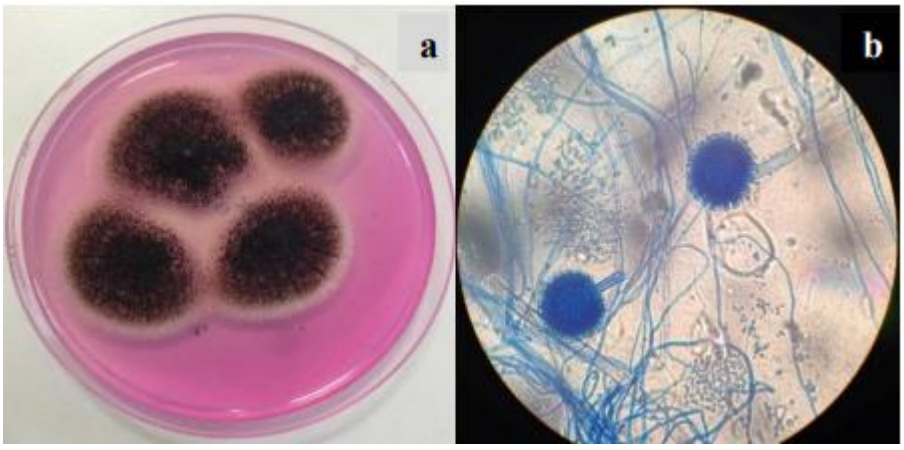


Figure 1. 3. a) Macroscopic and b) Microscopic image of *Aspergillus* spp. in DRBC medium.

1.3.6. *Penicillium*

Alexander Fleming discovered the *Penicillium* fungus in London in 1929, and the penicillin antibiotic was created against this fungus by the same scientist. *Penicillium* is a best-known and widespread fungus that may be found in a wide variety of situations, including soil, plants, air, indoor environments, and many food items [47]. *Penicillium* has a few hundred recognized species. Over 20 are frequently stated as being present in the indoor environment. Many *Penicillium* species adapt well to indoor conditions and grow effectively on construction materials. It can be found on walls, wood, painted surfaces, wallpaper, and various home materials, particularly in high-humidity environments [48].

Penicillium spp. is a form of a fungus with a cottony, woolly, velvety surface that can be found indoors and outdoors. Some species are produced via the degradation of nutrients, whereas others can be found on a range of organic substrates. *Penicillium* spp. is mesophilic fungi that thrive at pH 3–4.5, and temperatures range from 5–37°C (ideal, 20–30°C) [47].

Some specious of *Penicillium* fungi can cause serious health problems such as allergic reactions (skin), high fever, shortness of breath, and may induce hypersensitivity pneumonitis and allergy alveolitis. Some species are capable of producing carcinogens and mycotoxins, which are known to harm internal organs [26].

Indoor fungal concentrations are affected by the origins and degree of moisture in the building, as well as the wall and floor covering materials, frequency of ventilation, general cleaning frequency, and pet ownership. Keep in mind that seasonal variations and outside environmental factors played a significant role in the variability of indoor fungal concentrations. The macroscopic and microscopic image of *Penicillium* spp. were depicted in figure 1.3.

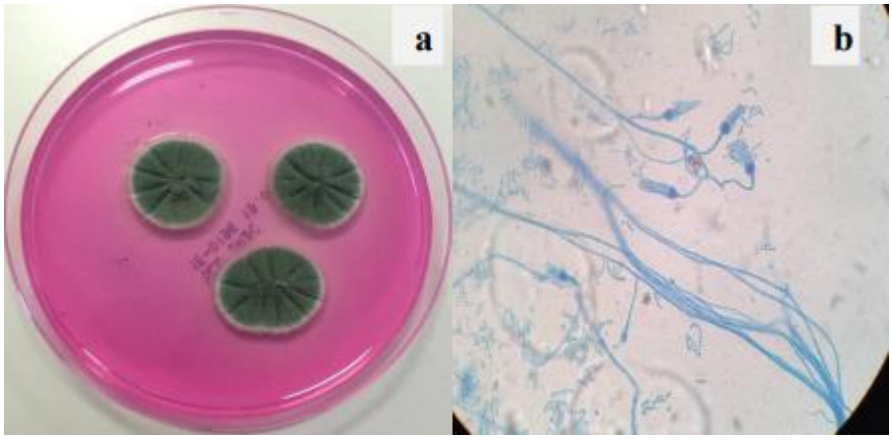


Figure 1. 4. a) Macroscopic and b) Microscopic image of *Penicillium* spp. in DRBC medium.

1.4. HEALTH EFFECTS OF BIOAEROSOLS

In most cases, people are exposed to diverse mixes of toxins, allergens, and chemicals. Therefore, an extensive variety of potential health impacts must be evaluated. Infectious problems, respiratory diseases, and cancer are the three primary types of diseases connected with bioaerosol exposure. Nevertheless, respiratory problems and lung function impairment have been the most extensively researched subjects; they are likely among the most severe health issues induced by bioaerosols. These categories are discussed in more detail in the following section.

1.4.1. Infectious Diseases

Exposure to biological markers such as bacteria, viruses, fungi, and other biological agents can cause infections and diseases. Transmissions of infectious agents can be direct, such as touching, and/or indirectly, like coughing or sneezing. Airborne and vector-borne transmission are other ways of exposure to infectious agents [49].

Around 200 distinct viral strains cause the common cold. Rhinovirus, human coronavirus, respiratory syncytial virus, and adenoviruses are known as the most typical viruses. Rhinovirus has been associated with 30-80% of colds [50]. These viruses emit airborne aerosols or through a variety of different channels, such as directly touching infected objects like phones, towels, keyboards, Etc. [51].

Some zoonotic illnesses such as swine influenza, Q-fever, anthrax, etc., are primarily associated with livestock farms, veterinary services, abattoirs, and animal shop employees [52]. Most of the seroepidemiological investigations were shown to be at an increased risk of zoonotic microbial infection [53]. Infected veterinarians spread zoonotic infections into the environment, such as their families and the animals they care for. Chlamydophila *psittaci* bacteria, for example, are common in psittacine birds and pigeons [54]. Persons become infected most often by inhalation and/or exposure to diseased birds, C. *psittaci*-infected aerosols, and touching contaminated avian materials [55].

When tiny infective aerosols are breathed, they cause pneumonic plague, another fatal infectious and highly contagious illness [56]. Between 2000 and 2009, 21,725 instances of human plague were documented worldwide, with 1612 fatalities [57]. Anthrax is an infectious illness caused by the Bacillus anthracis bacteria that can be transmitted through the intestines, lungs, or skin. The most common way for anthrax to reach people is to contact infected animals. [58].

Measles is another acute viral illness that can cause pneumonia, blindness, neurological disorders, and mortality [59]. Over 20 million measles infections were seen globally in 2013, resulting in approximately 145,000 fatalities. The diseases are believed to be spread by aerosol distribution from infected people's noses, throats, and mouths when they cough or sneeze [60]. Infected aerosols and droplets that settle on the surfaces could be infectious for a few hours. People who touch their eyes, nose, or mouth with virus-infected hands may become infected with the virus. Around 90% of unimmunized persons exposed to the virus will get the sickness [61].

Streptococcus pneumoniae bacteria can be attributed to infections in different parts of the body, such as the lungs, ears, nose, spinal marrow, and brain [62]. This bacterium was known to typically infect children, the elderly, and other persons with compromised immune systems. The WHO reported that Streptococcus pneumoniae causes the deaths of half a million children (under the age of five) globally each year [63]. Streptococcus pneumoniae germs are often transmitted via the air as aerosol droplets as a result of sneezing or coughing, as well as contact with an infected individual.

1.4.2. Respiratory Diseases

Among the diseases likely caused by exposure to bioaerosols, respiratory disorders and lung function diseases are likely the most well-studied. They can vary from acute mild disorders that have little impact on everyday life to severe chronic respiratory illnesses that need specialized treatment. In general, exposure to pro-inflammatory markers, toxins, and allergens induces airway inflammation. A differentiation between allergic and non-allergic respiratory disorders can be formed depending on the fundamental inflammatory processes and following symptoms. Non-allergic respiratory problems are caused by non-immune-specific airway inflammation. In contrast, allergic respiratory diseases are caused by immune-specific inflammation in which different antibodies, such as IgE and IgG, might play a significant part in the inflammatory process.

Chronic obstructive pulmonary diseases (COPD) are notably distinct from allergic asthma (induced by allergen exposure), in which chronic lung function decrease is often relatively mild. Furthermore, the cross-shift loss in lung function is frequently less severe than that seen in regular allergic asthma. Pre-existing respiratory disorders or other host variables such as atopy and smoke exposure may alter the chance of acquiring respiratory symptoms [64].

Exposure to organic dust might increase the risk of hypersensitivity pneumonitis (HP) and organic dust toxic syndrome (ODTS). ODTS is a non-allergic febrile sickness defined by a change in body temperature, shivering, dry cough, tightness in the chest, headache, muscle and joint aches, nausea, and overall malaise [65]. The symptoms are similar to influenza, although they usually go away the next day. The condition is widespread among employees exposed to much organic dust. HP is the general name for a dangerous pulmonary disorder [66]. The symptoms of HP are pretty similar to those of ODTS, but they are more significant and, in the chronic stage, can result in major lung problems and occupational impairment. The basic immunological processes of HP are complicated and only partially known; both allergic and non-allergic immune responses are thought to have a role [67].

1.4.3. Cancer

There have been reports of probable links between bioaerosol exposure and certain malignancies. Cancer is based on several reasons, such as oncogenic viruses and other biological markers. Mycotoxins are known as non-viral biological, environmental carcinogens. These arise

in industries that work with mold-contaminated products [68]. Aflatoxin from *Aspergillus flavus* may be the most well-known carcinogenic mycotoxin, and it is the best-known human carcinogen, notably concerning liver cancer [69].

Ochratoxin A is also suspected of being a human carcinogen. The most common exposure methods to aflatoxin and ochratoxin are ingestion and inhalation in workplaces such as peanut processing, livestock feed manufacturing, and industries where grain dust is present [70]. Farmers are more likely to have some cancers, such as hematological malignancies, stomach, prostate, and brain cancer [71]. People who work in the animal feed processing industry are more likely to develop liver cancer, as well as malignancies of the biliary system, salivary gland, and multiple myeloma [68]. One of the hypothesized reasons is exposure to pesticides, oncogenic virals, or other biological markers transported by farm animals. A review study reported lung cancer risk in highly affected employees in meat and poultry [72]. According to some studies, a continuous excess of lung cancer has been linked to slaughterhouse employees and butchers [73].

Several studies have discovered relations between wood dust exposure and different malignancies, including sinonasal cancer in furniture and cabinet manufacturing, carpentry and joinery, and other wood-related occupations such as sawmills [74]. Moreover, employees in numerous other sectors that treat biological materials, such as the textile and shoe industries, are at risk of different types of cancers. Nevertheless, it is still uncertain whether these increased hazards result from biological agent exposure or the numerous chemicals utilized in these businesses.

1.4.4. Asthma

Asthma is a chronic and non-communicable illness that affects both children and adults. Further, it is known as the most prevalent long-term condition among children. Asthma prevalence in adults is believed to be between 2% and 12%, while in children, it is estimated to be between 3% and 38% [75]. In 2019, an estimated 262 million individuals had asthma, resulting in 461,000 fatalities [76]. Asthma symptoms are caused by inflammation and constriction of the tiny airways in the lungs. Asthma symptoms include coughing, wheezing, breathing difficulty, and tightness in the chest. These sensations are irregular and are usually worse at night or during

activity. These conditions result in missed school and work days, restrictions on everyday activities, and sleep disruptions. Asthma is a mild annoyance for some people, but for others, it can be a severe issue that disrupts everyday activities and may result in a life-threatening asthma attack. Asthma is frequently misdiagnosed and undertreated, especially in low-and middle-income nations. People with untreated asthma may have sleep disturbances, fatigue during daily activities, and difficulty concentrating. Asthmatics and their families may miss school and work, causing financial hardship for the family and, in the enormous scope, for society. If symptoms worsen, persons with asthma may require emergency medical attention and be hospitalized for management and care. Asthma can be fatal in the most severe instances. Asthmatic diseases that are potentially fatal can impact adolescent psychosocial adjustment. Adolescents with asthma are much more able than their healthy counterparts to experience clinically severe anxiety symptoms. Ortega et al. discovered that children with asthma had a greater risk of separation anxiety disorder, overanxious disorder, and phobias than those with diabetes or other chronic diseases [77].

Many distinct variables have been linked to an increased chance of having asthma, while defining a single, direct cause is typically challenging. Other family members with asthma are more likely to have asthma, especially a close family member such as a father, mother, or sibling. People with other allergy disorders like eczema and rhinitis are more susceptible to developing asthma. Overweight or obese children and adults are at a higher risk of developing asthma. Early childhood events have an impact on the developing lungs and can raise the chance of asthma. Some of these events include low birth weight, preterm, exposure to environmental tobacco smoking and other causes of air pollution, as well as viral respiratory infections. Moreover, asthma prevalence rises with urbanization, most likely attributed to a combination of lifestyle variables. Exposure to a variety of environmental allergens and triggers, such as indoor and outdoor air pollution, home dust mites, molds, and exposure to chemicals, fumes, or household dust, is also known to raise the risk of asthma [78].

Asthma is a complex disease that can occur depending on genetic characteristics, lifestyle, and environmental factors. In most cases, asthma appears in childhood and will likely last a lifetime. It is unknown why some people get asthma, and others do not, but it is most likely because of environmental and genetic reasons. Exposure to numerous irritants and chemicals that cause

allergies can exacerbate asthma symptoms. Asthma triggers vary from individual to person. Respiratory infections like common colds, physical activity, cold air, air pollution, exposure to airborne allergens such as pollen, house dust mites, and molds are known as asthma triggers. In addition, some medicines, such as beta-blockers, aspirin, and nonsteroidal anti-inflammatory drugs such as ibuprofen and naproxen sodium, are included as asthma triggers. Strong emotional reactions, stress, gastroesophageal reflux disease, and some types of foods and beverages with sulfites and preservatives added are also known as asthma triggers.

Asthma symptoms and indicators can worsen in the following scenarios for certain people [79]:

- Asthma is caused by exercise, which may be exacerbated when the air is cold and dry.
- Irritants cause occupational asthma at work, such as chemical fumes, gases, or dust.
- Allergy-induced asthma is caused by airborne allergens such as pollen, mold spores, cockroach feces, or pet dander.

Asthma cannot be cured, but it may be controlled with inhaled drugs. Good management with medicines allows asthmatic people to live everyday and active life. Inhalers are classified into two types: bronchodilators (such as salbutamol), which expand the airways and ease symptoms, and steroids (such as beclometasone), which reduce inflammation in the airways; This minimizes the risk of severe asthma attacks and mortality while also improving asthma symptoms. Asthmatics may need to use their inhaler every day. Their therapy will be determined by the frequency of their symptoms as well as the many types of inhalers available. Coordination of breathing with an inhaler can be challenging, especially for youngsters in emergency cases. A "spacer" device makes using an aerosol inhaler easier and increases the drug's effectiveness in reaching the lungs. In many nations, access to inhalers is a concern. In 2019, just half of the asthmatic persons had access to a bronchodilator. Further, in low-income countries, less than one in five had access to a steroid inhaler in public health centers [80].

Asthmatics and their families require education to gain knowledge about their condition, medication, triggers to avoid, and how to manage their symptoms at home. It is also critical to increase community knowledge in order to dispel misunderstandings and stigma connected with asthma in various contexts.

1.5. PROTECTIVE EFFECTS OF MICROBIAL EXPOSURE

Recently, the "hygiene hypothesis" has shifted focus from the negative health impacts of microbial agents to the potential positive benefits of microbial agents [81]. In accordance with the hygiene hypothesis, early exposure to germs and some biological agents aids in the development of the immune system. This educates the body to distinguish between innocuous and dangerous compounds that cause asthma attacks. Exposure to some pathogens, in theory, educates the immune system not to respond [82].

Children who grow up in rural regions, near animals, or in bigger families appear to acquire asthma at a lower rate than other children. According to hygiene theory, this is attributed to increased exposure to certain viruses, germs, or parasites [82].

The difficulty with excessively clean settings, due to the "hygiene hypothesis," is that they do not allow being exposed to germs to teach the immune system so that it can learn to activate defense responses against microbial agents. Furthermore, its defense reactions become so ineffective that they lead to the development of asthma [83].

The "hygiene hypothesis" is validated by epidemiologic investigations that show allergic disorders and asthma are more likely to be present when bacterial endotoxin (LPS) concentrations in the house are low. Lipopolysaccharide is a bacterial molecule that activates and teaches the immune system by trigging signals via TLR_4, a molecular "switch" located on particular immune system cells [82].

Exposure to microbial agents, particularly endotoxin exposure early in life, is thought to protect against atopy and allergic asthma development, but the processes are not fully understood [84]. This hypothesis proposes that growing up in a cleaner and more hygienic environment may improve atopic (Th2) immune responses [81]. Some animal and in vitro research and population studies appear to back up this idea [85]. Furthermore, multiple investigations indicated that growing up on a farm had a protective effect against atopy and asthma [86].

1.6. ASTHMA AND INDOOR ENVIRONMENT

According to the WHO, environmental variables are responsible for 24 percent of the worldwide illness burden and 23 percent of all fatalities, according to the WHO [87]. The path from

exposure to sickness and mortality is frequently complicated and largely unknown. These dangers are not equally distributed among all age groups. Children are more vulnerable to the negative impacts of the environment, as the percent of mortality among children related to the environment is approximately 36% [87]. The high frequency of asthma in children [88] and adults [89] over the world cannot be explained only by genetic factors. Furthermore, differences between various regions with similar ethnicities cannot be explained [90]. Exacerbation of asthma is caused by a variety of allergens, airborne irritants, and infections. People spend most of their time indoors, so exposures detected in indoor environments such as houses, workplaces, and schools are critical [91]. Exposure to indoor contaminants, such as environmental tobacco smoke [92], air pollution [93], and allergens [94], is a major risk factor for asthma disease. Even though the significance of indoor environmental exposure in the formation of morbidities and exacerbations is relatively unclear, there is considerable evidence that exposure to indoor risk factors plays a crucial role in initiating and worsening asthma, allergy, and respiratory disorders.

A great deal of study has been done to examine the relationship between indoor environmental variables and asthma, allergy, and respiratory disorders in children's homes, schools, and childcare settings [95][96][97]. On the other hand, adults might be exposed to interior risk factors in variety of settings. Previous studies show that many houses and workplaces in different countries have important indoor environmental problems [98][99][100]. This is a critical issue since individuals spend the most of their time indoors.

There are several sources of indoor air pollutants in the house. Air pollution within houses is caused by a complicated mixture of compounds that enter from the outside air and agents created inside. Indoor air pollutants can differ in terms of their potential health impacts and severity and their distribution across geographical regions, cultural backgrounds, and socioeconomic positions. Indoor pollutants contain combustion byproducts such as particulate matter and nitrogen oxides, as well as airborne allergens and biological contaminants. Indoor air pollution can induce health consequences such as sneezing, coughing, cancer, and worsening chronic respiratory diseases such as asthma [96]. In comparison to outside surroundings, humans may have a better capacity to change indoor environmental exposures. Because of adjusting to indoor surroundings, indoor air pollution is an appealing target for illness prevention. Chemical and

biological compounds and their relationships with asthma morbidity were investigated in the following section.

1.6.1. Indoor Chemical Pollutants and Asthma

Volatile organic compounds (VOCs) are known as chemical contaminants found in indoor environments. VOCs are defined as organic substances mainly composed of carbon and hydrogen with a boiling temperature according to WHO [101]. VOCs have a high vapor pressure but poor water solubility. Volatile organic compounds are harmful gases or vapors generated by some solids or liquids at room temperature. Most of the VOCs are man-made substances that are utilized and manufactured in the production of paints, medicines, and refrigerants. Volatile organic compounds contain a diversity of chemicals such as benzene, perchloroethylene, and fuel oxygenates [102]. VOCs are commonly found in gasoline, Cigarette smoke, hydraulic fluids, paint thinners, dry cleaner chemicals, and floor and wall covering materials. In addition, VOCs are prevalent pollutants in groundwater and are found as byproducts of water treatment like chloroform. VOCs are released by a diverse range of items that number in the thousands, for example, building materials, furnishings, office equipment, Etc.

VOCs contain a wide range of compounds, some of which could have short and long-term negative health consequences. Many volatile organic compound concentrations are continuously greater indoors (up to ten times greater) than outside. Exposure to indoor VOCs causes some symptoms such as rhinitis, wheezing, ocular and respiratory irritation, shortness of breath, and asthma. Most of the VOCs are known to have negative effects on lung function. However, most research has investigated the effects of single VOCs rather than the overall effects on health [103][104]. In a study conducted in France, 490 homes were investigated for various VOC concentrations. According to this study, N-undecane and 1,2,4-trimethylbenzene levels were risk factors for asthma, and ethylbenzene, trichloroethylene, and m/p-and o-xylene were risk factors for rhinitis. VOC levels in houses were shown to be concentration-dependently related to the incidence of asthma and rhinitis in adults [105]. An investigation in the United States showed that exposure to aromatic chemicals was associated with physician-diagnosed asthma (OR = 1.63) and wheezing attacks (OR = 1.68) [103].

One of the most well-known VOCs is formaldehyde. At room temperature, formaldehyde is a colorless gas that is combustible and extremely reactive. Formaldehyde is also one indoor air

contaminant that can be easily detected. Exposure to high levels of formaldehyde causes cough and wheeze symptoms and could irritate the eyes and nose. Additional exposure may result in severe allergic reactions to the skin and respiratory disorders [106]. According to WHO, the highest level of formaldehyde in indoor settings could be 100 mg/m^3 in 30 minutes. In a study conducted in Japan, 998 pregnant women were investigated and reported that exposure to more than 47 ppb of formaldehyde was related to a higher frequency of atopic eczema [107]. Another study reported that exposure to low concentrations of formaldehyde (<100 $\mu g/m^3$) was positively associated with bronchial responsiveness to mite allergen in mite sensitized adults with asthma. [108].

Particulate matter, carbon monoxide, sulfur dioxide, nitrogen dioxide, and organic pollutants are released by coal or biomass-fueled stoves, which are essential sources of indoor combustion. It is thought that most people in the world, especially in developing countries, still utilize coal or biomass for heating systems and cooking [109]. Some studies in the literature investigate the associations between fuel combustion and asthma disease in the indoor environment. A cross-sectional study conducted in India represented that exposure to the combustion of biomass fuels was a risk factor for asthma morbidity in elderly persons [110]. Another study conducted in the United States involved 508 adults. This study reported that exposure for more than six months to coal and wood used for cooking was a risk factor for asthma severity. However, no association was reported for indoor heating using coal and wood with asthma [111]. A cross-sectional investigation was conducted among women in rural Nepal discovered substantial connections between biomass smoke consumption and cough, phlegm, shortness of breath, wheezing symptoms, and asthma [112]. According to a study in China, using coal fuel indoors has negative effects on forced vital capacity (FVC) and asthma in rural regions [113]. Unlike previous research, an indoor study from the United States showed no relation between gas stove usage and forced expiratory volume (FEV_1), FVC, or peak expiratory flow (PEF) with an increased risk of respiratory symptoms [114].

Carbon monoxide (CO) is a stifling gas that binds to hemoglobin, interfering with oxygen transfer. The elderly, children, fetuses, and asthmatics are among groups that are sensitive to increased carbon monoxide levels. The primary indoor sources of carbon monoxide include gas and wood stoves, tobacco consumption, and fireplaces. Elevated levels of CO in dwellings are

directly associated with using unclean fuels such as biomass fuels, especially for heating systems and indoor cooking [115]. A study conducted in China among farmers discovered that high CO levels harm pulmonary functions [116].

Nitrogen dioxide (NO_2) is a significant pollutant created by combustion, especially at high temperatures. Unvented gas appliances are the primary source of NO_2 indoors, such as stoves, furnaces, and tobacco smoking. Nitrogen dioxide could be especially serious in the inner city due to the everyday use of gas stoves, and appropriate venting is not available. NO_2 is an irritating gas that has been associated with respiratory problems. Even though some research has concluded that indoor NO_2 hurts respiratory health [117][118], others have been unable to corroborate that relationship [114]. Previously, studies reported that indoor NO_2 might enhance bronchial responsiveness in people with asthma and also increase the risk of respiratory disorders in the general population [119][120]. A study undertaken in eight inner cities throughout the US discovered a connection between elevated indoor NO_2 and reduced PEF and worse respiratory symptoms in children with asthma [121]. Another study in Singapore investigated the impact of exposure to indoor gas cooking on women with chronic asthma. Results of this study showed that exposure to NO_2 was found to be positively related to the frequency of cooking, which was attributed to the higher usage of rescue bronchodilators [122]. However, there is some evidence that NO_2 has a role in the pathophysiology of asthma, but the fundamental processes remain unknown. Bayram et al. showed that NO_2 controlled airway inflammation in people with asthma by enhancing the release of inflammatory mediators from bronchial epithelial cells [123]. In addition, another study reported that NO_2 acts like a pro-inflammatory air pollutant during repeated exposure settings and so is reduced in FEV_1 and FVC after first exposure in healthy nonsmokers [120].

Particulate matter (PM) contributes to indoor air pollution in dwellings. PM comes from a range of manufactured and natural sources. Pollen, spores, germs, plant and animal detritus are considered natural sources, and emissions from vehicles, burning byproducts, and power plants are examples of manufactured sources. Some indoor sources consist of cooking, stoves that use biomass or wood, cigarette consumption, and outdoor PM sources [124][125]. Particulate matter indoors and outdoors varies in terms of source, content, and concentration. For example, the health impacts of indoor PM could not be easily evaluated from outside air pollution research

[126][127]. Particulate matter concentration is significantly greater and more variable within the home compared to outdoor PM concentration. There have been some investigations into the relationship between indoor PM and respiratory diseases such as asthma. A study conducted in Seattle among school-age children found that exposure to indoor $PM_{2.5}$ was more effective than exposure to outdoor $PM_{2.5}$ in reducing lung function [128]. An investigation in California reported considerable decreases in lung function (FEV_1) linked with indoor PM concentration among 19 children. This study also discovered significant relationships between lung function and indoor PM concentrations compared to outdoor PM concentrations [129].

Indoor PM concentrations were significantly influenced by smoking [125]. In the last several years, smoking has been identified as a significant contributor of indoor particles. ETS (environmental tobacco smoke) is known as a dynamical, complex combination of over 4,000 compounds [130]. Environmental tobacco smoke is also found in vapor and particulate phases. A study conducted in the United States reported that ETS levels differed between various environments. ETS exposure was most significant at home, work, and outdoors, respectively [131]. Being with a smoker in the same place is related to a 20-30% higher risk of lung cancer [132]. Adults are often exposed to ETS, which has been linked to an elevated risk of asthma disease. Some studies estimated that more than 30 percent of children in the United States are exposed to secondhand smoke. The impacts of ETS on respiratory disorders, especially asthma disease, have been studied in several publications, and the data suggest that individuals who have been exposed to ETS are more likely to acquire asthma. A study in India found that exposure to environmental tobacco smoke was a risk factor for asthma exacerbation [133]. In another investigation, Hersoug et al. found that exposure to environmental tobacco smoke for 0.5–5 hours was related to a higher incidence of rhinitis symptoms [134]. 3471 persons (aged 18–69 years old) participated in a study in Denmark. As a result of this study, exposure to ETS for more than 5 hours per day leads to an elevated risk of chronic cough, wheezing, and reduced lung function [135]. In addition, ETS exposure was investigated with other probable risk factors for asthma in various studies [136][137]. In a study conducted in China, 31704 people from six cities participated. Exposure to environmental tobacco smoke, workplace dust and gas, and irritant smoke during cooking were risk factors for chronic cough, wheezing, and asthma [137].

1.6.2. Indoor Biological Pollutants and Asthma

Several investigations have indicated that biological pollutants could be found in the environment. Microbial pollutants are routinely found indoors and outdoors; however, exposure levels vary greatly. Biological markers have many reservoirs, such as bacteria, mold spores, and many other fungi, dust mites, pets like cats and dogs, pests (cockroaches, rats, rodents), plants like pollen endotoxins, beta-glucans, microbial fragments, as well as humans. Numerous studies have investigated the association between exposure to allergens from dust mites, cats, dogs, cockroaches, and rodents, as well as molds and asthma development and aggravation. The detectable concentrations of biological pollutants might vary considerably because of differences in sampling methodology and analysis techniques utilized. Several experimental research studies have employed antibody-based enzyme-linked immunosorbent assays to determine allergen levels.

In many regions of the globe, house dust mites (HDM) are detected indoors as a significant source of inhalant allergens. Some studies have found increased levels of house dust mite allergens in residential areas [138][139][140]. In house dust, cat and dog allergens were found in concentrations ranging from 0.11 to 24 μg/g. Concentrations of HDM in houses depend on home characteristics and behavioral factors. Gross and coworkers represented that older mattresses, old carpets, homes without central heating systems, lower floors, and keeping dogs at home are the factors that increase levels of house dust mite allergens in homes [139].

Cockroach and mouse allergens are common in indoor environments and may be associated with asthma morbidity. Previously, some studies have investigated levels of cockroach and rodent allergens in homes. For example, 82 percent of US households (n = 831) had detectable levels of mouse allergen, with kitchen floor values exceeding 1.6 μg/g [141]. Another study reported that cockroaches were found in 77 percent of flats (total of the flats were 324), and flats with elevated levels of *B Germanica 2* were a risk factor for having an asthmatic occupant [142]. Similarly, 107 homes were included in a study conducted in China. The results showed that cockroach allergens were found in 93% of the houses and were more prevalent in living room samples than in mattress samples. The allergen levels of cockroaches were more significant in the winter than in the summer [143]. A well-known indoor allergen, mouse allergen, is a frequent domestic allergen. Matsui et al. reported that mouse allergen was identified in house

dust samples from 100% of residences in inner-city Baltimore [144]. Furthermore, airborne mouse allergen was found in more than 80% of the bedrooms examined [145]. This study reported that houses with more than 0.5 μg/g mouse allergen in their bedrooms were associated with increased asthma morbidity and asthma medication usage among mouse sensitized people [144].

Dogs and cats seem to be the most well-liked pets throughout many regions of the world, while having such animals at home is linked to an increased risk of allergic sensitization. Some investigations reported levels of cat and dog allergens in the house, and increased amounts of *C familiaris 1* and *F domesticus 1* were found in settled house dust and airborne samples in houses that kept these pets [138][146]. Allergen levels varied greatly amongst residences, ranging from 0.1 to 200 μg/g [147]. In a study conducted in 831 US houses, dust samples were collected from different locations of homes. *C familiaris 1* and *F domesticus 1* were found in 100% and 99.9% of households, respectively, even though only 49.1% of residences had domestic pets [148]. A study conducted in Sweden investigated the relationship between exposure to indoor allergens and the frequency of allergy sensitization. This study reported that exposure to cat allergens was a risk factor for asthma symptoms [100].

Fungal allergen concentrations in house dust samples are often higher. Fungal allergen exposure has often been evaluated through fungal culture techniques. In general, indoor fungi are a combination of outdoor and indoor sources [149]. Although *Aspergillus* spp. and *Penicillium* spp. are less frequent outdoors, they are the most common indoor fungi [150]. An investigation in Iran found that the most frequent indoor fungus species were *Cladosporium* spp. (29.2 percent), *Aspergillus* spp. (19 percent), and *Penicillium* spp. (18.3 percent), respectively [151]. Fungal sensitivity can be induced through exposures in the outdoors, indoors, or at work. Several investigations have found that exposing sensitized asthmatics to elevated amounts of allergens worsens their lung function [152][153]. A study discovered that numerous asthma triggers, particularly mold, were as ubiquitous or more frequent in the homes of people with asthma in New York than in control families. The presence of mold was found as a risk factor for current asthma. [154]. Other research in the UK found that adults who were sensitized or exposed to extreme amounts of allergens had substantially lower predicted FEV_1 percent than unsensitized or unexposed persons [155]. On the other hand, cohort research in New York revealed no

relation between sensitivity to indoor allergens and higher asthma prevalence in inner-city individuals [156].

1.7. LITERATURE REVIEW

Various studies in the literature have found a link between several biological markers and respiratory illnesses. The findings of these investigations are contradictory. The following section is a summary of important studies in the literature.

Three European countries, Spain (n = 481), the Netherlands (n = 553), and Germany (n = 395), contributed to a birth cohort study. The purpose of this study was to determine if early exposure to biological agents such as endotoxin, β-(1→3)-D-glucan, and extracellular polysaccharide (EPS) in house dust is related to asthma and allergies later in life in children from suburban regions. House dust samples were collected from the living room a short time after birth. High endotoxin levels were found as a risk factor for current asthma at six years of age in the Netherlands, whereas elevated levels of endotoxin were inversely associated with ever asthma up to 10 years of age in Spain. No relation was found between asthma and endotoxin levels in German samples. In all cohorts, no relationships with atopic sensitization were found. All of the relationships between β-(1→3)-D-glucan and fungal EPS were statistically insignificant [157].

In a cross-sectional study in five European countries, microbial agents in house dust were measured to investigate the associations between these markers and atopic wheeze in farm children. The study population consisted of 270 atopic and 441 non-atopic children. Average concentrations of mattress dust endotoxin, fungal EPS, and β-(1→3)-D-glucan in the study group were somewhat greater in control children than atopic wheezers. As a conclusion of this study, mold components and bacterial endotoxin may have some protection against atopic wheeze in children. However, this study cannot explain the protective effect of growing on-farm and mattress biomarker concentrations detected in the study population [158].

The relationship between exposure to biological agents and asthma and allergies in 6 years old children was investigated in a case-control study conducted across three European birth cohorts. The study population consisted of two birth cohorts in Germany (n = 358) and one in the Netherlands (n = 338). Exposure to house dust endotoxin and EPS from *Aspergillus* and *Penicillium* spp. has an inverse association with physician-diagnosed asthma in the two German subgroups. Furthermore, EPS exposure was found to be inversely associated with physician-

diagnosed allergic rhinitis. In the Dutch children, no associations were found between exposure to biological markers and respiratory disorders. This study cannot explain the reasons for the mixed results between countries. [159].

A multi-center cross-sectional study was carried out in Albania, New Zealand, Sweden, and the United Kingdom. The study population consisted of 840 children (9–12 years old). Dust samples were collected from living rooms to determine the endotoxin levels. Combined across countries, endotoxin concentrations were inversely related to childhood asthma. In addition, current wheeze and atopy have inverse associations with endotoxin levels [160].

A case-control study was carried out in the rural region of Humboldt, Canada, from 2005 to 2007. The children in the study ranged in age from age 6 to 18. Within the last year before sampling, cases (n = 102) reported doctor-diagnosed asthma or wheezing. Children in the control group (n = 208) were chosen randomly among children who did not have asthma or wheeze. Mattress endotoxin levels were negatively linked with asthma in children aged 6–12 years (OR = 0.44, 95% CI: 0.20–0.98). However, these associations were not found in older children (12–18 years old). The results of this study imply that exposure to endotoxin has a protective effect on asthma and wheezing in younger and non-allergic schoolchildren [161].

Three European countries (Germany, Sweden, and the Netherlands) contributed to the data in a case-control study. One hundred eighty sensitized children for the case group and 180 non-sensitized children for the control group were selected per country. The purpose of this study was to examine the links between biological markers in household dust and allergy sensitization in children aged 2-4 years. Findings of this study showed that elevated levels of mattress dust and higher amounts of endotoxin, β-(1→3)-D-glucan, and extracellular polysaccharide loads in mattress dust were related to a considerably lower incidence of sensitization to inhalant allergens [162].

The association between exposure to biological agents such as endotoxin and β -(1→3)-D-glucan with asthma severity in children aged 7–17 was investigated in a clinical cross-sectional study carried out in the province of Saskatchewan, Canada. Asthmatic children (n = 116) included 75.9% with mild asthma and 24.1% with moderate/severe asthma. House dust samples were collected from the play area and mattresses of children. Elevated endotoxin levels in children's mattresses were a risk factor for asthma severity (aOR = 11.40, 95% CI: 1.45–89.43).

On the other hand, higher β -(1→3)-D-glucan levels in play areas were negatively related to moderate/severe asthma (aOR = 0.16, 95% CI: 0.03–0.89). Moreover, high amounts of mattress endotoxin were linked to decreased FVC and FEV_1. These relationships were not observed for β-(1→3)-D-glucan [163].

Another cross-sectional study of children aged 7–17 years was conducted in the region of Saskatchewan, Canada. The purpose of the study was to determine the association between endotoxin exposure in household dust with atopy and exercise-induced bronchospasm (EIB) in children with asthma. Skin prick testing (SPT) was completed by 99 of the 116 asthmatic children, while all completed exercise challenge testing (ECT). 71.7% of children were atopic, while 22.4% had EIB. Atopy was shown to be adversely related to high endotoxin concentrations and loads in play areas. On the other hand, EIB was linked to high endotoxin levels in mattresses [164].

In a study conducted in the Netherlands, 148 schoolchildren (7–11 years old) contributed. Half of the children in this study had self-reported or parent-reported chronic respiratory problems. The association between peak expiratory flow (PEF) variability with endotoxin and β-(1→3)-D-glucan levels in house dust was determined in this study. Endotoxin and β-(1→3)-D-glucan levels per square meter of living room floor were substantially linked with PEF-variability in unadjusted analyses, notably in atopic children with asthma symptoms. Adjusted analysis revealed the same relationship for β-(1→3)-D-glucan but not for endotoxin [29].

Another study investigated exposure to indoor pollutants such as endotoxin, Der p 1, damp, ETS, and $PM_{2.5}$ among asthma and non-asthmatic children. The children were chosen from two primary care facilities based on their responses to a questionnaire survey. Children with asthma were matched for gender, age, and sibling size with children from asthma-free homes. Elevated endotoxin concentrations were identified in the living room floors of the asthmatic children, but not in the bedroom carpets or mattresses, compared to the control homes in 90 matched pairs. Furthermore, children with asthma were likely to reside in a single-parent household, in a home in which the parents reported dampness at home, and in a home where the living room had been redecorated within the last year of the sampling. According to this study endotoxin in urban dwellings may be a risk factor for the presence of asthma [165].

In a study conducted in New York City, 301 participants contributed. Endotoxin levels were determined in dust samples taken from bedroom floors at ages 12 and 36 months. Lower endotoxin levels have been linked to wet mop cleaning and specific neighborhoods. Endotoxin concentrations in the dust corresponded only slightly with cockroach and mouse allergies. Children in families with elevated endotoxin levels were less likely to develop eczema at one year of age and more likely to wheeze at two years of age. These correlations were significant in children whose mothers had asthma. According to the findings of this study, exposure to endotoxin in the inner-city neighborhood may be linked to wheezing in children. Nevertheless, negative relations were observed with eczema [166].

A case-control study was conducted in Sweden among pre-school children to determine the association between endotoxins and their effects on asthma. There were 198 children with asthma and 202 non-asthmatic children in the study. Dust samples were collected from the child's bedroom and living room. The amounts of endotoxin in the child's bedroom and living rooms were 479–188×10^3 EU/g and 138–942×10^3 EU/g, respectively. Pet ownership and agricultural activities were shown to be substantially linked with higher endotoxin concentrations in indoor dust. This study showed that exposure to endotoxin in the indoor environment was not related to asthma and allergy in pre-school children. Although this study discovered an association between endotoxin and disease symptoms in a subset of households that did not have indoor pets [167].

A study was conducted in Germany among 272 schoolchildren to investigate the relationship between viable mold levels at home and allergy sensitization. Dust samples were collected from the living room to determine fungal spore counts. According to the results of this study, high levels of *Cladosporium* and *Aspergillus* (> 90th percentile) were a risk factor for allergic sensitization. Additionally, no significant association was observed for *Penicillium* and allergic sensitization [168].

Another study investigated the association of exposure to indoor β-(1→3)-D-glucan and the incidence of allergen sensitization and wheezing within the first year of life. This study was conducted among 574 infants born to atopic parents. Endotoxin exposure was considered a potential confounder. Results of this study showed that exposure to high levels of β-(1→3)-D-

glucan had a lower risk of recurrent wheeze among infants born to atopic parents. This impact was particularly significant in the allergen-sensitized newborn category [169].

A cross-sectional study was conducted in the rural regions of Germany, Switzerland, and Austria. 812 school children (6–13 years old) were included in the study. Blood samples were collected from the youngsters and analyzed for atopic sensitization. Exposure to high endotoxin levels from children's mattresses was inversely associated with hay fever, atopic asthma, and atopic sensitization. Although, high endotoxin levels were not found to be a risk factor for non-atopic wheeze [170].

House dust samples were collected from 422 homes of children (7-10 years old) to determine β -(1→3)-D-glucan concentration. At ages 7–10 and 11–14, each child's health outcome information (asthma, atopy, and bronchial hyperresponsiveness) was evaluated. This study showed that exposure to β -(1→3)-D-glucan at age 7–10 was a risk factor for persistent atopic asthma at age 11–14, regardless of exposure to endotoxin and *Alternaria* or *Cladosporium* sensitization. Elevated levels of BDG in house dust were associated with bronchial hyper-responsiveness in children with asthma. Among children without asthma, exposure to high levels of β -(1→3)-D-glucan was a risk factor for new-onset atopic asthma, although the association was not statistically significant [171].

Three European countries contributed to a study to determine the level of β -(1→3)-D-glucan and EPS from *Aspergillus* and *Penicillium* spp. in mattresses and living rooms among pre-school children. Dust samples were collected from 1065 German, Dutch, and Swedish homes. A survey questionnaire was used to examine the determinants. Amounts of house dust and concentrations of β-(1→3)-D-glucan and extracellular polysaccharide vary between regions. Floor dust and concentrations of β-(1→3)-D-glucan and EPS in mattresses were very slightly associated with those in living room flooring. Carpeted floors were significantly associated with floor dust and levels of β-(1→3)-D-glucan and EPS. None of the other factors were substantially linked with levels of dust β-(1→3)-D-glucan and extracellular polysaccharide on living floors and mattresses [172].

A particular enzyme immunoassay method was used to assess β -(1→3)-D-glucan in settled house dust from the living room flooring of 395 residences in two German cities, Erfurt and Hamburg. β -(1→3)-D-glucan loads from living room floors were correlated with endotoxin,

mite and cat allergens, and culturable mold spores. Carpeting in the living room, visible mold within the last year, the number of occupants (> 4), keeping a dog at home, and a lower frequency of cleaning are all significant determinants associated with elevated β -(1→3)-D-glucan levels [173].

The association of endotoxin and β-(1→3)-D-glucan concentrations in house dust with certain household features was investigated in a study that was conducted in Germany. Dust samples were collected in 25 homes from the living room and bedroom floors and mattresses. Endotoxin and BDG concentrations in dust samples varied from 200-48×10^3 EU/g and 182-3507 µg/g. Microbial agents' levels were high on living room flooring, while the lowest concentrations were found in mattresses. Endotoxin and β-(1→3)-D-glucan levels in house dust were significantly related to the heating system and dwelling age. There were no relationships detected between biological agents' levels and other determinants such as temperature, relative humidity, and dampness. Furthermore, β-(1→3)-D-glucan levels in home dust were linked to total culturable fungus and the fungal species *Alternaria* [174].

Another study conducted in Brittany (western France) was investigated to determine the concentrations of frequent molds and allergens in house dust and indoor air. Airborne and house dust samples were collected from 150 houses that contributed to the study. *Cladosporium*, *Penicillium*, and *Aspergillus* were the most common molds found in the air. The same species were found in dust samples as dominant types of mold. Dwelling age, aeration behavior, pet ownership, smoking at home, dampness, temperature, and season are determinants that are significantly associated with mold concentrations at home. Furthermore, house dust allergens were associated with pet keeping and bedsheet washing techniques [175].

House dust endotoxin and β-(1→3)-D-glucan were measured in 317 Danish children's homes. The geometric mean of endotoxin and β-(1→3)-D-glucan were 31.1×10^3 EU/g and 0.71×10^3 µg/g, respectively. High correlations were observed among floor dust, endotoxin, and BDG. Having a carpet at home was related to a higher dust load, as well as higher endotoxin and β-(1→3)-D-glucan concentrations. Pet ownership, building type, and residence location influenced endotoxin concentrations. There were no additional factors linked with β-(1→3)-D-glucan concentrations. In comparison to other European investigations, this research reported lower BDG but greater endotoxin concentrations [40].

The purpose of another study was to examine the amounts of indoor endotoxin in air and dust samples from randomly chosen urban and rural residences. Endotoxins were measured in farmhouses and nonfarm houses in rural areas, as well as dwelling features, lifestyle variables, and agricultural methods that might impact air and dust endotoxin levels. Endotoxin concentrations in farmhouse floors and mattress dust were much higher than in other rural and urban residences. Nevertheless, no variation in endotoxin levels in the air of urban and rural residences was identified. Also, no correlation was observed between airborne and house dust endotoxin levels. Direct entrance into the home and lack of ventilation is associated with a high endotoxin concentration in house dust. Findings from the study demonstrate that dairy farming is related to a significant endotoxin level. There was no difference in indoor airborne concentrations between urban and rural dwellings. These findings imply that detecting endotoxin in house dust is the most appropriate approach for determining endotoxin exposure [176].

2. MATERIALS AND METHODS

The following sections explain the selection of case and control groups, survey questionnaires, dust sampling, and dust extraction, analyzing the biological markers, and statistical analysis used in this study.

2.1. STUDY POPULATION

A case-control study was conducted in the province of Ankara, Turkey, from 2018 to 2020. This study included both asthmatic and non-asthmatic schoolchildren. The children in the study ranged in age from 6 to 11. There were 109 asthmatic children and 130 non-asthmatic children in the study (without asthma). Hacettepe University Pediatric Clinic tracked patients in the case group who had been diagnosed with asthma. The follow-up duration of asthmatic children was carried out by Hacettepe University Department of Child Health and Disease Allergy and Asthma Unit. We obtained authorization from the Ankara Provincial Directorate of National Education to select the control group from schools for this study. The children in the control group are between the ages of 6 and 11 years old and do not have any asthma or allergic sensitization that a doctor has identified. For the case and control groups, the distribution of volunteer families is in Ankara. The families participating in the research in the asthma and control groups are similar in terms of socioeconomic status. All families who participated in this study were informed about the research, and parents completed consent forms. The local Ethical Committees in Turkey approved the study (26/02/2016-E.7061). The ethical committee approval is given in appendix A and the parental consent form is given in appendix B.

2.2. SURVEY QUESTIONNAIRES

A comprehensive survey questionnaire was prepared to collect data, which was filled in by our research team during home visits. The survey questionnaire included questions about housing characteristics, environmental exposure, lifestyle and habits of occupants, building characteristics, and general health information. A total of 239 families completed the survey questionnaire. Table 3.1 presents characteristics for the study population for asthma and control groups. The home characteristics of children in asthma and control groups and the lifestyles of their family members are compared in Table 3.1. The Table is presented in section 3.1.1.

2.3. DUST SAMPLING

Settled house dust samples were collected during home visits in the winter of 2019 as a part of a study in both case and control houses. According to a standardized protocol, House dust samples were collected from carpets ($2m^2$) and smooth floors ($4m^2$) of the living room and bedroom of children, using a vacuum cleaner equipped with a socks filter for 4 min. Combination living room and bedroom floor dust samples were collected at each participant's home using a Dyson Ball Multi-Floor vacuum cleaner fitted with a socks filter. Dust samples were sieved using a 200μm mesh steel filter and were stored -20°c until analyzed. Figure 2.1 shows the socks filter used during house dust sampling.

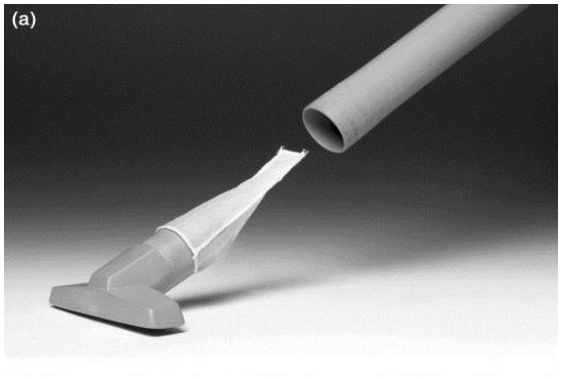


Figure 2. 1. Socks filter for house dust sampling

2.4. ANALYZE OF BIOLOGICAL MARKERS

2.4.1. Analyze of Endotoxin

The bacterial endotoxins test (BET) uses amoebocyte lysate from the horseshoe crab (*Limulus* polyphemus or Tachypleus tridentatus) to identify or measure endotoxins from gram-negative bacteria. This test can be done in three ways. The first method is the gel-clot technique, which depends on gel-forming. The second technique is the turbidimetric method, which depends on the turbidity improvement after the separation of an endogenous substrate. The third technique is the chromogenic method, which depends on the color change after the separation of a synthetic peptide-chromogen compound. A turbidimetric technique was used in this study to determine the bacterial endotoxin. This method is a photometric test that measures changes in the turbidity of the reactant. This approach can be divided into either an endpoint-turbidimetric

test or a kinetic-turbidimetric test, depending on the assay principle used. The measurable association between the amounts of endotoxin and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation time is the basis of the endpoint-turbidimetric test. The kinetic-turbidimetric technique is a method for determining the time (onset time) required to obtain a predefined absorbance or transmission of the reaction mixture, as well as the rate at which turbidity develops. The assay is carried out at the lysate manufacturer's specified incubation temperature (typically 37°C).

After sieving (0.2 mm) and weighing up the house dust samples collected on the filter, samples were extracted at room temperature. Dust samples were extracted with pyrogen-free water (endotoxin-free water). 10 mg of dust were extracted with 5 ml of pyrogen-free water and sonicated for 1 hour at room temperature. After centrifuging at 3000 rpm for 15 minutes, the supernatants were collected for endotoxin analysis. The samples were kept at -20 °C until they were analyzed. The first supernatant was used to measure endotoxin in the Turbidimetric kinetic *Limulus* Amoebocyte Lysate (LAL) test, using one single lot of the LAL reagent.

Before starting the endotoxin analysis, validation tests are required to determine the accuracy of the Kinetic Bacterial Endotoxin Tests. When a new batch of lysate is used or when the test conditions can alter the results, the lysate sensitivity confirmation test should be performed. The greatest permitted dilution of a sample at which the endotoxin limit may be determined is known as the Maximum Valid Dilution (MVD). Calculate the MVD using the following formula:

$$MVD = \text{Endotoxin Limit} \times \text{Concentration of sample solution} / \lambda$$

The limit of endotoxin depends on the product and its administrative route and is outlined in the monograph. The concentration of the sample solution is expressed as mg/ml. λ is the lowest concentration used in the standard curve for the turbidimetric or chromogenic methods.

Materials for endotoxin assay:

- Control Standard Endotoxin (CSE)
- Limulus Amebocyte Lysate (Pyrotell®-T)
- Pyrosol buffer
- LAL Reagent Water (LRW) (Pyrogen-free water)
- Pyros Kinetix® Flex tube reader

All the above materials were obtained from Associates of Cape Cod, Inc. Except for the standard, all of those mentioned above are devoid of interfering amounts of endotoxin. All other materials used in this method, such as glassware, pipettes, and test tubes, were free of interfering endotoxin.

Turbidimetric endotoxin testing may be used for an extensive and varied range of tests, including water testing and samples that require great sensitivity, like intrathecal products, and those that require large dilutions to overcome interference. This method allows the user to create a wide range of standard curves. The limit of detection in this assay was 0.001 EU/ml.

For endotoxin analysis, Control Standard Endotoxin (CSE) (*E. coli* 0113:H10) and Pyrotell®-T were prepared in the first step. According to the manufacturer's protocol, Pyrotell®-T was reconstituted with 5 mL of LRW. Different endotoxin concentrations were prepared to generate the standard curve. To perform the test, each standard endotoxin solution was measured twice. By mixing CSE with LRW, 500 EU/ml, 50 EU/ml, 5 EU/ml, 0.5 EU/ml, 0.05 EU/ml, and 0.005 EU/ml standards were prepared, respectively. The standard coefficient of correlation should be greater than or equal to 0.99. Figure 2.2 shows the standard curve for endotoxin analysis.

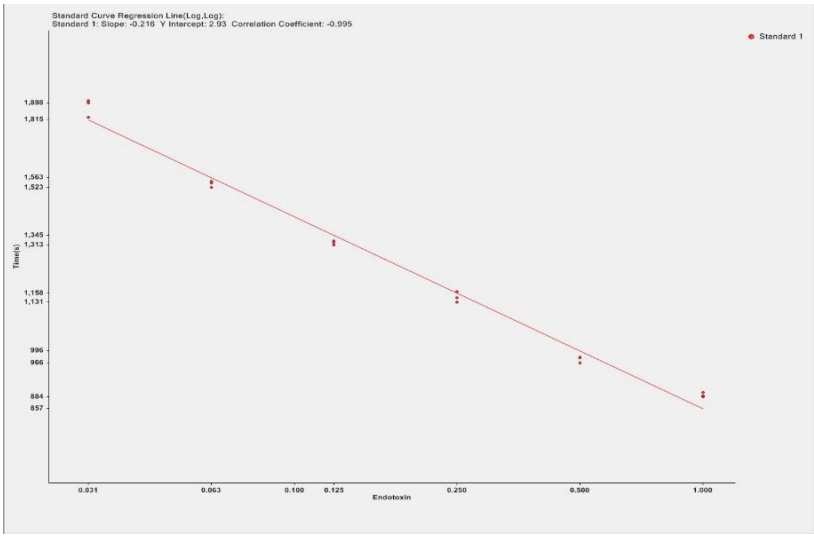


Figure 2. 2. Standard curve for endotoxin

Controls are required to ensure an accurate test. The positive control's endotoxin concentration should be the same as a standard from the center of the standard curve. The appropriate concentration for positive control in this study was 5 EU/ml. Positive sample controls are inhibition and enhancement controls that include the sample or dilution of the sample to which

standard endotoxin is added. The increased endotoxin concentration in the test sample should be the same as in the positive control. The additional endotoxin is referred to as a "spike." In addition, each test contained LRW as negative controls.

All endotoxin tests were evaluated according to three criteria determined as the acceptance limit. These criteria are as follows:

-The recovery of endotoxin should be between 50-200%.

-In linear regression, the correlation coefficient (r) must be greater than or equal to 0.98.

-The coefficient of variation (CV) for each standard solution should be less than 20%.

All samples were placed in the Pyros Kinetix® Flex tube reader. Optical densities were read by incubating the samples at 37°C ± 0.5°C and 600 nm wavelength. The standard curve's lowest concentration determines the period of incubation. Endotoxin concentration was calculated according to a standard curve by the software. Figure 2.3 shows Pyros Kinetix® Flex tube reader and Limulus Amebocyte Lysate (Pyrotell®-T).

Figure 2. 3. Pyros Kinetix® Flex tube reader and Limulus Amebocyte Lysate (Pyrotell®-T)

2.4.2. Analyze of β -(1→3)-D-glucan

The frozen residues and remaining supernatants were used for further processing and β-(1→3) -D-glucan analysis. After the first extraction, the frozen residues were vortexed for 30-60 seconds at room temperature and centrifuged at 3000 rpm for 15 minutes to remove the liquid

part. The samples were then autoclaved for 60 minutes at 120 °C. Autoclaved samples were prepared at specific dilutions. For the analysis of β-(1→3) -D-glucan, two procedures are available: the *Limulus* Amebocyte Lysate assay (LAL) and the inhibition Enzyme Immunoassay (EIA). The LAL test is more reliable, specific, and sensitive than the EIA in quantifying linear and branched β-(1→3)-D-glucan. β-(1→3)-D-glucan was measured in the second supernatant with LAL assay.

The Fungitell (Associates of Cape Cod, East Falmouth, MA) test detects β -(1→3)-D-glucan. This method depends on a shift to the Limulus Amebocyte Lysate (LAL) pathway. The Fungitell reagent has been modified to remove bacterial endotoxin reactions and, as a result, exclusively respond to β -(1→3)-D-glucan via the Factor G-mediated route. Factor G, a serine protease zymogen, is activated by β -(1→3)-D-glucan. When Factor G is activated, it changes the inactive pro-clotting enzyme to the active clotting enzyme, which then cleaves para-nitroanilide (pNA) from the chromogenic peptide substrate, BocLeu-Gly-Arg-pNA, producing a chromophore, para-nitroaniline, which absorbs at 405 nm. The Fungitell kinetic test, which is detailed further below, is based on determining the rate of optical density increase caused by a sample. This rate is compared to a standard curve to get estimates of β -(1→3)-D-glucan concentration in the sample. Figure 2.4 shows the *Limulus* Amebocyte Lysate (LAL) pathway.

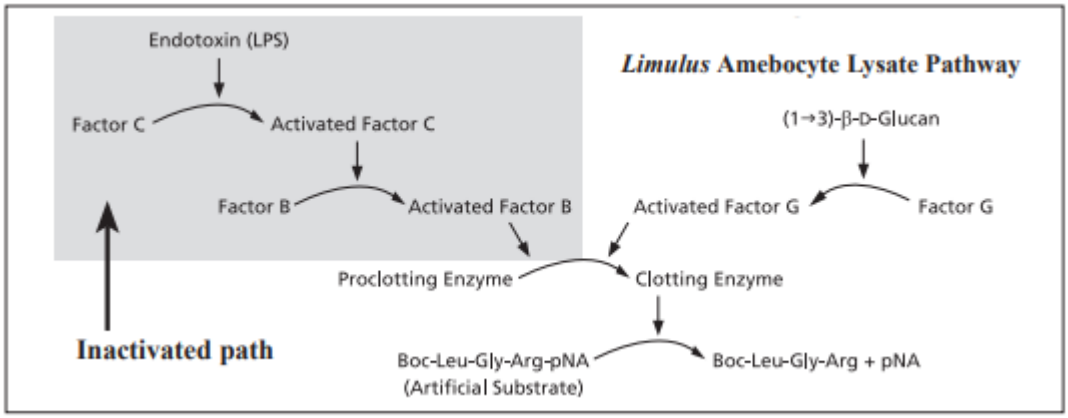


Figure 2. 4. Limulus Amebocyte Lysate (LAL) pathway

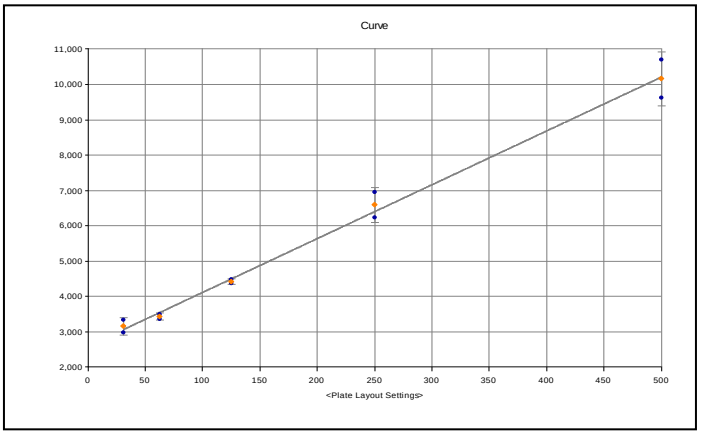
Materials for β -(1→3)-D-glucan assay:

- Fungitell Reagent, a lyophilized β -(1→3)-D-glucan specific LAL
- Pyrosol Reconstitution Buffer

- Glucan standard, with the β -(1→3)-D-glucan content stated on the label
- Reagent Grade Water (RGW)
- Alkaline Pre-treatment Solution

Except for the standard, all of those mentioned above are devoid of interfering amounts of β -(1→3)-D-glucan. All other materials used in this method, such as glassware, pipettes, and test tubes, were free of interfering glucan.

One vial of the glucan standard was dissolved with the RGW (glucan-free water) specified in the vial to make a 100 pg/mL solution. The solution was vortexed for 30 seconds. The standard glucan solution was kept between 2-8°C and was utilized within three days. By mixing glucan solutions with RGW, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, and 6.25 pg/mL standards were prepared, respectively. The standard coefficient of correlation should be greater than or equal to 0.99. The standard curve obtained in the analysis is given in Figure 2.5. To prepare the Fungitell reagent, 2.8 mL of Pyrosol buffer was added after dissolving a vial of Fungitell reagent with 2.8 mL of RGW. Figure x shows the standard curve for β -(1→3)-D-glucan assay.



	Curve Name	Curve Formula	A	B	R²
	Curve	Y=A*X+B	0.0152	2.6	0.998

Figure 2. 5. Standard curve for β -(1→3)-D-glucan

After vortexing the samples for 30 seconds, 5 µl of each sample was added to the designated wells in at least one duplicate. The procedure was repeated for each sample. If the samples have a pH issue, 20 µl of KCL+KOH is added to each well with the sample. If there is no pH issue, 20 µl of RGW is added to each well, including the sample. 25 µl of negative controls and glucan standards were added in the next step. After adding 100 µL of the Fungitell reagent to each well, the plate was inserted into the microplate reader. The plate reader software was configured to collect data in the mean mode. The software wavelength was set up at 405 nm, and the incubation temperature was to be set at 37 °C for 40 min. The shaking plate reader was to be set for 5 – 10 seconds. The concentration of β -(1→3)-D-glucan in each sample was determined according to the standard curve. Figure 2.6 shows the microplate reader and Fungitell test kits.

Figure 2. 6. Microplate reader and Fungitell test kits

2.4.3. Analyze *Aspergillus* and *Penicillium* Spp.

The dust samples we collected from 239 houses were weighed initially, and 0.025 g of sieve dust was separated for extraction and identification of molds. 25 mg of dust was diluted in 5 ml of pyrogen water. After shaking for 1 hour at room temperature, the samples were centrifuged at 2500 rpm for 15 minutes. Sabouraud-Antibiotic media were used to prevent bacterial growth. Plates were incubated for seven days at 25° C. Dichloran Rose-Bengal Chloramphenicol (DCBR) agar base were used for *Aspergillus* and *Penicillium* spp. All the samples were repeated 2 or 3 times in the same dilution, and the average amount was considered. The plates were incubated at 25° C for ten days. Colonies were counted for Aspergillus and Penicillium using

optical methods. The number of colony-forming units (CFU) was counted by a colony counter, and the results were expressed as CFU per gram of dust.

CFU/gram of dust = average of parallel plates × dilution factor

2.5. STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS statistical software version 23.0 (SPSS Inc. Armonk, NY: IBM Corp.). Distributions of the biological marker concentrations and floor dust loading were skewed and, so, were described using the median (interquartile range (IQR)). The independent sample chi-square ($\chi 2$) test was used for comparing categorical variables between asthma and control groups. Concentrations of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* spp. levels were expressed as median (IQR) and compared between asthma and control groups.

Since endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations were not normally distributed, non-parametric tests were performed to compare concentration differences among children with various family lifestyles and home characteristics. Mann–Whitney U test was used when we had two options for independent variables. For more than two options, the Kruskal–Wallis test was performed. Home characteristics and the lifestyles of families were categorized according to residence characteristics, dampness indicators in homes, different family lifestyle habits, and some lifestyle behaviors.

In the ANOVA test analyses, twelve determinants had at least one significant association with floor dust and β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations. These determinants include house floor, residential area, heating system, water damage at home within the last year, dampness at home, visible mold spots at home, houseplants, having new furniture, type of rug, frying frequency, where to dry clothes, and the frequency of changing coverlets and bedsheets. Of these, water damage at home, dampness, and visible mold spots have a moderate correlation. Therefore, in the multivariate logistic regression models, visible mold spots were chosen because they had more significant associations with β-(1→3) -D-glucan, *Aspergillus*, and *Penicillium* concentrations than water damage and dampness in the non-parametric test. Heating systems are another factor not included in multivariate logistic models because the number of houses that use coal or wood for heating systems is only four when compared to other

groups with different heating systems. In this case, the multivariate models had nine determinants. There was a low or slight correlation between these factors.

Families were divided into two subgroups (≤ 50 percentile (median) vs > 50 percentile) based on the amount of floor dust and concentrations of endotoxin, β-(1→3) -D-glucan, *Aspergillus* and *Penicillium,* and then bivariate and multivariate logistic regressions were used to look for relationships between higher (> median) concentrations for biological markers and household determinants. First, bivariate logistic regression models were used to investigate crude associations. After that, in the bivariate logistic regression analysis, those determinants that had at least one significant relationship with the higher floor dust or concentration of biological markers in the non-parametric tests were selected for multivariate modeling. In the multivariate logistic models, the selected determinants were mutually adjusted. Multivariate results were expressed as add ratios (OR) and adjusted odds ratios (aOR) with a 95% confidence interval (Cl).

The multivariate logistic regression model was used to determine the association between microbial exposure, home characteristics, the lifestyle of families, and asthma risk (control group as reference). The parameters included in this model were sex, age, potential determinants such as dwelling age, location of house floor, having a separate kitchen at home, damp smells, pet ownership, smoking at home, frying frequency at home, cleaning frequency, frequency of changing coverlets, repair and paint, new furniture at home, number of people in the household, and the material of the child's mattress. The variables included in this model were identified based on statistical significance in univariate analysis. In the logistic regression analysis, the amounts of floor dust and concentrations of microbial markers were divided into two categories. The reference category consisted of levels below the 75th percentile. In addition, a multiple logistic regression model was fitted to determine the association between microbial exposure and asthma severity. Also, logistic regression analysis was used to determine risk factors for uncontrolled asthma. The parameters included in these models were sex, age, potential determinants, and health outcomes of asthmatic children. The results are presented as univariate and multivariate odds ratios (ORs) and 95% confidence intervals (95% CI). Statistical significance was determined by an alpha value of $p \leq 0.05$. GraphPad Prism version 9 was used for figures (GraphPad Software, Inc., San Diego, CA, USA).

3. RESULTS AND DISCUSSION

In this study, house dust samples were collected during the winter of 2019 for asthma and control groups as a case-control study to determine endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* spp. Furthermore, the associations of microbial markers with asthma in school-age children were evaluated. A survey questionnaire about home characteristics and lifestyles of families and also health information for asthmatic children were compared with the results.

3.1. Characteristics of Study Population

3.1.1. Results of Survey Questionnaire

A comprehensive survey questionnaire was prepared to collect data, which was filled in by our research team during home visits. The survey questionnaire included questions about housing characteristics, environmental exposure, lifestyle and habits of occupants, building characteristics, and general health information. A total of 239 families completed the survey questionnaire. Table 3.1 presents characteristics for the study population for asthma and control groups. The home characteristics of children in asthma and control groups and the lifestyles of their family members are compared (Table 3.1). The distribution of control and asthma groups shows that the characteristics of the houses and the living habits of family members are generally similar. Distributions for children's age and gender were similar between asthma and control groups ($p > 0.05$). The median age for the asthma group is 7, and for the control group, it is 8.5. 49.79% of the asthma group are male, and 50.21% of them are female. The distribution of gender for the control group is equal for males and females. Most of the characteristics, including residential area, floor covering material, type of rug, cleaning frequency, water damage, and visible mold at home, do not have a statistically significant difference between asthma and control groups. The house floor level and the house's age are the most important determinants in the home characteristics of the study population. The participants of the asthma group lived more frequently on the ground and first floor (73%) than the control group (40%). 33% of the asthma group and 60% of the control group lived on upper grounds (≥2). Additionally, asthma group participants more frequently lived in old dwellings (>20 years) (42.2%) compared to the control group (28.5%). The type of wall paint is another factor that had statistically significant differences between asthma and control groups. Plastic paint was the most preferred wall paint in both asthma (66.1%) and control groups (74.6%). Oil paint is more commonly used in the

asthma group (11.9%) compared to the control group (6.9%). 13.8% of the control group and 9.2% of the asthma group prefer limewash for wall paint in their houses. Wallpaper is more frequent in the asthma group (12.8%) compared to the control group (4.6%). Parents of the control group participants were more likely to report having a separate kitchen at home (97.7%) compared to the asthma group (89%). The number of occupants living in the house is also one of the important factors. 71.5% of the control group declared that there were four or fewer members in the household. This rate increased to 84.4% in families of children with asthma. 28.5% of the control group had more than four people in the household. This rate in the asthma group is 15.6%. There are statistically significant differences between asthma and control groups for the heating system. The Naturel gas-combi boiler was the most preferred heating system in both asthma (64.2%) and control groups (80%). 18.5% of non-asthmatic parents reported using a central heating system. This rate is higher in asthmatic children's homes (33.9%). Using coal or wood as a heating system is low in both groups. 1.8% of asthmatic children and 1.5% of non-asthmatic children lived in houses with a coal or wood heating system. 67.8% of parents reported frying at least once per week in the control group. This rate for the asthma group is 48.6%. Once every two weeks, the frying frequency at home for people with asthma and control groups is 32.1% and 13.8%, respectively. 19.3% of parents in the asthma group declared a once per month frying frequency at home. This rate was 18.4% in the control group. Parents of asthmatic children included in this study reported significantly less smoking at home (3.7%) compared to the control group. However, 42.2% of participants in the asthma group reported smoking on their house balconies. 36.7% of parents in the asthma group reported having new furnishings at home within the last year; this rate for the control group is 22.3%. Pet ownership was more often reported among the control group (27.7%) compared to the asthma group (11.9%). 15.4% of the control group and 14.7% of the asthma group never used bleach for home cleaning. The use of bleach for 33% of the asthma group is once per week. This rate for the control group is 16.9%. The use of bleach at home 2 or 3 days per week for asthma and control groups is 33% and 34.6%, respectively. 19.3% of parents in the asthma group declared using bleach more than four days per week at home. This rate is 33.1% in the control group. 77.1% of the asthma group and 67.7% of the control group prefer using viscoelastic mattresses for children. 9.2% of non-asthmatic parents reported using cotton mattresses. This rate is higher in asthmatic children's homes (12.8%). 10.1% of asthmatic children and 23.1% of non-asthmatic

children use wool mattresses. The majority of the other characteristics, such as window frame material, number of toilets and bathrooms at home, fireplace at home, proximity to the main street, repair and painting within the last year, houseplants, bugs at home, spraying for bugs at home, air conditioning, ventilation at home, changing coverlets and bedsheets, using softener for clothes, and drying clothes, do not show a statistically significant difference between asthma and control groups.

Table 3. 1. Characteristics of study population

Factor		Total n (%)	Asthma group n (%)	Control group n (%)	*P* value
Gender	Male	119 (49.79)	54 (49.5)	65 (50.0)	0.688
	Female	120 (50.21)	55 (50.5)	65 (50.0)	
Age, years	Median (IQR)		8 (7-10)	8.5 (7-10)	0.366
Type of dwelling	<5 floor	144 (60.3)	66 (60.6)	78 (60.0)	0.666
	5-10 floor	49 (18.3)	20 (18.3)	29 (22.3)	
	>10 floor	46 (21.1)	23 (21.1)	23 (17.7)	
House floor	≤1	125 (52.3)	73 (67.0)	52 (40.0)	**<0.001**
	≥2	114 (47.69)	36 (33.0)	78 (60.0)	
Dwelling age	≤20	156 (65.27)	63 (57.8)	93 (71.5)	**0.019**
	>20	83 (34.72)	46 (42.2)	37 (28.5)	
Residential area (m^2)	≤100	75 (31.4)	31 (28.4)	44 (33.8)	0.480
	>100	164 (68.6)	78 (71.6)	86 (66.2)	
Floor covering material	Laminated wood	206 (86.2)	97 (89)	109 (83.8)	0.692
	Solid wood	25 (10.5)	10 (9.2)	15 (11.5)	
	Stone	8 (3.3)	2 (0.81)	6 (4.6)	
Type of rug	Synthetic rug	91.6 (219)	99 (90.8)	120 (92.3)	0.680
	Wool rug	8.4 (20)	10 (9.2)	10 (7.7)	
Type of wall paint	Plastic paint	169 (70.7)	72 (66.1)	97 (74.6)	**0.043**
	Oil paint	22 (9.2)	13 (11.9)	9 (6.9)	
	Lime wash	28 (11.7)	10 (9.2)	18 (13.8)	
	wallpaper	20 (8.4)	14 (12.8)	6 (4.6)	
Window frame material	Plastic-steel	174 (72.8)	79 (72.5)	95 (73.1)	0.917
	Wood	65 (27.2)	30 (27.5)	35 (26.9)	
Separate kitchen	No	15 (6.3)	12 (11)	3 (2.3) (3)	**<0.001**
	Yes	224 (93.7)	97 (89)	127 (97.7)	
Number of toilets at home	1	67 (28.1)	26 (23.9)	41 (31.5)	0.336
	2	133 (55.6)	66 (60.5)	67 (51.6)	
	3	39 (16.3)	17 (15.6)	22 (16.9)	
Number of bathrooms at home	1	164 (68.6)	74 (67.9)	90 (69.2)	0.577
	2	69 (28.9)	31(28.4)	38 (29.2)	
	3	6 (2.5)	4 (3.7)	2 (1.5)	
Fireplace at home	No	237 (99.2)	107 (98.2)	130 (100.0)	0.121
	Yes	2 (0.8)	2 (1.8)	0 (0.0)	
Near main street	No	70 (29.3)	32 (29.4)	38 (29.2)	0.983
	Yes	169 (70.7)	77 (70.6)	92 (70.8)	
Number of occupants	≤4	185 (77.4)	92 (84.4)	93 (71.5)	**0.018**

	>4	54 (22.6)	17 (15.6)	37 (28.5)	
Heating system	Naturel gas-Combi boiler	174 (72.8)	70 (64.2)	104 (80.0)	**0.022**
	Central heating system	61 (25.5)	37 (33.9)	24 (18.5)	
	Coal or wood	4 (1.7)	2 (1.8)	2 (1.5)	
Frying frequency	Once or more / Week	141 (59)	53 (48.6)	88 (67.8)	**<0.001**
	Once/ 2 Weeks	53 (22.2)	35 (32.1)	18 (13.8)	
	Once / month	45 (18.8)	21 (19.3)	24 (18.4)	
Smoking	No	115 (48.1)	59 (54.1)	56 (43.1)	**<0.001**
	Yes	32 (13.4)	4 (3.7)	28 (21.5)	
	House balcony	92 (38.5)	46 (42.2)	46 (35.4)	
Repair and paint	No	173 (72.4)	84 (77.1)	89 (68.5)	0.138
	Yes	66 (27.6)	25 (22.9)	41 (31.5)	
Water damage	No	187 (78.2)	88 (80.7)	99 (76.2)	0.393
	Yes	52 (21.8)	21 (19.3)	31 (23.8)	
Damp smells	No	179 (74.9)	87 (79.8)	92 (70.8)	0.108
	Yes	60 (25.1)	22 (20.02)	38 (29.2)	
Visible mold	No	182 (76.2)	84 (77.1)	98 (75.4)	0.762
	Yes	57 (23.8)	25 (22.9)	32 (24.6)	
New furniture	No	170 (71.1)	69 (63.3)	101 (77.7)	**0.014**
	Yes	69 (28.9)	40 (36.7)	29 (22.3)	
Houseplants	No	204 (85.3)	93 (85.3)	111 (85.5)	0.984
	Yes	35 (14.7)	16 (14.7)	19 (14.6)	
Pet ownership	No	190 (79.5)	96 (88.1)	94 (72.3)	**0.003**
	Yes	49 (20.5)	13 (11.9)	36 (27.7)	
Bugs at home	No	203 (84.9)	91 (83.5)	112 (86.2)	0.566
	Yes	36 (15.1)	18 (16.5)	18 (13.8)	
Spraying home for bugs	No	210 (87.9)	93 (85.3)	117 (90.0)	0.270
	Yes	29 (12.1)	16 (14.7)	13 (10.1)	
Spraying building for bugs	No	136 (56.9)	62 (56.9)	74 (56.9)	0.995
	Yes	103 (43.1)	47 (43.1)	56 (43.1)	
Air conditioning	No	227 (95)	125 (93.6)	125 (96.2)	0.364
	Yes	12 (5)	7 (6.4)	5 (3.8)	
Ventilation at home	No	39 (16.3)	22 (20.2)	22 (13.1)	0.139
	Yes	22 (83.7)	87 (79.8)	113 (86.9)	
Cleaning home	Once a week or less	77 (32.2)	42 (38.6)	35 (26.9)	0.110
	2-4 days a week	78 (32.6)	36 (33)	42 (32.3)	
	More than 4 days a week	84 (35.1)	31 (28.4)	31 (40.8)	
	Never	36 (15)	16 (14.7)	20 (15.4)	**0.023**

Frequency of using bleach	One day per week	58 (24.3)	36 (33)	22 (16.9)	
	2 or 3 days a week	81 (33.9)	36 (33)	45 (34.6)	
	More than 4 days a week	45 (26.8)	45 (19.3)	43 (33.1)	
Changing coverlets and bedsheets	Once / Week	96 (40.2)	49 (45)	47 (36.2)	0.310
	Once / 2 Weeks	108 (45.2)	47 (43.1)	61 (46.9)	
	Once / month	35 (14.6)	13 (11.9)	22 (16.9)	
Using softener for clothes	No	114 (47.7)	57 (52.3)	57 (43.8)	0.193
	Yes	125 (52.3)	52 (47.7)	73 (56.2)	
Where to dry clothes	Indoor	152 (63.6)	72 (66)	80 (61.6)	0.081
	Outdoor	63 (26.4)	22 (20.2)	41 (31.5)	
	Dryer machine	24 (10)	15 (13.8)	9 (6.9)	
Materials of child's mattress	Viscoelastic	172 (72)	84 (77.1)	88 (67.7)	**0.026**
	Cotton	26 (10.9)	14 (12.8)	12 (9.2)	
	Wool	41 (17.2)	11 (10.1)	30 (23.1)	
New furnishing at child's room	No	188 (78.8)	83 (76.1)	105 (80.8)	0.385
	Yes	51 (21.3)	26 (23.9)	25 (19.2)	
Repair and paint at child's room	No	187 (78.2)	91 (78.2)	96 (73.8)	0.720
	Yes	52 (21.8)	18 (21.8)	34 (26.2)	

3.1.2. Characteristics of The Asthma Group

The 109 children with doctor-diagnosed asthma were classified for health outcomes in Table 3.2. The frequency of gender in the asthma group was approximately equal (female: 50.5%, male: 49.5%). Asthma study children were, on average, eight years of age. Of the children with asthma diagnosed, 44.7% had asthma exacerbation within last month, 84.5% had asthma exacerbation within last year, and 35.9% reported emergency department visits within last year. 37.9% of asthma children had atopy disease, and 19.4% of children participating in the asthma group had doctor-diagnosed atopic dermatitis during the follow-up. 44.7% of children including in this study, had a history of atopic disease in their families. Only 11.7% of children in the asthma group reported having wheezing. In addition, 44.7% of the asthmatic group reported having night cough. The mean FEV_1 (Forced Expiratory Volume) value for the asthma group was 91.23%. According to Asthma Control Test (ACT), asthma symptoms of more than two-thirds (74.8%) of the children may be controlled as well as it could be (ACT score >19). The 109 children with asthma were classified into two asthma severity groups based on the GINA 2006 guidelines: mild asthma versus moderate/ severe asthma. The dose and combination of the

drugs and spirometry test were used as parameters in the GINA guidelines for the determination of asthma severity. Within the asthma group, 59.8% of them have mild asthma, and 40.2% of them have moderate/severe asthma.

Table 3. 2. Characteristics of the asthma group

	n=109
Female, n (%)	**55 (50.5)**
Age, mean (± SD)	**8.32 (±1.74)**
Age of symptom, mean (± SD)	**2.98 (±1.87)**
Age of asthma diagnose, mean (± SD)	**4.24 (±1.89)**
Follow up duration, mean (± SD)	**4.82 (±2.18)**
Family history of atopic disease, n (%)	**46 (44.7)**
Asthma exacerbation within last month, n (%)	**46 (44.7)**
Asthma exacerbation within last year, n (%)	**87 (84.5)**
Emergency department visit within last year, n (%)	**37 (35.9)**
Allergic Rhinitis, n (%)	**23 (22.3)**
Atopy, n (%)	**39 (37.9)**
Atopic dermatitis, n (%)	**20 (19.4)**
FEV_1, mean (± SD)	**91.23(±13.61)**
Wheezing, n (%)	**12 (11.7)**
Night cough, n (%)	**46 (44.7)**
Asthma severity	
Mild asthma, n (%)	**64 (59.8)**
Moderate/ Severe asthma, n (%)	**43 (40.2)**
ACT Score, (Median IQR)	**23 (19-25)**
ACT	
Controlled, n (%)	**83 (76.14)**
Uncontrolled, n (%)	**26 (23.85)**

ACT: Asthma Control Test, FEV_1: Forced Expiatory Volume

3.2.GENERAL EVALUATION OF BIOLOGICAL MARKERS

Microbial markers were determined in 239 dust samples collected from the homes of school-aged children. Table 3.3 shows the levels of floor dust, endotoxin, β-(1→3)-D-glucan *Aspergillus*, and *Penicillium* spp. expressed in load (per m^2) and concentration (per gram).The geometric mean was 508.10 mg per m^2 (GSD = 1.76) for dust; 11.47×10^3 EU/g (GSD = 3.54) for endotoxin; 42.18 µg/g (GSD = 2.34) for β-(1→3)-D-glucan, 2259 CFU/g (GSD = 3.20) for *Aspergillus*, and 3603 CFU/g (GSD = 2.89) for *Penicillium* spp.

Table 3. 3. Floor dust and concentration of biological markers

n=239	LOD	GM	GSD	25th	50th	75th
Floor dust (mg/m^2)	-	508.10	1.76	359	561	764
Endotoxin (10^3 Eu/g)	-	11.47	3.54	5.22	11.40	25.30
β-(1→3)-D-glucan (μg/g)	27	42.18	2.34	22.44	42.65	75.83
***Aspergillus* (CFU/g)**	24	2259	3.20	880	1960	5010
***Penicillium* spp. (CFU/g)**	40	3603	2.89	1300	3075	7210

*LOD: Limit of Detection

All of the house dust samples had measurable amounts of endotoxin. Endotoxin ranges for all dust samples were 0.28 $\times 10^3$ EU/g – 33.7$\times$ 10^4 EU/g. Overall, β-(1→3)-D-glucan levels were under the limit of detection for 27 house dust samples. BDG levels range from 10.33 μg/g to 250 μg/g for all dust samples. Of the 239 house dust samples, 24 and 40 samples were not at detectable levels for *Aspergillus* and *Penicillium* spp., respectively. *Aspergillus* and *Penicillium* spp. ranges were 1000 CFU/g – 44500 CFU/g and 1000 CFU/g – 58000 CFU/g, respectively.

Biological markers have been measured in indoor environments in a wide range all over the world. Reasons for this wide range are generally considered to be geographical differences, various climates, diverse sampling methods, home characteristics, and lifestyles of families in different countries. Figure 3.1 presents the results of some studies for endotoxin levels. Eight hundred twelve children were participants in a study conducted in Austria, Germany, Switzerland. Endotoxin ranges were 44.9×10^3- 81.8×10^3 EU/g in this study [86]. That is a higher endotoxin concentration compared with other European countries. In one study conducted in Denmark, 317 dust samples were collected to determine endotoxin concentration. The geometric mean was found 31.1×10^3 EU/g for endotoxin [40]. The median endotoxin concentration reported by Heinrich et al. (2003) was 27.8×10^3 EU/g in 745 homes in Germany [177]. In another study performed in Sweden, dust samples were collected from 390 houses. The endotoxin median was determined as 6.22×10^3 EU/g in this study [167]. In a study conducted by 10 European countries, samples were taken from 974 houses. The endotoxin level in this study ranges between 0.82×10^3 - 4.81×10^3 EU/g [178]. In a study performed by Germany, Finland, Spain, and the Netherlands, samples were collected from 1572 homes. The endotoxin

concentration ranges were 3.20×10^3 - 23×10^3 EU/g [179]. Another study performed at 150 homes in France reported a 3.75×10^3 - 5×10^3 EU/g range for endotoxin [176]. In this study, endotoxin ranges for all dust samples were 0.28×10^3 EU/g – 33.7×10^3 EU/g.

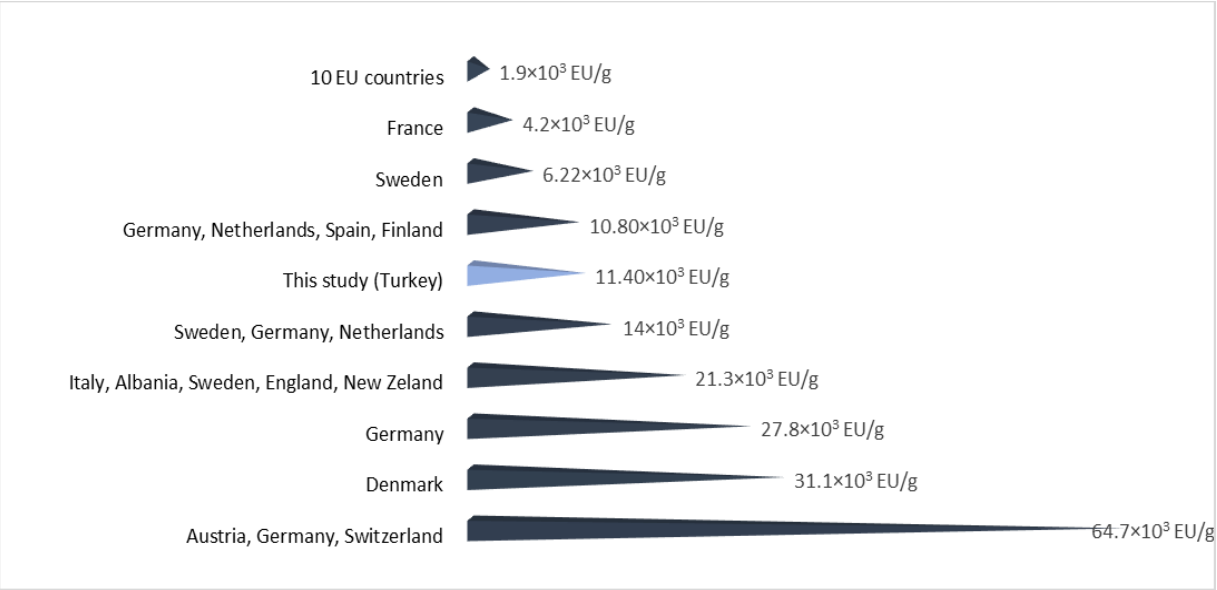


Figure 3. 1. Comparison of Endotoxin levels in different studies

Figure 3.2 presents the results of some studies for β-(1→3)-D-glucan levels. In one study conducted in Denmark, 317 dust samples were collected to determine β-(1→3)-D-glucan concentration. The geometric mean was found 0.71×10^3 µg/g for β-(1→3)-D-glucan [40]. In a study conducted by Germany, Finland, Spain, and the Netherlands, samples were taken from 1572 houses. In this study, the β-(1→3)-D-glucan level varies between 0.9×10^3 -2.4×10^3 µg/g [179]. A study including 1065 houses was conducted in Sweden, Germany, Netherlands. β-(1→3)-D-glucan ranges were 2×10^3- 2.8×10^3 µg/g in this study. That is a higher β-(1→3)-D-glucan concentration compared with other European countries [172]. The β-(1→3)-D-glucan concentration reported by Gehring et al. (2001) was 1.71×10^3 µg/g in 395 homes in Germany [173]. Another study in Germany reported a 0.76×10^3 µg/g concentration for β-(1→3)-D-glucan [174]. In a study conducted in Canada, dust samples were collected from 116 homes to determine BDG levels. The geometric mean was 9.7 µg/g for β-(1→3)-D-glucan concentration in this study [163]. In this study conducted in Turkey, dust samples were collected from 239 houses. The geometric mean was 42.18 µg/g for β-(1→3)-D-glucan concentration. BDG concentration in this study seems to be closer to the studies conducted in Canada.

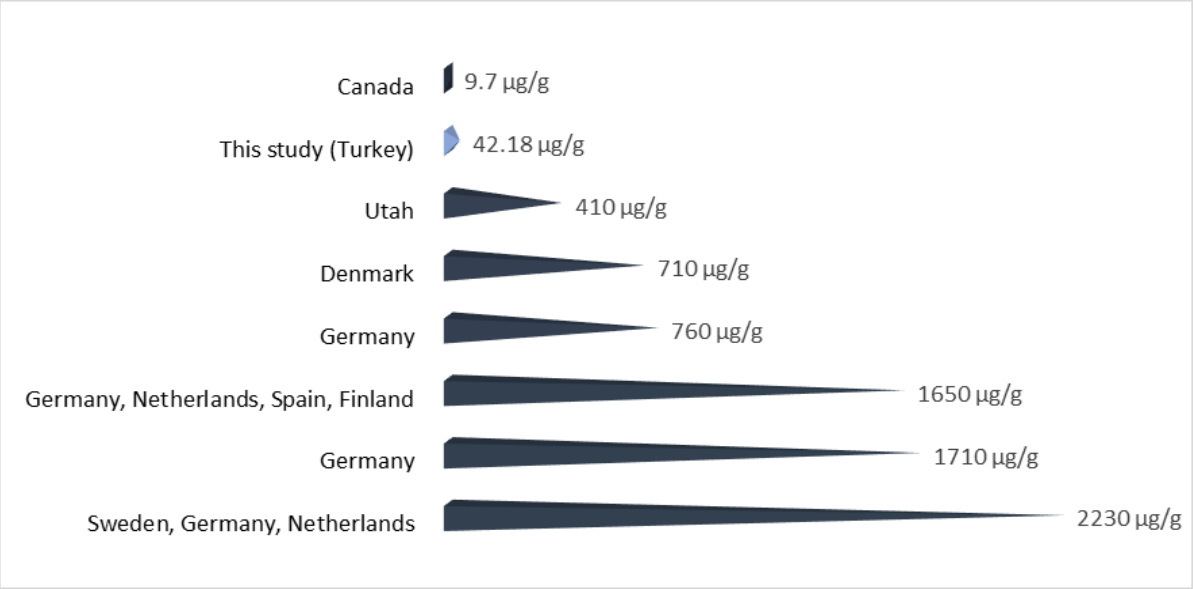


Figure 3. 2. Comparison of β-(1→3)-D-glucan levels in different studies.

Figure 3.3 presents the results of some studies for *Aspergillus* levels. In one study conducted in Arabia, 36 dust samples were collected to determine *Aspergillus* concentration. The median *Aspergillus* was 5400 CFU/g [180]. In one study performed in Poland, 16 dust samples were collected from homes to determine *Aspergillus* concentration. The median *Aspergillus* count was 5200 CFU/g [181]. In a study conducted in Germany, dust samples were collected from the homes of 115 asthmatic and 157 non-asthmatic children. In this study, the *Aspergillus* level was determined as 5000 CFU/g in both groups [168]. In another study conducted in the United States, California, dust samples were collected from 26 dwellings. The median was 1450 CFU/g for *Aspergillus* concentration [182]. The *Aspergillus* concentration reported by Dallongeville et al. (2015) was 4300 CFU/g in 150 homes in France [175]. In this study, dust samples were collected dust samples in 239 homes. The geometric mean was 2259 CFU/g for *Aspergillus* concentration.

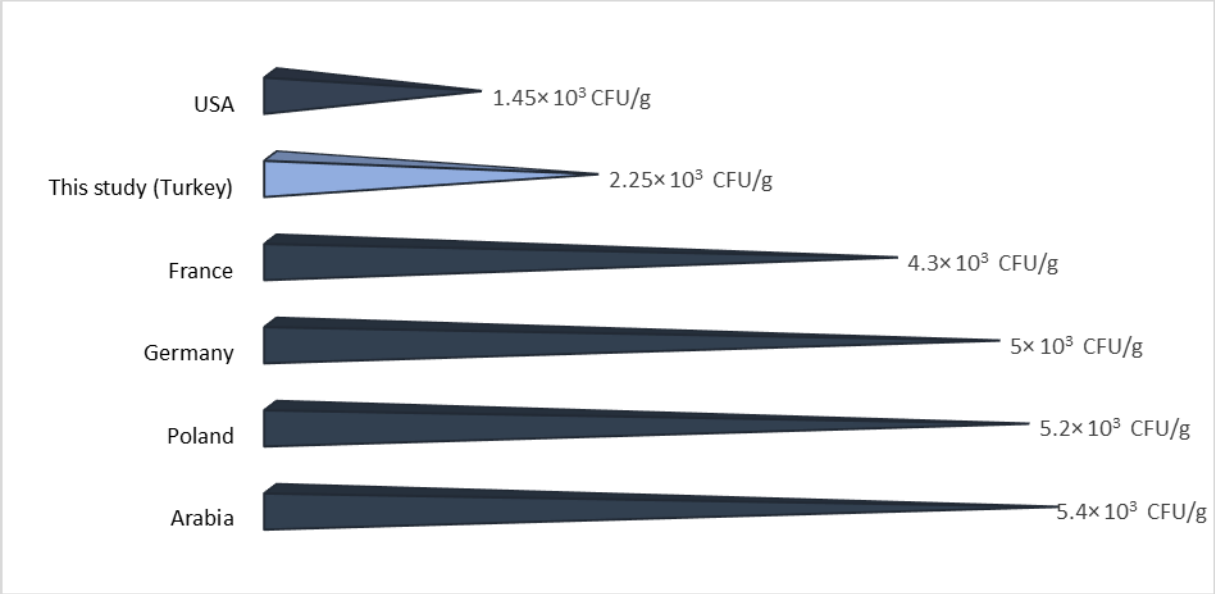


Figure 3. 3. Comparison of *Aspergillus* levels in different studies.

Figure 3.4 presents the results of some studies for *Penicillium* spp. levels. In one study conducted in Poland, 16dust samples were collected to determine *Penicillium* spp. concentration. The median *Penicillium* spp. was 21200 CFU/g [181]. In a study conducted in Germany, dust samples were collected from the homes of 115 asthmatic and 157 non-asthmatic children. In this study, the *Penicillium* spp. level was determined as 15000 CFU/g in both groups [168]. In another study conducted in the United States, California, dust samples were collected from 26 dwellings. The median was 9000 CFU/g for *Penicillium* spp. concentration [182]. The *Penicillium* spp. concentration reported by Dallongeville et al. (2015) was 20000 CFU/g in 150 homes in France [175]. That is a higher *Penicillium* spp. concentration compared with other studies. In this study, we collected dust samples in 239 homes. The geometric mean was 3603 CFU/g for *Penicillium* spp. concentration.

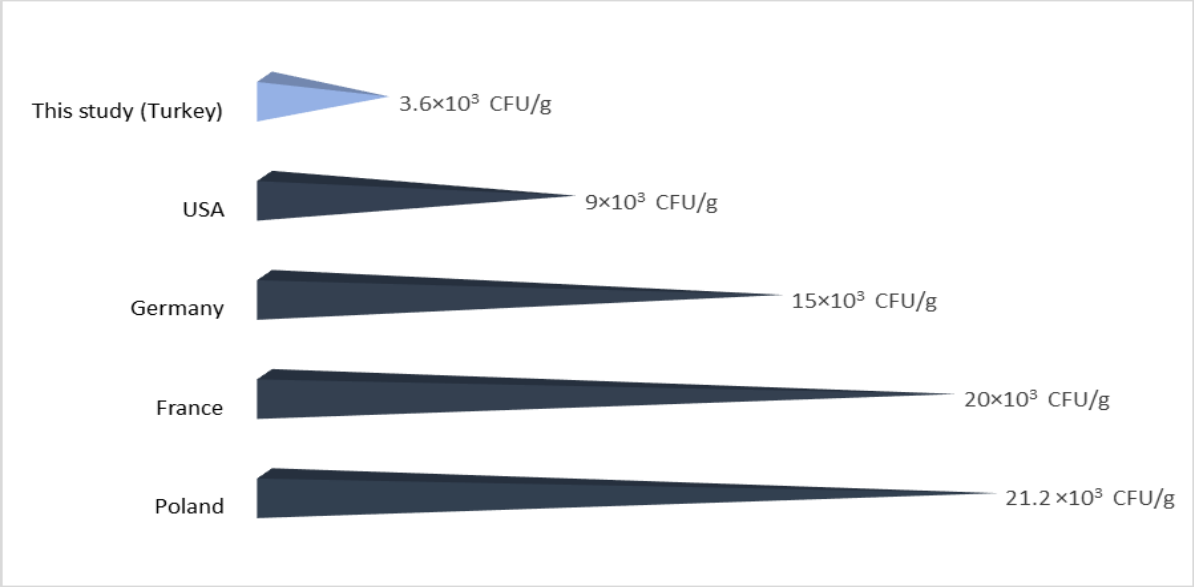


Figure 3. 4. Comparison of *Penicillium* spp. levels in different studies.

3.2.1. Distribution of Floor Dust and Biological Markers in the Control Group

Microbial markers were determined in 130 dust samples collected from the homes of non-asthmatic children in the control group. Table 3.4 shows the levels of floor dust, endotoxin, β-(1→3)-D-glucan *Aspergillus*, and *Penicillium* spp. expressed in load (per m^2) and concentration (per gram) for the control group. The geometric mean was 570.66 mg per m^2 (GSD = 1.69) for dust; 16.11 × 10^3 EU/g (GSD = 3.01) for endotoxin; 43.08 µg/g (GSD = 2.18) for β-(1→3)-D-glucan, 2470 CFU/g (GSD = 2.63) for *Aspergillus*, and 3493 CFU/g (GSD = 2.45) for *Penicillium* spp.

Table 3. 4. Floor dust and concentration of biological markers in the control group

n=130	LOD	GM	GSD	25th	50th	75th
Floor dust (mg/m^2)	-	570.66	1.69	419.50	644.50	841.25
Endotoxin (10^3 EU/g)	-	16.11	3.01	7.54	15.10	33.32
β-(1→3)-D-glucan (µg/g)	9	43.08	2.18	22.57	42.67	74.22
***Aspergillus* (CFU/g)**	12	2470	2.63	1100	2600	5400
***Penicillium* spp. (CFU/g)**	20	3493	2.45	1200	3400	7600

All of the house dust samples in the control group had measurable amounts of endotoxin. The median endotoxin for the control group was 15.10×10^3 EU/g (interquartile range (IQR), 7.54×10^3 -33.32×10^3). In general, β-(1→3)-D-glucan levels were under the limit of detection for 9 houses in the control group. The BDG median for the control group was 42.67 µg/g (IQR: 22.57-74.22). Of the 130 house dust samples in the control group, 12 and 20 samples were not at detectable levels for *Aspergillus* and *Penicillium spp.*, respectively. The median *Aspergillus* was 2600 CFU/g (IQR: 1100-5400) in the control group. The median *Penicillium* spp. was 3400 CFU/g (IQR: 1200-7600) in the control group.

Figures 3.5, 3.6, 3.7, and 3.8 show the distribution of biological markers in the control group. Distributions are shown as endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* Spp., respectively.

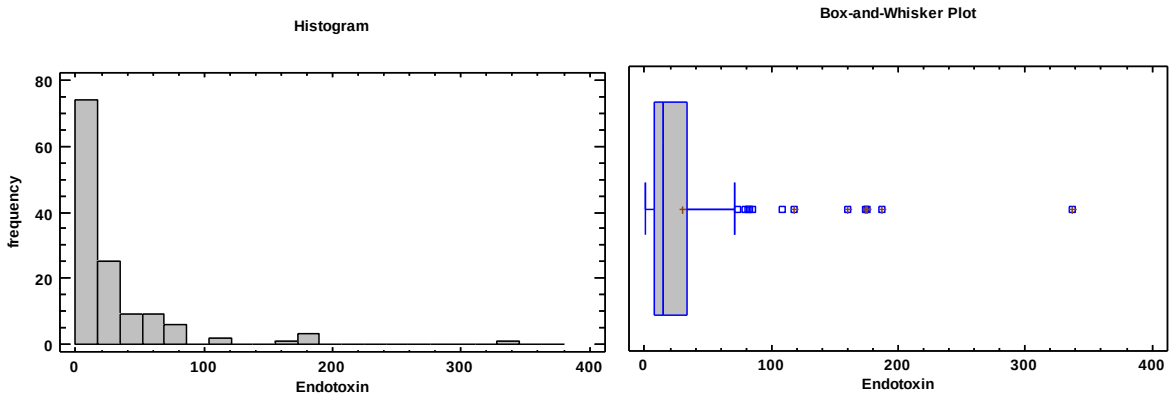


Figure 3. 5. Distribution of endotoxin for the control group

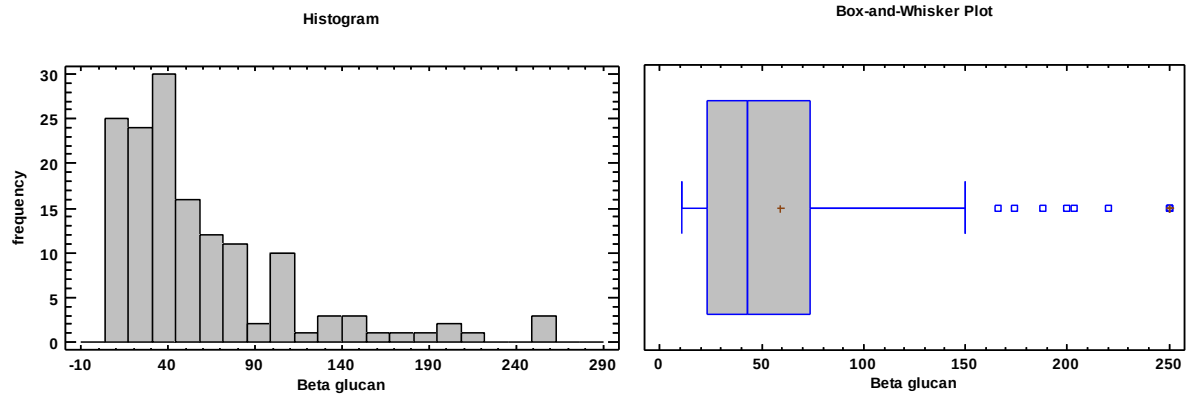


Figure 3. 6. Distribution of β-(1→3)-D-glucan for the control group

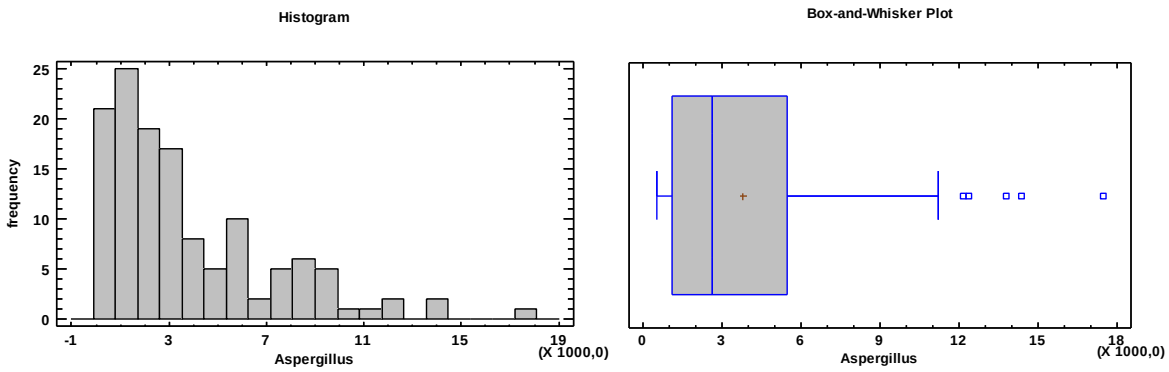


Figure 3. 7. Distribution of *Aspergillus* for the control group

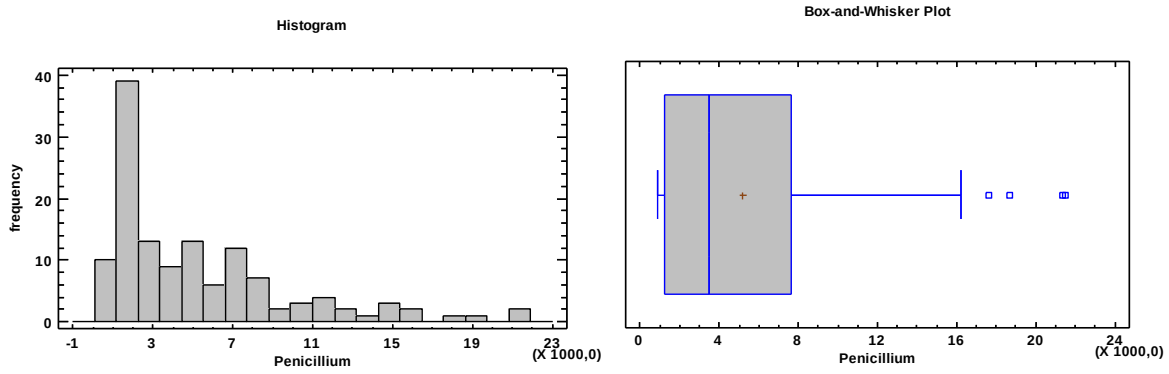


Figure 3. 8. Distribution of *Penicillium* Spp. for the control group

Table 3.5 shows characteristics of homes that have high endotoxin levels (>75 percentile) in the control group. The highest endotoxin concentration was measured in the control group. The house with the highest endotoxin level is slum style and uses wood in the heating system. Douwes et al. reported the association of using coal, wood, or other biomass as a heating system with high endotoxin levels [174]. The floors are covered with mosaic stone, and two birds are kept as pets in the house. Keeping pets like birds at home is considered to be one of the reasons for high endotoxin levels. Type of floor covering is another parameter associated with endotoxin concentration. In different studies, endotoxin levels were associated with a type of flooring such as carpet, rug, stone, wood [40][172]. In the control group, the typical characteristics of homes with high endotoxin levels are moisture and dampness at home and drying of the clothes at home. In addition, there are chicken coops near three houses with high endotoxin levels in the control group. Previous studies represent that farming and contact with animals are associated with high endotoxin levels [86][158][183].

Table 3. 5. Characteristics of homes with high endotoxin levels in the control group

Endotoxin level	Home characteristics
337×10^3 EU/g	Slum style house with a heating system based on coal and wood, Stone mosaic floor covering, domestic birds, drying clothes at home
187 ×10^{3}EU/g	Near to chicken coop, water damage at home, smoking at home, drying clothes at home, house floor is ≤ 1
175 ×10^{3}EU/g	Repair and house painting, humidity, and dampness at home, mouse at home
174 ×10^{3}EU/g	Dwelling age is 40, smoking at the balcony, near to chicken coop, drying clothes at home
160×10^{3}EU/g	Humidity and dampness and mold spores at home, domestic bird, drying clothes at home, house floor is ≤ 1
108 ×10^{3}EU/g	Near to chicken coop, a home without a separate kitchen, humidity at home, drying clothes at home.

The characteristics of the houses with high β-(1→3)-D-glucan levels (>75 percentile) for the control group are presented in Table 3.6. Water damage within last year and smoking at home are the characteristics of the two houses with high β-(1→3)-D-glucan levels in the control group. In the control group, the typical characteristics of homes with high β-(1→3)-D-glucan levels are water damage, visible mold, humidity, and dampness at home. Cases et al. reported the association of dampness with high β-(1→3)-D-glucan levels in a study conducted in 1572 homes [179]. A study performed in New Zealand reported higher β-(1→3)-D-glucan levels in homes with water damage [184]. In addition, in the control group, pests and insects were seen in some of the houses with high β-(1→3)-D-glucan levels.

Table 3. 6. Characteristics of homes with high β-(1→3)-D-glucan levels in the control group

β-(1→3)-D-glucan level	Home characteristics
250 µg/g	Water damage at home, humidity, and dampness, painting home within last year, smoking at home
220 µg/g	Water damage at home, repair and house painting, smoking at home, new furniture, drying clothes at home
203.74 µg/g	Visible mold at home, humidity, and dampness
187.76 µg/g	Visible mold at home, humidity, and dampness, smoking at home, drying clothes at home
173.79 µg/g	Visible mold and dampness at living room, smoking at house balcony, painting home within last year
135.18 µg/g	Humidity and dampness at home, repair, and house painting
101.53 µg/g	Keeping bird at home, cockroach at home

92.90 µg/g	Water damage at home, drying clothes at home

The characteristics of the houses with high *Aspergillus* levels (>75 percentile) for the control group are presented in Table 3.7. Humidity and dampness at the toilet and kitchen (house floor ≤ 1) are the characteristics of the house with the highest *Aspergillus* level in the control group. Generally, in the control group, the typical characteristics of homes with high *Aspergillus* levels are visible mold, humidity, and dampness at home. Humidity, dampness, and mold spores are known to be the reasons for the high level of *Aspergillus* [182]. Water damage at home is considered to be one of the reasons for high *Aspergillus* levels [185].

Table 3. 7. Characteristics of homes with high *Aspergillus* levels in the control group

***Aspergillus* level**	**Home characteristics**
17500 CFU/g	Humidity and dampness at toilet and kitchen, house floor is ≤ 1
14300 CFU/g	Visible mold at bedroom, humidity, and dampness
13800 CFU/g	Keeping pet (dog and bird), visible mold, humidity, and dampness, house floor is ≤ 1
12200 CFU/g	Heating system based on coal and wood, water damage at the kitchen, humidity and visible mold at bedroom, keeping bird at home
11200 CFU/g	Water damage at home, repair, and house painting, house floor is ≤ 1, smoking at home
9800 CFU/g	Dwelling age is 41, humidity and dampness at home, drying clothes at home
8200 CFU/g	Water damage, humidity, and dampness, smoking at home
7700 CFU/g	Humidity and dampness at the living room and bedroom, keeping bird at home, drying clothes at home

The characteristics of the houses with high *Penicillium* spp. levels (>75 percentile) for the control group are presented in Table 3.8 The house with the highest *Penicillium* spp. in the control group has a heating system based on coal and wood, water damage in the kitchen, humidity, and visible mold in the bedroom. Generally, in the control group, the common characteristics of homes with high *Penicillium* spp. levels are visible mold, humidity, and dampness at home. Humidity, dampness, and mold spores are known to be the reasons for the high level of *Penicillium* spp. [182]. Water damage at home is considered to be one of the reasons for high *Penicillium* spp. levels [185]. In addition, some pet ownership houses have high *Penicillium* spp. levels.

Table 3. 8. Characteristics of homes with high *Penicillium* spp. levels in the control group

***Penicillium* spp. level**	**Home characteristics**
21500 CFU/g	Heating system based on coal and wood, water damage in the kitchen, humidity and visible mold at bedroom, keeping bird at home
21300 CFU/g	Humidity and dampness at toilet and kitchen, house floor is ≤ 1
18700 CFU/g	Visible mold at bedroom, humidity, and dampness
17600 CFU/g	Humidity and visible mold at home keeping dog and bird at home, drying clothes at home
15400 CFU/g	Water damage at home, repair, and house painting, house floor is ≤ 1, smoking at home
14500 CFU/g	Water damage, humidity, and dampness at home, drying clothes at home
12400 CFU/g	Visible mold in the living room, humidity, and dampness, smoking at home
8700 CFU/g	Humidity and dampness at the living room and bedroom, keeping bird at home, drying clothes at home

3.2.2. Distribution of Floor Dust and Biological Markers in the Asthma Group

Microbial markers were determined in 109 dust samples collected from the homes of asthmatic children as a case group. Table 3.9 shows the levels of floor dust, endotoxin, β-(1→3)-D-glucan *Aspergillus,* and *Penicillium* spp. expressed in load (per m^2) and concentration (per gram) for the asthma group. The geometric mean was 442.39 mg per m^2 (GSD = 1.73) for dust; 7.63 × 10^3 EU/g (GSD = 3.71) for endotoxin; 41.13 µg/g (GSD = 2.51) for β-(1→3)-D-glucan, 2032 CFU/g (GSD = 3.80) for *Aspergillus*, and 3738 CFU/g (GSD = 3.38) for *Penicillium* spp.

Table 3. 9. Floor dust and concentration of biological markers in the asthma group

n=109	**LOD**	**GM**	**GSD**	**25th**	**50th**	**75th**
Floor dust (mg/m^2)	-	442.39	1.73	274	464	686
Endotoxin (10^3 EU/g)	-	7.63	3.71	3.81	8.23	15.05
β-(1→3)-D-glucan (µg/g)	18	41.13	2.51	20.10	37.39	87.23
***Aspergillus* (CFU/g)**	8	2032	3.80	740	1350	3525
***Penicillium* spp. (CFU/g)**	15	3738	3.38	1500	2780	6200

All of the house dust samples in the asthma group had measurable amounts of endotoxin. The median endotoxin for the asthma group was 8.23×10^3 EU/g (interquartile range (IQR), 3.81×10^3

-15.05×10^3). In general, β-(1→3)-D-glucan levels were under the detection limit for 18 houses in the asthma group. The BDG median for the asthma group was 37.39 µg/g (IQR: 20.10-87.23). Of the 110 house dust samples in the asthma group, 8 and 15 samples were not at detectable levels for *Aspergillus* and *Penicillium* spp., respectively. The median *Aspergillus* was 1350 CFU/g (IQR: 740-3525) in the asthma group. In the asthma group, the median *Penicillium* spp. was 2780 CFU/g (IQR: 1500-6200).

Figures 3.9, 3.10, 3.11, and 3.12 show the distribution of biological markers in the asthma group. Distributions are shown as endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* Spp., respectively.

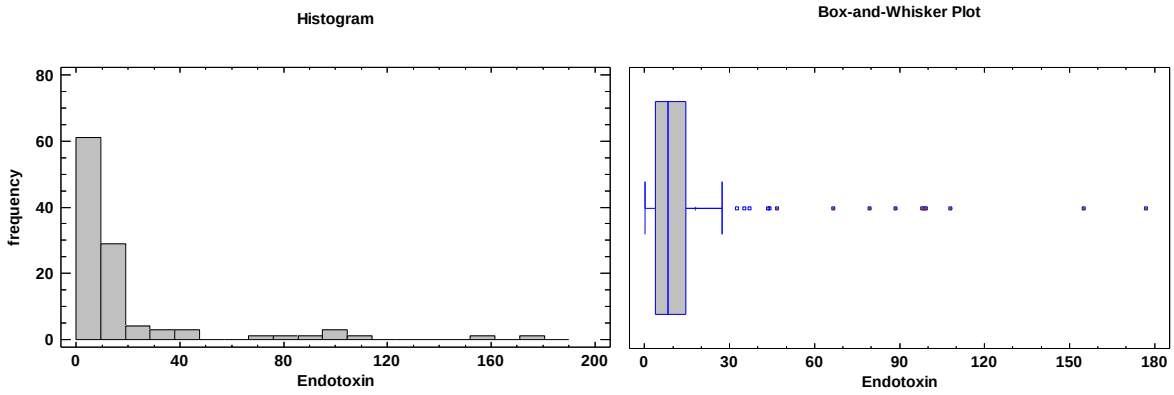


Figure 3. 9. Distribution of endotoxin for the asthma group

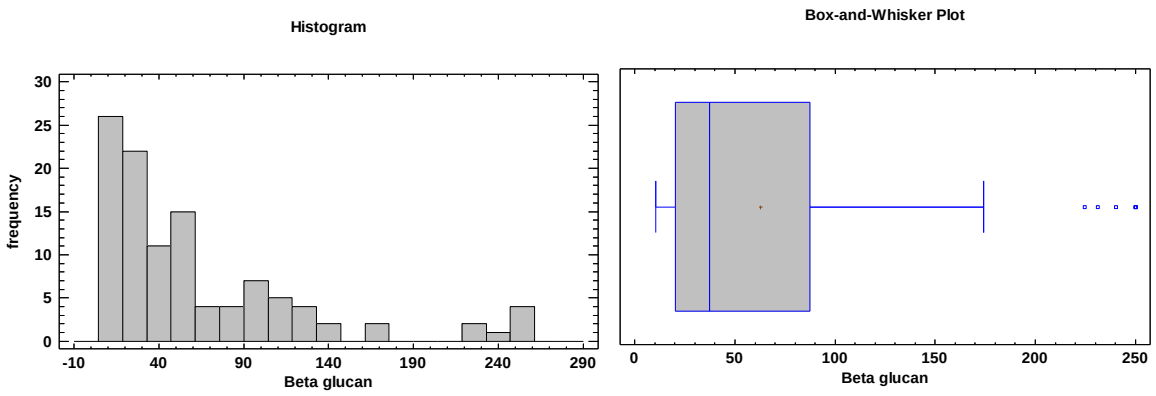


Figure 3. 10. Distribution of β-(1→3)-D-glucan for the asthma group

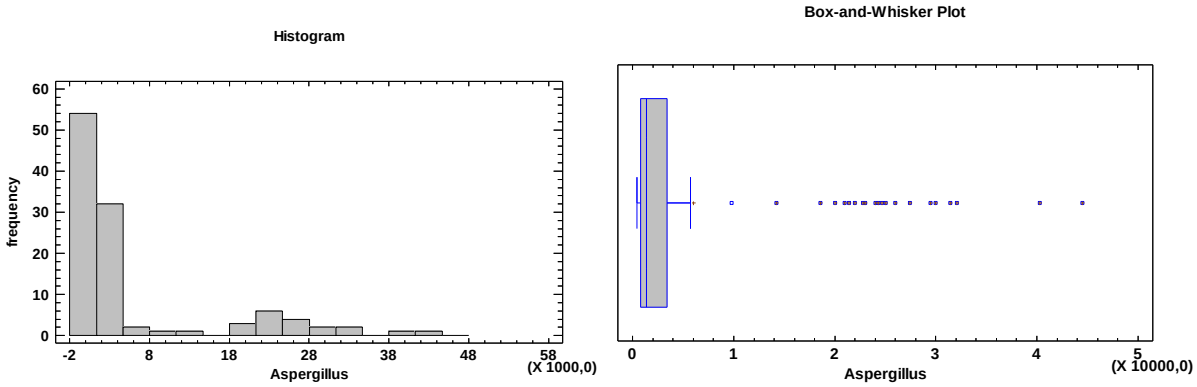


Figure 3. 11. Distribution of *Aspergillus* for the asthma group

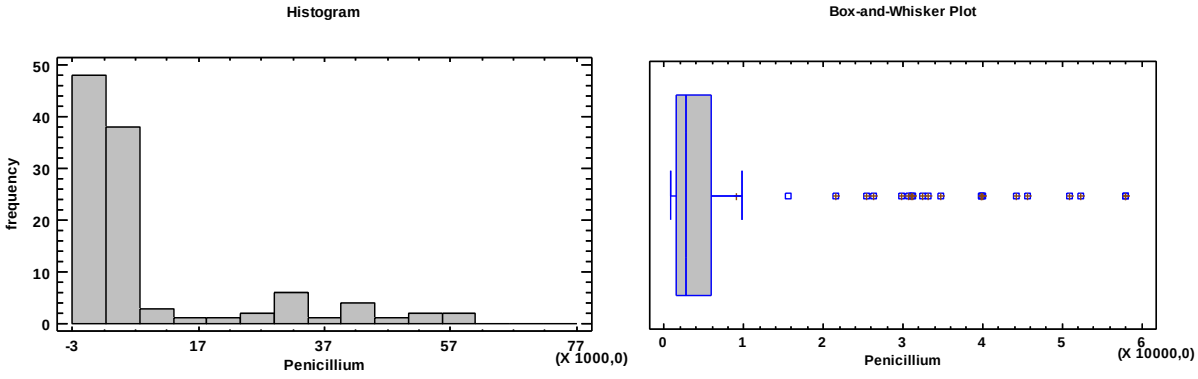


Figure 3. 12. Distribution of *Penicillium* Spp. for the asthma group

Table 3.10 shows characteristics of homes that have high endotoxin levels (>75 percentile) in the asthma group. The house with the highest endotoxin level in the asthma group has water damage in the kitchen, and the dwelling age is 40. Two houses with high endotoxin levels in the

asthma group have a heating system based on coal and wood. Douwes et al. reported the association of using coal, wood, or other biomass as a heating system with high endotoxin levels [174]. In the asthma group, the typical characteristics of homes with high endotoxin levels are older dwellings, dampness, and homes without a separate kitchen. In addition, there are chicken coops near houses with high endotoxin levels in the asthma group. Previous studies represent that farming and contact with animals are associated with high endotoxin levels [86][158][183].

Table 3. 10. Characteristics of homes with high endotoxin levels in the asthma group

Endotoxin level	**Home characteristics**
177×10^3 EU/g	Dwelling age is 40, water damage in the kitchen, drying clothes at home
155×10^3EU/g	A home without a separate kitchen, using an air conditioner at home, house painting within last year, house floor is ≤ 1
108×10^3EU/g	A home without a separate kitchen, repair and house painting, humidity and dampness at home
99.41×10^3EU/g	Slum style house with a heating system based on coal and wood, near to chicken coop
98.7×10^3EU/g	Near to chicken coop, humidity and dampness at bedroom, a heating system based on coal and wood
98.1×10^3EU/g	Dwelling age is 60, humidity, dampness, visible mold at home, mouse at dwelling.

The characteristics of the houses with high β-(1→3)-D-glucan levels (>75 percentile) for the asthma group are presented in Table 3.11. Water damage within last year and cockroaches at home are the characteristics of the three houses with high β-(1→3)-D-glucan levels in the asthma group. In the asthma group, the typical characteristics of homes with high β-(1→3)-D-glucan levels are water damage, visible mold, humidity, dampness at home, and older dwellings. Cases et al. reported the association of dampness with high β-(1→3)-D-glucan levels in a study conducted in 1572 homes [179]. A study performed in New Zealand reported higher β-(1→3)-D-glucan levels in homes with water damage [184]. In addition, pests and insects were seen in some houses with high β-(1→3)-D-glucan levels in the asthma group.

Table 3. 11. Characteristics of homes with high β-(1→3)-D-glucan levels in the asthma group

β-(1→3)-D-glucan level	Home characteristics
250 µg/g	Dwelling age is 65, water damage at the toilet, visible mold and dampness, cockroach at home, aquarium at home
250 µg/g	Water damage at home, smoking at home, drying clothes at home
240.20 µg/g	cockroach at home, drying clothes at home
231.14 µg/g	Dwelling age is 60, visible mold at home, humidity and dampness, painting home within last year, mouse at dwelling
224 µg/g	Dwelling age is 40, smoking at house balcony, drying clothes at home
174.23 µg/g	Humidity and dampness at home, visible mold especially in the bathroom, cockroach at home
141.69 µg/g	Dwelling age is 35, water damage, keeping bird at home, visible mold at home, humidity and dampness, the aquarium at home
129.7 µg/g	Water damage at home, humidity, and dampness
110.44 µg/g	Near to chicken coop, humidity and dampness at bedroom, a heating system based on coal and wood.

The characteristics of the houses with high *Aspergillus* levels (>75 percentile) for the asthma group are presented in Table 3.12. Visible mold, humidity, and dampness at home (dwelling age is 60) are the characteristics of the house with the highest *Aspergillus* level in the asthma group. Generally, in the asthma group, the typical characteristics of homes with high *Aspergillus* levels are visible mold, humidity, and dampness at home. Humidity, dampness, and mold spores are known to be the reasons for the high level of *Aspergillus* [182]. Water damage at home is considered to be one of the reasons for high *Aspergillus* levels [185]. Furthermore, pests and insects were seen in some houses with high *Aspergillus* levels in the asthma group.

Table 3. 12. Characteristics of homes with high *Aspergillus* levels in the asthma group

***Aspergillus* level**	**Home characteristics**
44500 CFU/g	Dwelling age is 60, visible mold at home, humidity and dampness, painting home within last year, mouse at dwelling
40300 CFU/g	Dwelling age is 35, water damage, keeping bird at home, visible mold at home, humidity and dampness, the aquarium at home
32000 CFU/g	Water damage at home, house floor is ≤ 1, smoking at house balcony, drying clothes at home
31400 CFU/g	Dwelling age is 65, water damage at the toilet, visible mold and dampness, cockroach at home, aquarium at home
30000 CFU/g	Near to chicken coop, humidity and dampness at bedroom, a heating system based on coal and wood
29500 CFU/g	Water damage at home, humidity, and dampness

25000 CFU/g	Dwelling age is 30, humidity and dampness at home, cockroach at home.

The characteristics of the houses with high *Penicillium* spp. levels (>75 percentile) for the asthma group are presented in Table 3.13. The house with the highest *Penicillium* spp. in the asthma group has visible mold at home, humidity, and dampness, and dwelling age is 60. Generally, in the asthma group, the common characteristics of homes with high *Penicillium* spp. levels are visible mold, humidity, and dampness at home. Humidity, dampness, and mold spores are known to be the reasons for the high level of *Penicillium* spp. [182]. Water damage at home is considered to be one of the reasons for high *Penicillium* spp. levels [185]. In addition, pests and insects were seen in some houses with high *Penicillium* spp. levels in the asthma group.

Table 3. 13. Characteristics of homes with high *Penicillium* spp. levels in the asthma group

***Penicillium* spp. level**	**Home characteristics**
58000 CFU/g	Dwelling age is 60, visible mold at home, humidity and dampness, painting home within last year, mouse at dwelling
57400 CFU/g	Dwelling age is 65, water damage at the toilet, visible mold and dampness, cockroach at home, aquarium at home
52300 CFU/g	Humidity and dampness, cockroach at home
50500 CFU/g	Dwelling age is 35, water damage, keeping bird at home, visible mold at home, humidity and dampness, the aquarium at home
44200 CFU/g	Near to chicken coop, humidity and dampness at bedroom, a heating system based on coal and wood
39800 CFU/g	Dwelling age is 30, humidity and dampness at home, cockroach at home
34600 CFU/g	Water damage at home, humidity, and dampness
26340 CFU/g	Dwelling age is 45, water damage at the toilet, humidity and dampness, cockroaches at home.

3.3. COMPARISON OF BIOLOGICAL MARKERS BETWEEN ASTHMA AND CONTROL GROUPS

A total of 239 settle dust samples were analyzed for biological markers, including 109 children for the asthma group and 130 children for the control group. Table 3.14 shows the level of biological markers and their distributions. Floor dust loadings in the control group (644.5 mg/m^2) were significantly more than the asthma group (464 mg/m^2). The median endotoxin concentration was lower in the asthma group (8.23×10^3 EU/g), compared to 15.1×10^3 EU/g in the control group. There were no statistically significant differences between asthma and control

groups in median β-(1→3) -D-glucan levels. The median concentration of *Aspergillus* (2600 CFU/g) was higher in the control group compared to the asthma group (1350 CFU/g). No differences were shown in *Penicillium* spp. between the two groups ($p > 0.05$).

Table 3. 14. Comparison of microbial markers between asthma and control groups

	Asthma Group (n=109)					**Control Group (n=130)**					***P*-Value**
	<LOD	**GM**	**Median**	**25th**	**75th**	**<LOD**	**GM**	**Median**	**25th**	**75th**	
Floor Dust (mg/m^2)	-	442.39	464	273	686	-	570.66	644.5	419.5	841.25	**<0.001**
Endotoxin ($10^3EU/g$)	-	7.63	8.23	3.8	15.05	-	16.11	15.1	7.54	33.32	**<0.001**
β-(1→3)-D-glucan (μg/g)	18	41.13	37.39	19.99	89.45	9	43.08	42.67	22.57	74.22	0.653
***Aspergillus* (CFU/g)**	12	2031.95	1350	713.5	3525	12	2470.09	2600	1080	5486.75	**0.012**
***Penicillium* (CFU/g)**	20	3738.55	2780	1556	6297	20	3493.75	3456.5	1235.25	7655.5	0.468

Bold indicates that differences between asthma and control groups were statistically significant.

In recent years, exposures to indoor endotoxin, fungal β-(1→3) -D-glucan, and molds such as *Aspergillus* and *Penicillium* spp. have been discussed in some studies. Respiratory disorders such as asthma have been influenced by indoor microbial exposure, but the associations are not clear [186]. For example, endotoxin in house dust has been known to have protective [159][161], harmful [165], and no associations [166][160] with childhood asthma. Recent epidemiological studies suggest that exposure to microorganisms and their components, especially endotoxin and fungal beta-glucan, can significantly influence asthma prevalence in children. Some studies show that exposure to the high level of microbial markers in early life has a protective effect on asthma prevalence at later ages in childhood [184]. Indeed, endotoxin has been shown to exacerbate asthma symptoms in children with the disease, including increased wheezing and the use of asthma medications [187][188]. Endotoxin has been associated with allergic sanitization in previous studies [159][189][162]. Endotoxin exposure has also been inversely associated with atopic asthma and atopic wheeze in school-aged children in the general population [158][85]. Approximately half of the asthma cases in the general population can be linked to allergic sensitization, so it is unclear if this association holds for children with asthma [84].

β-(1→3)-D-glucan is a marker of fungal and bacterial exposure, and its function in asthma exacerbation is less well understood with previous studies [190]. In one study, respiratory and general health of school-aged children were compared with β-(1→3)-D-glucan level, and a positive association was found [191]. Furthermore, elevated levels of β-(1→3)-D-glucan have been reported an increase in PEF variability in asthmatic children, as well as chronic atopic asthma and new-onset bronchial hyper-responsiveness [171]. Another study in Canada shows an inverse association between high BDG and asthma severity among school-aged children [164]. β-(1→3) -D-glucan was found to be significantly associated with increasing FEV_1 as a measure of asthma severity in a study conducted in Australia [192]. In a study conducted in the United States, high β-(1→3)-D-glucan levels were found to be inversely related to the frequency of wheezing in infants [169].

Penicillium was the most common indoor mold genus, followed by *Cladosporium* and *Aspergillus*, according to previous studies [193]. Mold sensitivity is a risk factor for allergic diseases, and molds can be severe indoor allergens [193]. Visible mold in the indoor environment has increased the risk of asthma and wheezing in children. A case-control study conducted in Germany reported that exposure to the high level of *Aspergillus* (above 90th percentile) increased the risk of allergic sensitization. However, exposure to *Penicillium* was not a risk factor for allergic sensitization [168]. The built environment, socioeconomic parameters, and lifestyle of people have been shown to affect the fungal concentration in household dust [194].

Few studies in the literature investigate the effect of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* on human health [158]. According to previous studies, higher levels of endotoxin, β-(1→3) -D-glucan, and fungal species were found in rural areas[173][178]. In turkey, there is only one study measuring the endotoxin level in house dust [195]. There is not any study in Turkey about the level of BDG, *Aspergillus*, and *Penicillium* in house dust. This study determined endotoxin levels among 100 children with and without allergy diagnosis. Endotoxin level ranges from 0.05 EU/ml-209 EU/ml, and the geometric mean was 61.8 EU/ml. There were no statistically significant differences in endotoxin levels between allergic and non-allergic groups. In this study, the highest Endotoxin levels were measured in the rural areas of non-allergic children [195].

In general, the association between indoor microbial markers and childhood respiratory diseases, especially asthma, was discussed in the literature. According to previous studies, the association between asthma and microbial exposures is not clear yet [163]. Some studies reported beneficial [161][159], some harmful [165], and some found no association[160][166] between indoor microbial exposure and respiratory diseases. The origins of the diverse outcomes are unknown, but geographic differences, variation in sampling procedures, and children's allergic sensitivity are thought to be the causes. A summary of several studies conducted in various nations is shown in Table 3.15.

Table 3. 15. Summary of some studies about indoor microbial agents and asthma

References	Country	Population	Agents	Determinants
Tavernier et al. (2005)	England	90 children	Endotoxin	Endotoxin is a risk factor for asthma
Tavernier et al. (2005)	England	90 children	Fungal genus	No association was found between the fungal genus and asthma.
Lawson et al. (2012)	Canada	310 children	Endotoxin	Endotoxin has a protective effect on asthma
Gehring et al. (2008)	Italy, Albania, New Zealand, Sweden, UK	840 children	Endotoxin	Endotoxin and asthma were inversely associated
Braun et al. (2001)	Germany, Belgium, Switzerland	812 children	Endotoxin	Endotoxin and asthma were inversely associated
Tischer et al. (2011)	Germany, Netherlands	690 children	Fungal genus	Fungal genus and asthma were inversely associated
Oluwole et al. (2018)	Canada	116 homes	Endotoxin	Endotoxin and asthma are associated
Oluwole et al. (2018)	Canada	116 homes	β-(1→3) -D-glucan	High Beta Glucan level is inversely associated with asthma
Jacob et al. (2002)	Germany	272 homes	*Aspergillus, Penicillium*	High *Aspergillus* level is a risk factor for respiratory diseases.

3.4. ASSOCIATIONS OF HOME CHARACTERISTICS AND LIFESTYLE OF FAMILIES WITH BIOLOGICAL MARKERS

Dust samples were collected from 239 homes in Ankara, Turkey, and all samples were analyzed for endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium*. Since endotoxin, β-(1→3)-D-

glucan, *Aspergillus*, and *Penicillium* concentrations were not normally distributed, non-parametric tests were performed to compare concentration differences among children with various family lifestyles and home characteristics. Mann–Whitney U test was used when we had two options for independent variables; Kruskal–Wallis test was performed for more than two options. Home characteristics and lifestyle of families were categorized according to residences characteristics, dampness indicators in homes, different family lifestyle habits, and some lifestyle behavior.

3.4.1. Biological Markers Comparisons in Residences with Different Characteristics

Table 3.16 shows the associations between floor dust, biological markers, and houses with various dwelling types. Level of house floor, dwelling age, residential area, house wall covering material, house floor covering material, house window frame material, heating system, and having separate kitchen are residence characteristics that were analyzed. The level of house floor was significant, as we found that houses on the ground and first floor (≤1) had significantly higher floor dust loading, endotoxin, and *Aspergillus* concentrations (*p*-value <0.05) than other houses (house floor ≥2). No significant differences in β-(1→3)-D-glucan and *Penicillium* concentrations were found among residences from the different house floors. Larger residences (> 100m^2) had significantly lower concentrations of β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* than smaller residences (≤ 100 m^2). There are no differences in loading floor dust and endotoxin concentration among residences with different areas. Buildings with natural gas and the central heating system showed lower floor dust loading and endotoxin concentration compared with houses that use coal or wood for the heating system. No associations were monitored for β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* with houses with different heating systems. No significant differences in loading dust, endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations were observed between residences with different dwelling ages, house wall covering material, house floor covering material, house window frame material, and having a separate kitchen or not (*p*-value >0.05).

Table 3. 16. Floor dust, endotoxin, β-(1→3) -D-glucan, *Aspergillus* and *Penicillium* concentration comparisons in residences with different characteristics

	n (%)	Floor Dust (mg/m^2)		Endotoxin ($10^3EU/g$)		β-(1→3)-D glucan (μg/g)		*Aspergillus* (CFU/g)		*Penicillium* (CFU/g)	
		GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
(1) Level of house floor											
≤1	125(52.3)	**540.51**	**1.77***	**13.57**	**3.54***	46.94	2.34	**2592**	**3.23***	3942	3.01
≥2	114(47.7)	**48025**	**1.73**	**9.83**	**3.46**	38.26	2.29	**1993**	**3.09**	3320	2.75
(2) Dwelling age											
≤10	84(35.1)	511.32	1.81	11.89	3.71	45.12	2.39	2205	3.01	3600	2.81
11-29	131(54.8)	511.25	1.69	11.47	3.16	40.36	2.23	2141	3.09	3432	2.69
≥30	24(10)	480.51	1.73	10.05	5.37	42.38	2.63	3296	4.16	4711	4.07
(3) Residential area											
≤100 m^2	75(31.4)	546.05	1.90	12.52	3.63	**51.54**	**2.39***	**3118**	**3.31****	**4828**	**3.09****
>100 m^2	164(68.6)	491.66	1.65	11.01	3.46	**38.49**	**2.29**	**1950**	**3.01**	**3151**	**2.69**
(4) House wall covering											
Plastic Paint	169(70.7)	514.13	1.69	11.95	3.23	40.95	2.34	2139	3.09	3454	2.75
Oil Paint	22(9.2)	467.86	1.69	9.94	3.98	47.35	1.99	2444	3.38	4196	3.16
Lime wash	28(11.7)	505.84	2.23	12.03	5.37	44.67	2.39	2887	3.16	4382	2.81
Wallpaper	20(8.4)	506.77	1.65	8.81	3.23	44.05	2.81	2331	3.80	3313	3.16
(5) House floor covering material											
Laminated wood	206(86.2)	508.59	1.77	11.37	3.63	42.81	2.39	2292	3.16	3744	2.88
Solid wood	25(10.5)	493.70	1.69	12.02	2.51	32.56	2.13	1810	3.09	2621	2.45
Stone	8(5.5)	536.63	1.73	12.12	4.26	39.31	1.86	2950	3.23	3505	2.95
(6) House window frame material											
Plastic- Steel	174(72.8)	509.4	1.77	11.60	3.80	42.63	2.34	2207	3.16	3496	2.81
Wood	65(27.2)	504.66	1.73	11.11	2.88	40.99	2.34	2407	3.31	3905	3.09
(**7)** Heating system											
Naturel gas-Combi boiler	174(72.8)	**508.88**	**1.73**	**11.07**	**3.23**	45.57	2.23	2133	2.95	3432	2.69
Central heating system	61(25.5)	**484.78**	**1.73**	**10.84**	**3.98**	39.87	2.57	2429	3.63	3788	3.23
Coal or wood	4(1.7)	**937.49**	**1.34***	**119.89**	**2.04****	66.64	2.63	9043	2.63	13974	2.63
(8) Separate kitchen											
No	15(6.3)	601.60	1.77	16.22	3.01	49.67	1.90	1848	2.81	3680	2.45
Yes	224 (93.7)	502.39	1.73	11.20	3.54	41.72	2.34	2290	3.16	3598	2.88

n, numbers; GM, geometric mean; GSD, geometric standard deviation. Bold indicates that the differences between the compared items were statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

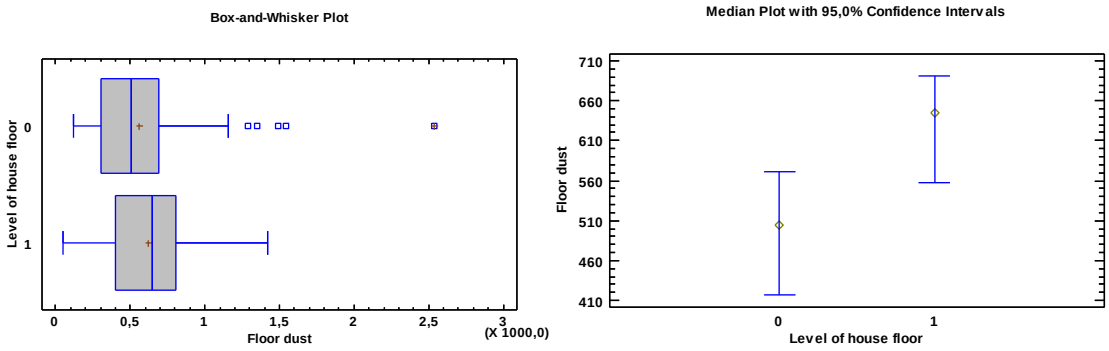


Figure 3. 13. Distribution plots for the association of floor dust and level of house floor

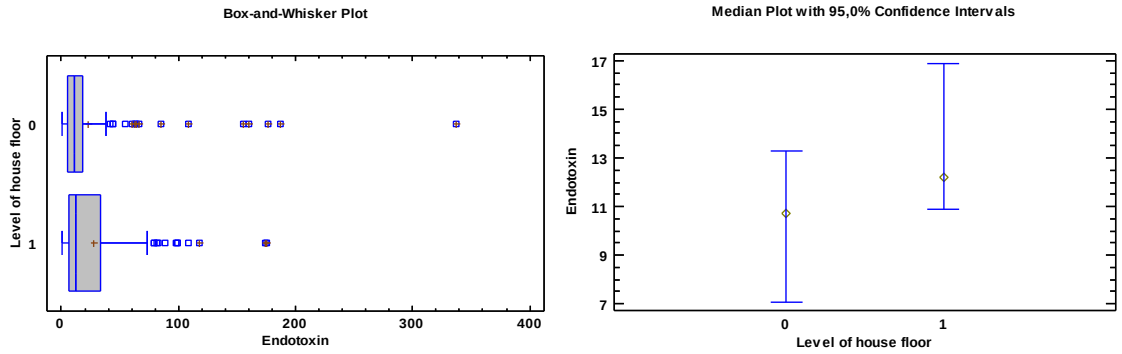


Figure 3. 14. Distribution plots for the association of endotoxin and level of house floor

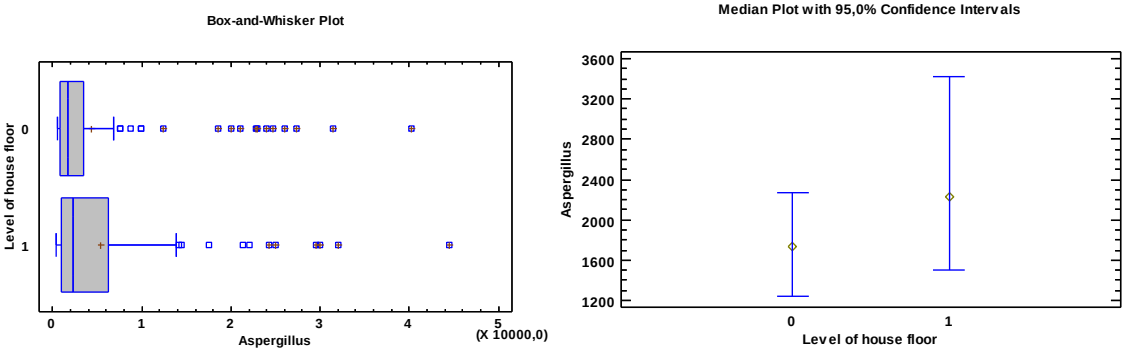


Figure 3. 15. Distribution plots for the association of *Aspergillus* and level of house floor

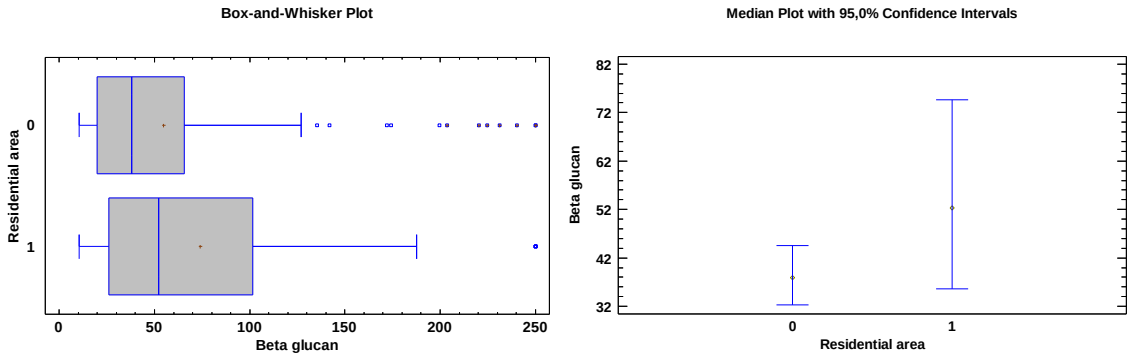


Figure 3. 16. Distribution plots for the association of β-(1→3) -D-glucan and residential area

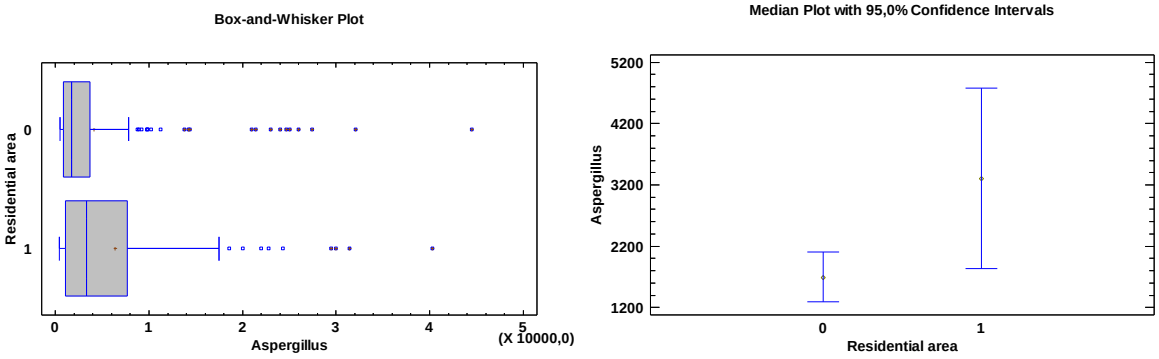


Figure 3. 17. Distribution plots for the association of *Aspergillus* and residential area

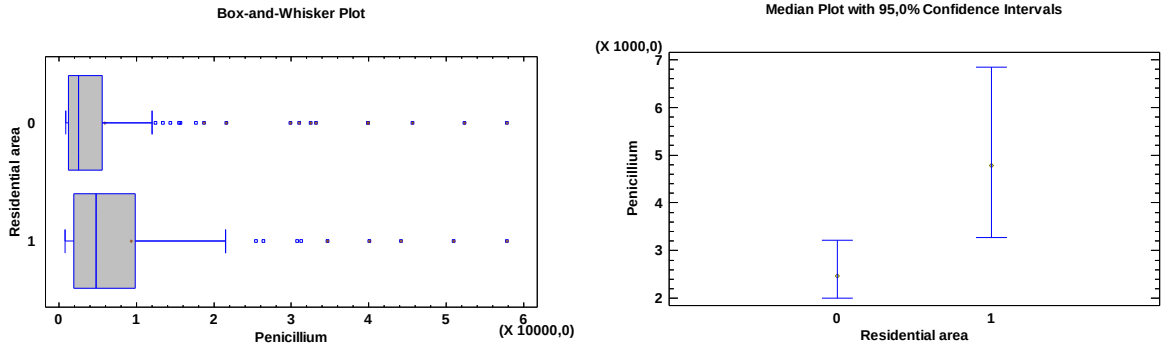


Figure 3. 18. Distribution plots for the association of *Penicillium* and residential area

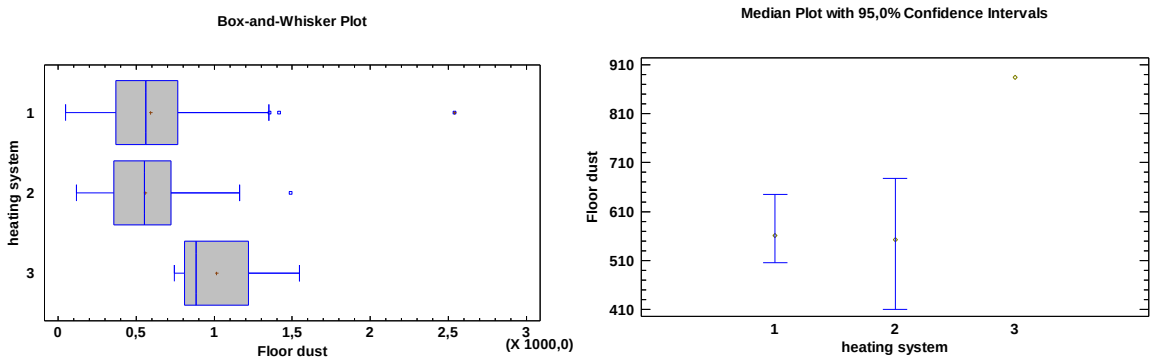


Figure 3. 19. Distribution plots for the association of floor dust and heating system

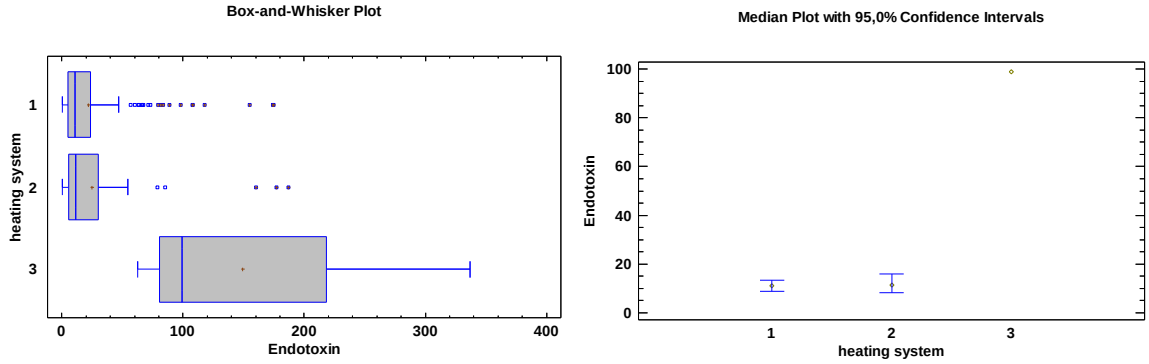


Figure 3. 20. Distribution plots for the association of endotoxin and heating system

3.4.2. Biological Markers Comparisons in Residences with Different Dampness Indicators

Table 3.17 shows the associations between floor dust, biological markers, and houses with dampness-related indicators. There were no significant variations in floor dust loading and endotoxin concentration between houses with and without dampness-related indicators (Table 3.17). However, there were many significant differences in β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations between houses with and without these indicators. Houses that had water damage within last year had significantly higher β-(1→3)-D-glucan (p-value < 0.05), *Aspergillus* and *Penicillium* concentrations (p- value< 0.0001) than houses without any water damage within last year. Houses with damp smells and visible mold spots had notably higher *Aspergillus* and *Penicillium* concentrations (p-value < 0.0001) than houses without these indicators.

Table 3. 17. Floor dust, endotoxin, β-(1→3) -D-glucan, *Aspergillus* and *Penicillium* concentration comparisons in residences with different dampness indicators

	n (%)	Floor Dust (mg/m^2)		Endotoxin ($10^3Eu/g$)		β-(1→3)-D glucan (μg/g)		*Aspergillus* (CFU/g)		*Penicillium* (CFU/g)	
		GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
(1) Water damage											
No	187(78.2)	503.66	1.73	10.88	3.63	**39.25**	**2.23**	**1911**	**2.81**	**3083**	**2.51**
Yes	52(21.8)	524.40	1.73	13.80	3.23	**54.62**	**2.51***	**4125**	**3.98*****	**6310**	**3.63*****
(2) Damp smells											
No	179(74.9)	500.86	1.73	10.46	3.54	40.65	2.29	**1904**	**3.01**	**3121**	**2.69**
Yes	60(25.1)	530.34	1.81	14.31	3.46	47.08	2.45	**3764**	**3.31*****	**5529**	**3.09*****
(3) Visible mold spots											
No	182(76.2)	509.11	1.73	11.30	3.63	41.69	2.29	**1949**	**3.01**	**3159**	**2.75**
Yes	57(23.8)	504.89	1.81	12.01	3.16	43.78	2.51	**3621**	**3.31 ***	**5486**	**3.01*****

n, numbers; GM, geometric mean; GSD, geometric standard deviation. Bold indicates that the differences between the compared items were statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

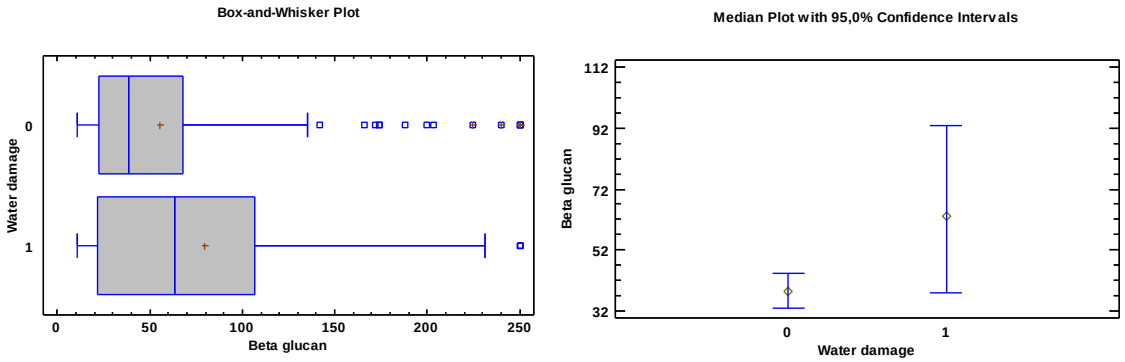


Figure 3. 21. Distribution plots for the association of β-(1→3)-D glucan and water damage

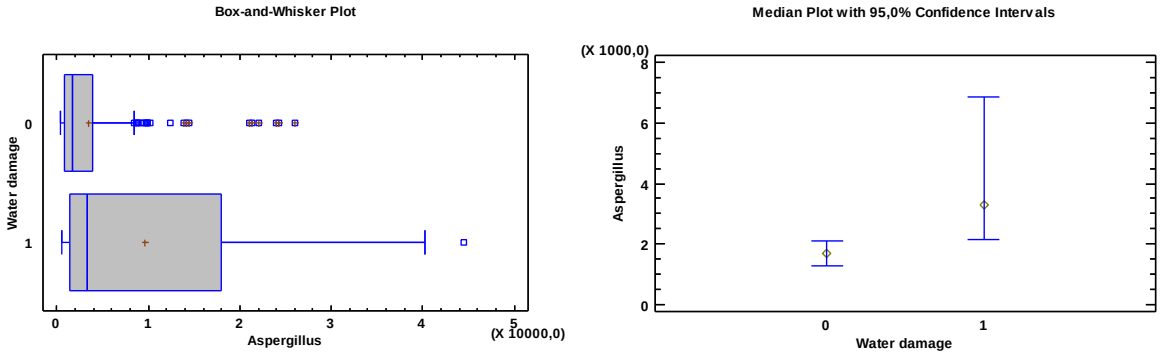


Figure 3. 22. Distribution plots for the association of *Aspergillus* and water damage

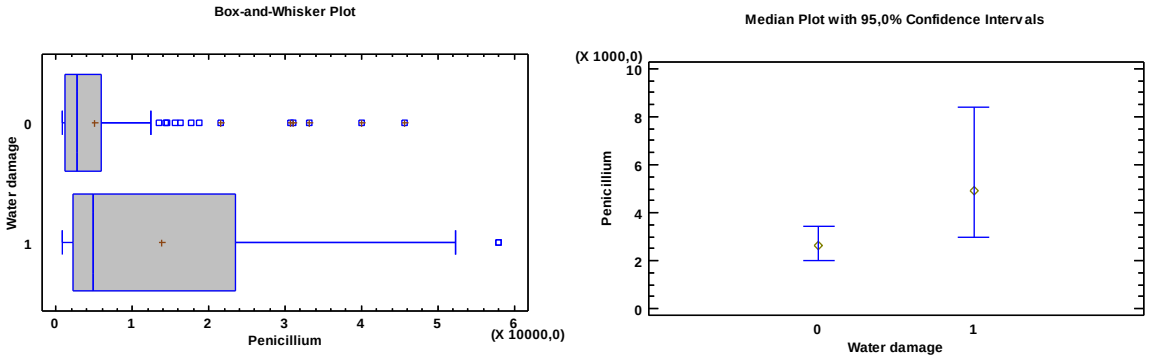


Figure 3. 23. Distribution plots for the association of *Penicillium* and water damage

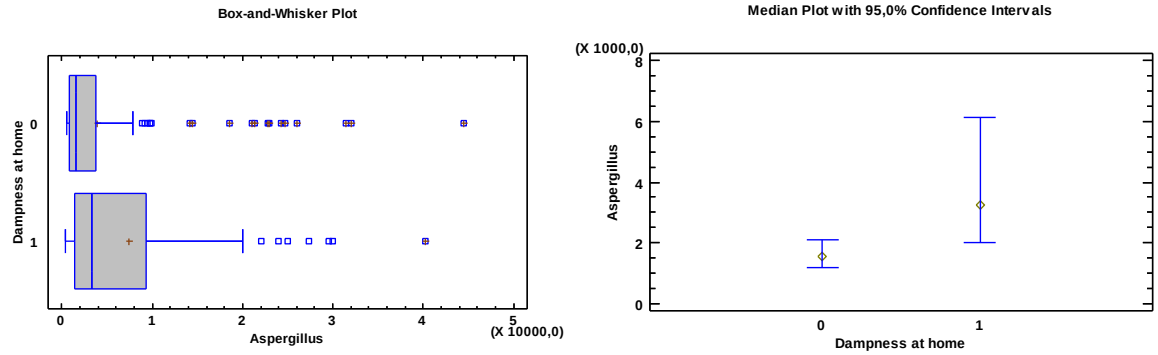


Figure 3. 24. Distribution plots for the association of *Aspergillus* and dampness

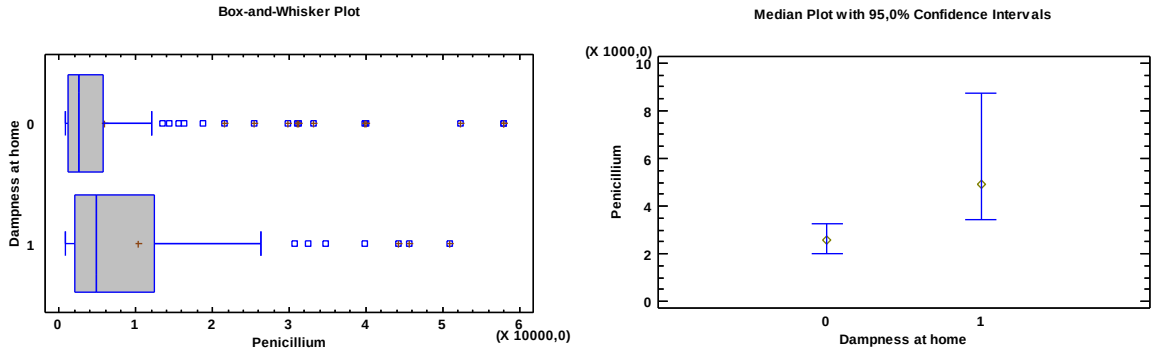


Figure 3. 25. Distribution plots for the association of *Penicillium* and dampness

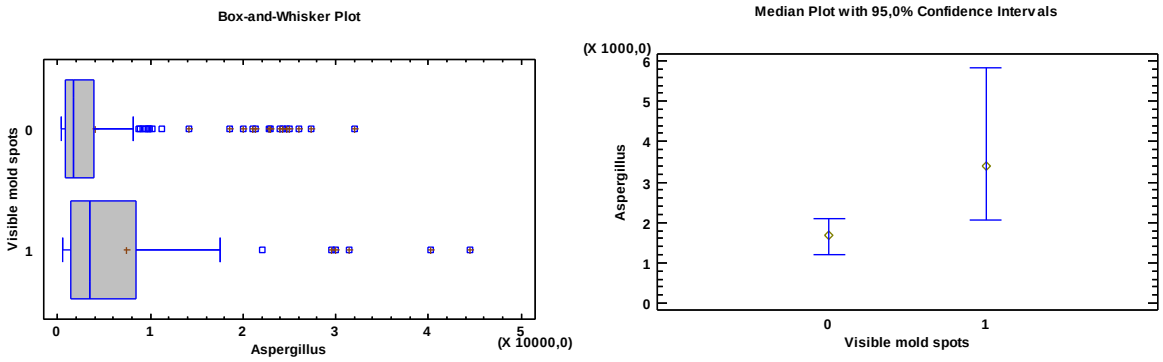


Figure 3. 26. Distribution plots for the association of *Aspergillus* and visible mold

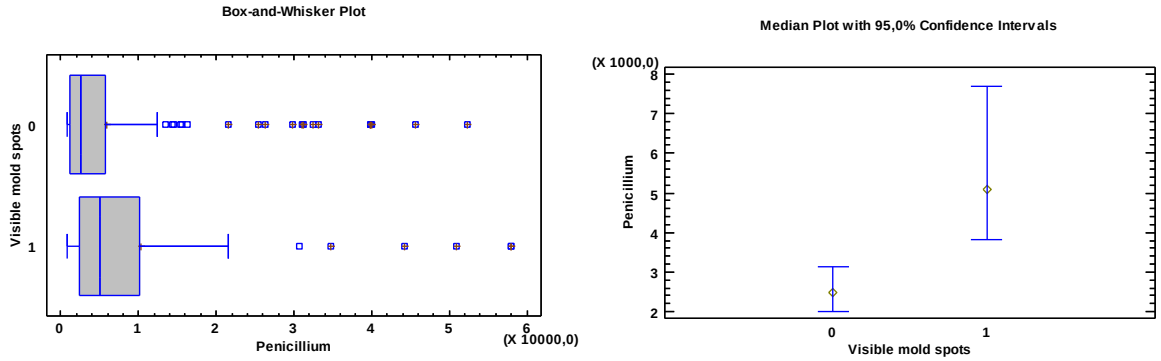


Figure 3. 27. Distribution plots for the association of *Penicillium* and visible mold

3.4.3. Biological Markers Comparisons in Families with Different Lifestyles

Table 3.18 shows the associations between floor dust, biological markers, and families with different habits. Some significant differences were detected in floor dust loading, endotoxin, β-(1→3) -D-glucan, *Aspergillus*, and *Penicillium* concentrations in houses from families with diverse lifestyle patterns. Higher β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations were discovered in the synthetic rugs compared to wool carpets. No significant differences in floor dust loading and endotoxin concentration were found for the type of rug. Significantly higher endotoxin, β-(1→3)-D-glucan and *Penicillium* concentrations were monitored from families that had houseplants at home compared to those who did not have (*p*-value < 0.05). Higher floor dust loading and endotoxin concentration were found in houses with old furniture. No significant differences were discovered for β-(1→3)-D-glucan, *Aspergillus*,

and *Penicillium* concentrations in the presence of new furniture at home. Significantly lower *Aspergillus* concentrations (p-value < 0.05) were determined in families with a lower frying frequency. Significantly lower floor dust loads and endotoxin concentration were discovered in the families that used dryer machines, as opposed to families who dried clothes indoors and outdoors (house balcony). Furthermore, there were no significant differences in floor dust loading, endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations between houses from families with various habits in relation to smoking and pet keeping (p-value >0.05).

Table 3. 18. Floor dust, endotoxin, β-(1→3) -D-glucan, *Aspergillus* and *Penicillium* concentration comparisons in families with different lifestyle

	n (%)	Floor Dust (mg/m^2)		Endotoxin ($10^3EU/g$)		β-(1→3)-D glucan (μg/g)		*Aspergillus* (CFU/g)		*Penicillium* (CFU/g)	
		GM	**GSD**	**GM**	**GSD**	**GM**	**GSD**	**GM**	**GSD**	**GM**	**GSD**
(1) Smoking											
No	115(48.1)	490.78	1.81	12.65	3.89	41.07	2.45	2239	3.46	3665	3.09
Yes	32(13.4)	594.93	1.51	11.53	2.51	39.47	2.13	2632	2.81	3332	2.51
House balcony	92(38.5)	579.81	1.73	10.11	3.38	44.63	2.23	2167	3.01	3624	2.69
(2) Houseplants											
No	204(85.3)	470.09	1.51	**7.04**	**3.46***	**31.99**	**2.13***	1780	3.09	**2606**	**2.81***
Yes	35(14.7)	508.78	1.77	**12.46**	**3.46**	**44.23**	**2.34**	2353	3.38	**3809**	**3.01**
(3) Pet ownership											
No	190(79.5)	494	1.77	11.24	3.63	43.17	2.34	2297	3.23	3752	2.88
Yes	49(20.5)	566.68	1.54	12.37	3.01	38.54	2.18	2118	2.95	3079	2.75
(4) New furniture											
No	170(71.1)	**542.51**	**1.73****	**12.91**	**3.46***	44.40	2.23	2102	3.01	3816	2.75
Yes	69(28.9)	**432.36**	**1.73**	**8.55**	**3.46**	41.17	2.57	2358	3.46	3528	3.23
(5) Type of rug											
Synthetic	219(91.6)	504.99	1.77	11.52	3.54	**43.98**	**2.34**	**2397**	**3.16**	**3815**	**2.81**
Wool	20(8.4)	543.5	1.62	10.83	3.23	**26.65**	**2.13***	**1179**	**2.88****	**1941**	**2.57****
(6) Frying frequency											
Once or more / Week	141(59.0)	519.65	1.77	12.59	3.31	43.90	2.23	**2425**	**3.09**	3779	2.81
Once / 2 Weeks	53(22.2)	470.61	1.73	9.21	3.63	45.90	2.63	**2644**	**3.89**	4194	3.31
Once / month	45(18.8)	518.30	1.69	11.06	4.07	33.66	2.18	**1503**	**2.45***	2594	2.29
(7) Where to dry clothes											
Outdoors	63(26.4)	**517.19**	**1.69**	**12.40**	**3.71**	42.76	2.45	2630	3.01	3927	2.88
Indoors	152(63.6)	**530.56**	**1.73**	**12.79**	**3.38**	41.88	2.23	2109	3.16	3403	2.81
Dryer machine	24(10)	**368.81**	**1.69***	**5.21**	**3.01****	42.46	2.75	2343	3.80	4126	3.23

n, numbers; GM, geometric mean; GSD, geometric standard deviation. Bold indicates that the differences between the compared items were statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

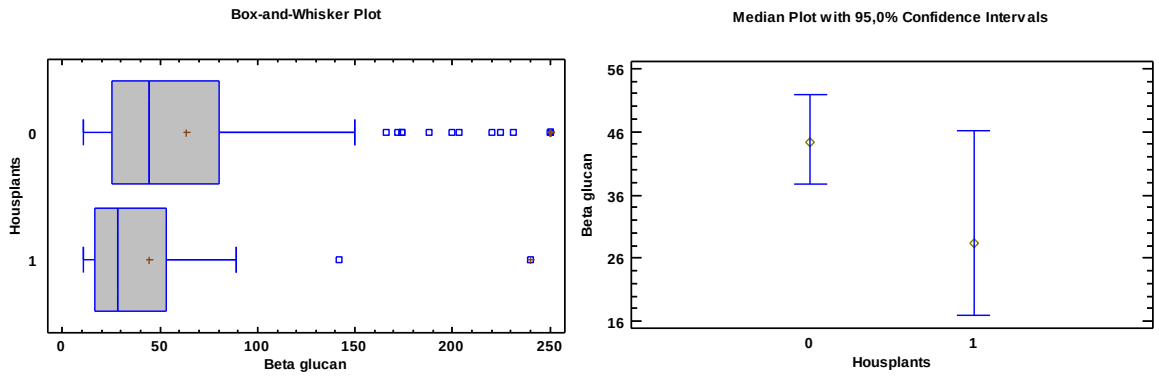


Figure 3. 28. Distribution plots for the association of β-(1→3)-D glucan and houseplants

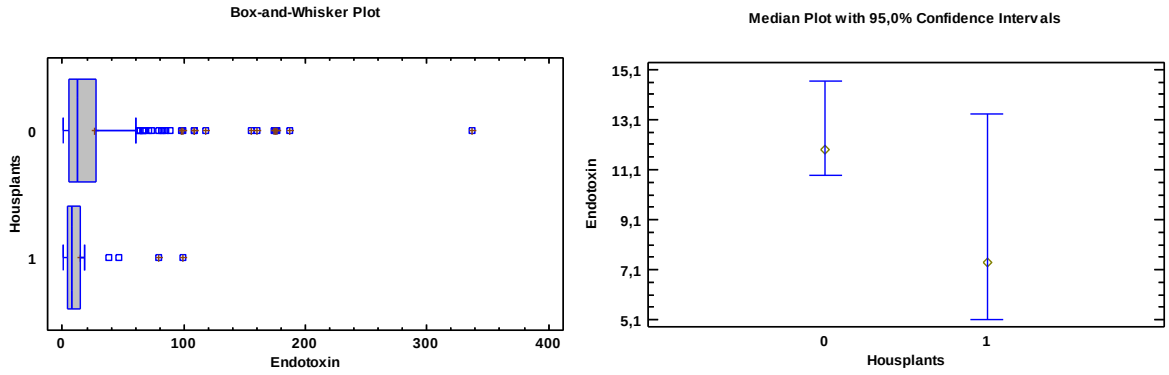


Figure 3. 29. Distribution plots for the association of endotoxin and houseplants

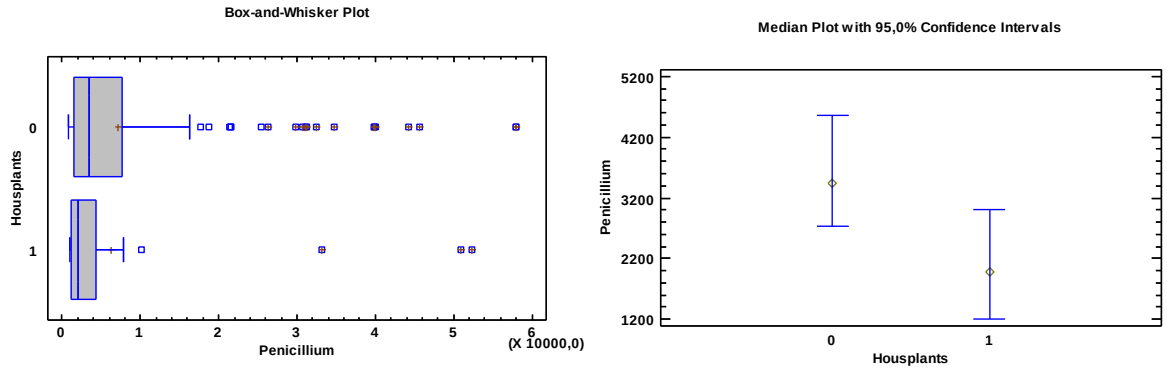


Figure 3. 30. Distribution plots for the association of *Penicillium* and houseplants

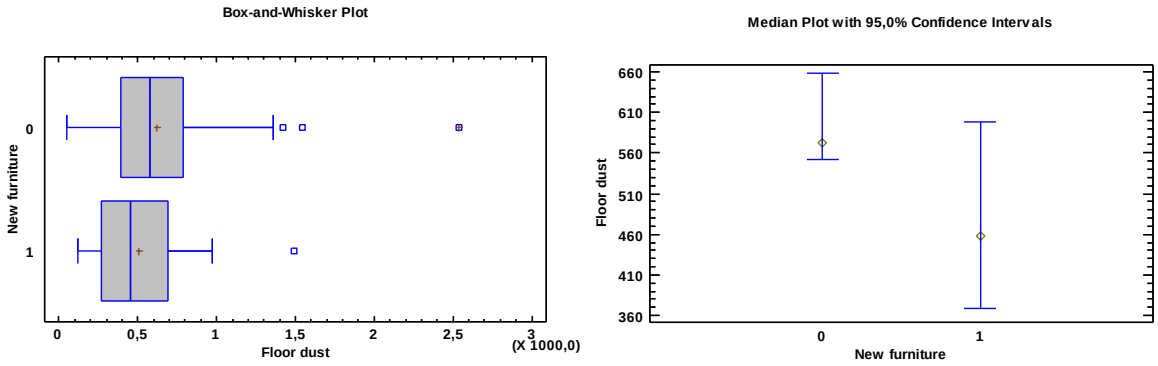


Figure 3. 31. Distribution plots for the association of floor dust and new furniture

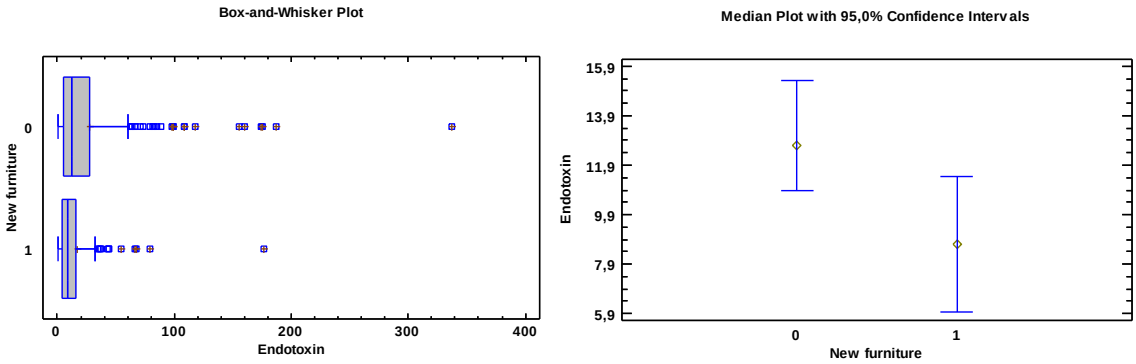


Figure 3. 32. Distribution plots for the association of endotoxin and new furniture

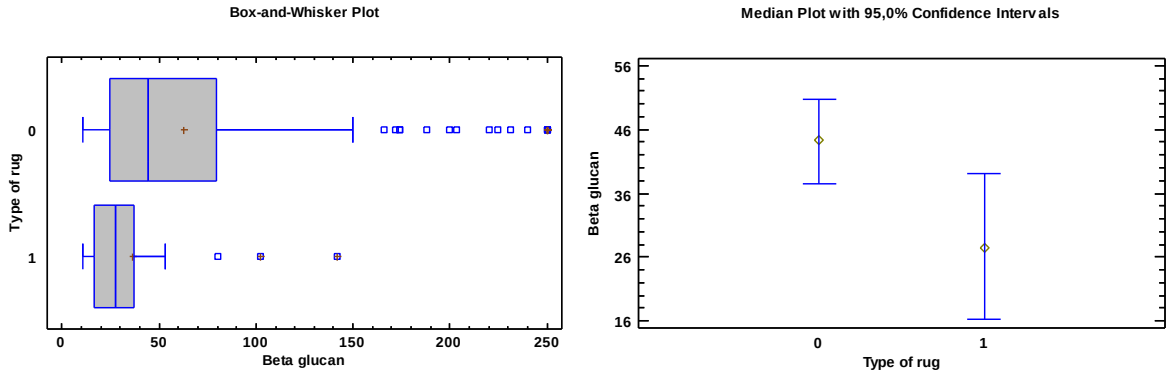


Figure 3. 33. Distribution plots for the association of β-(1→3)-D glucan and type of rug

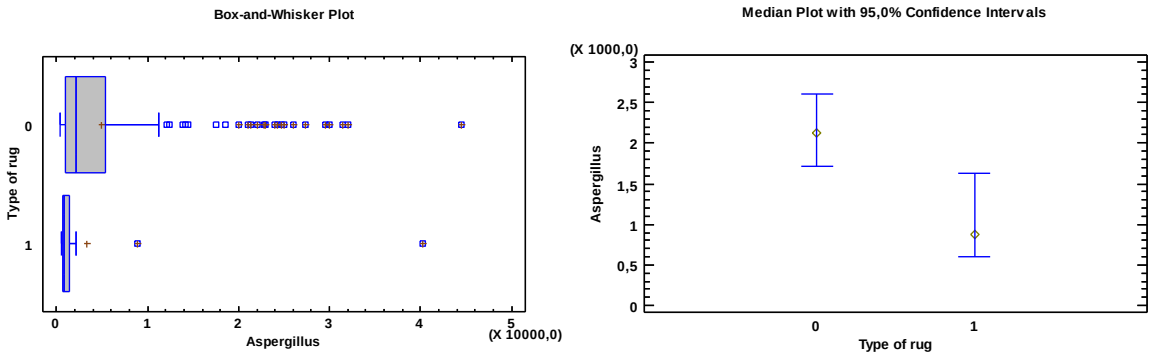


Figure 3. 34. Distribution plots for the association of *Aspergillus* and type of rug

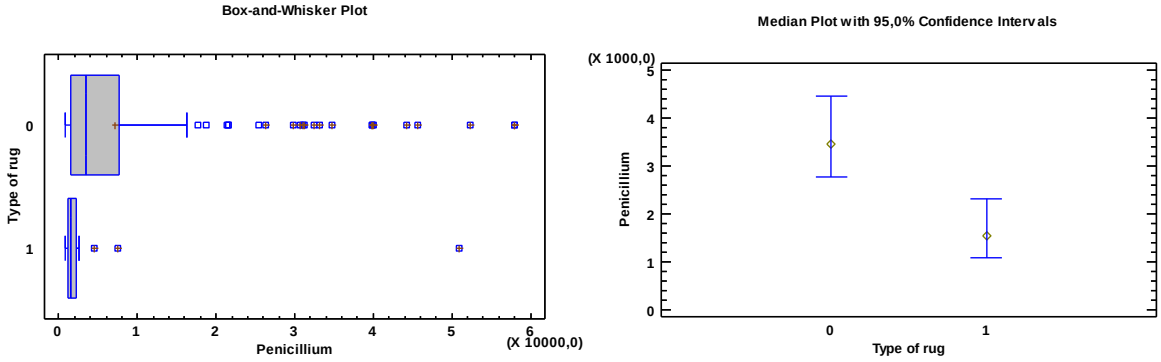


Figure 3. 35 Distribution plots for the association of *Penicillium* and type of rug

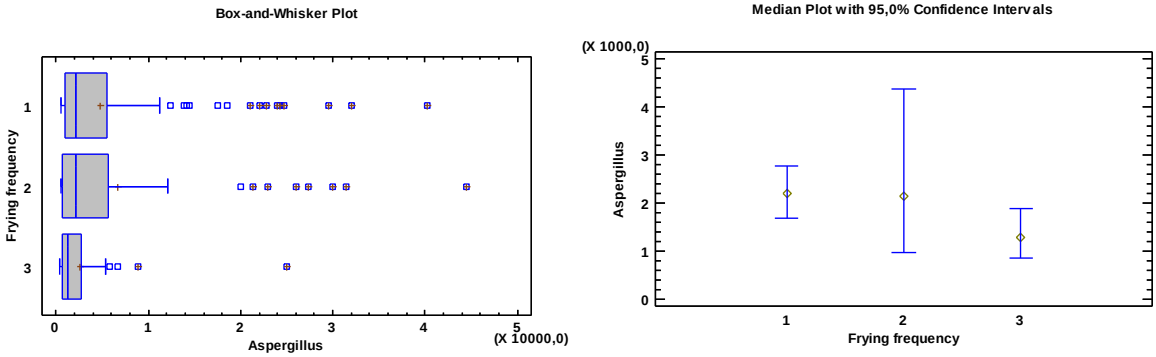


Figure 3. 36. Distribution plots for the association of *Aspergillus* and frying frequency

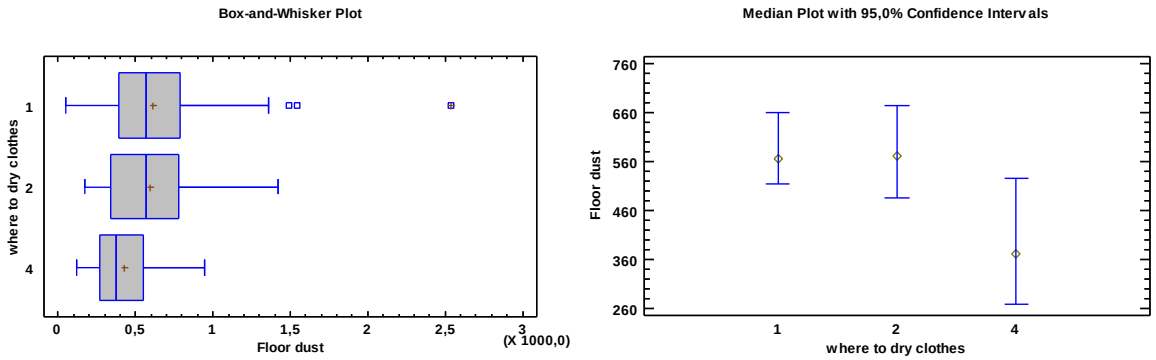


Figure 3. 37. Distribution plots for the association of floor dust and location of drying clothes

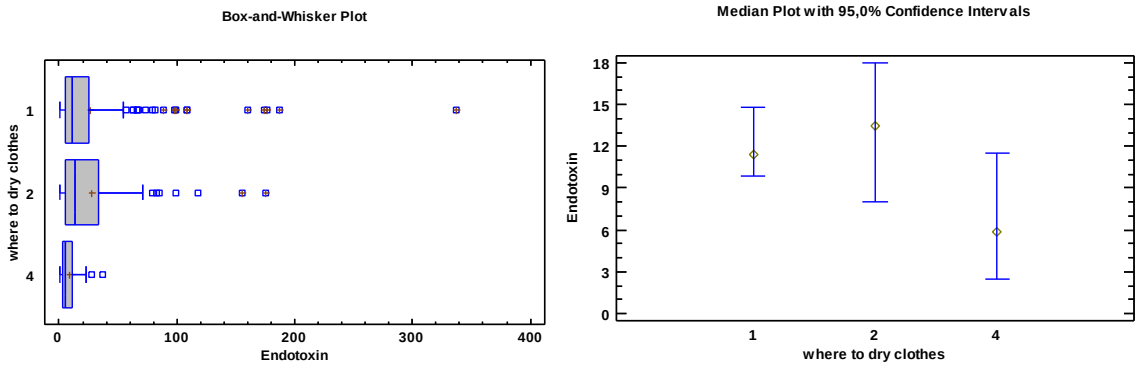


Figure 3. 38. Distribution plots for the association of endotoxin and location of drying clothes

3.4.4. Biological Markers Comparisons in Families with Different Lifestyle Habit Regarding Cleaning and Bedding

Table 3.19 shows the associations between floor dust, biological markers, and families with different lifestyle habits regarding cleaning and bedding. In terms of lifestyle behaviors, families that changed their bedsheets and coverlets less frequently (once a month) had significantly higher concentrations of β-(1→3)-D-glucan (p-value < 0.0001) and *Penicillium* (p-value < 0.001) in their houses. No significant differences were found in floor dust loads, endotoxin, and *Aspergillus* concentrations for the frequency of changing sheets and coverlets. Moreover, no significant differences (p-value >0.05) in floor dust loads, endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations were found across families with diverse behaviors in regards to cleaning frequency, use of bleach, and materials of mattress.

Table 3. 19. Floor dust, endotoxin, β-(1→3) -D-glucan, *Aspergillus* and *Penicillium* concentration comparisons in families with different lifestyle habit regarding cleaning and bedding

	n (%)	Floor Dust (mg/m^2)		Endotoxin ($10^3EU/g$)		β-(1→3)-D glucan (µg/g)		*Aspergillus* (CFU/g)		*Penicillium* (CFU/g)	
		GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
(1) Cleaning frequency											
Once a week or less	77(32.2)	481.30	1.81	11.81	3.31	39.27	2.23	2019	3.09	3139	2.69
2-4 days a week	78(32.6)	505.99	1.69	10.09	3.54	44.13	2.29	2282	3.09	3819	2.95
More than 4 days a week	84(35.1)	536.05	1.73	12.55	3.71	42.18	2.45	2358	3.31	3873	3.01
(2) Use of bleach											
Never	36(15.1)	527.23	1.62	14.52	3.46	32.13	2.08	1896	2.75	3080	2.57
One day per week	58(24.3)	504.74	1.77	12.29	3.31	43.35	2.08	2371	3.31	3544	3.16
2 or 3 days a week	81(33.9)	518.47	1.69	9.80	3.80	43.69	2.51	2235	3.38	3477	2.95
More than 4 days a week	64(26.8)	574.79	1.86	11.48	3.38	45.87	2.18	2419	3.09	4178	2.69
(3) Frequency of changing coverlets											
Once / Week	96(40.2)	531.53	1.69	9.97	3.80	**33.22**	**2.34**	1884	2.81	**2864**	**2.63**
Once / 2 Weeks	108(45.2)	485.67	1.81	12.55	3.38	**48.82**	**2.18**	2160	2.95	**3616**	**2.81**
Once / month	35(14.6)	516.14	1.58	12.71	3.23	**52.31**	**2.29*****	2817	3.46	**4658**	**3.01****
(4) Materials of mattress											
Viscoelastic	172(72)	507.10	1.65	10.68	3.23	41.25	2.08	2102	3.23	3409	2.88
Cotton	26(10.9)	480.54	1.81	12.05	4.67	39.65	1.99	2456	2.81	3274	2.63
Wool	41(17.2)	530.79	1.99	14.95	3.89	48.15	2.23	2902	2.95	4827	2.75

n, numbers; GM, geometric mean; GSD, geometric standard deviation. Bold indicates that the differences between the compared items were statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

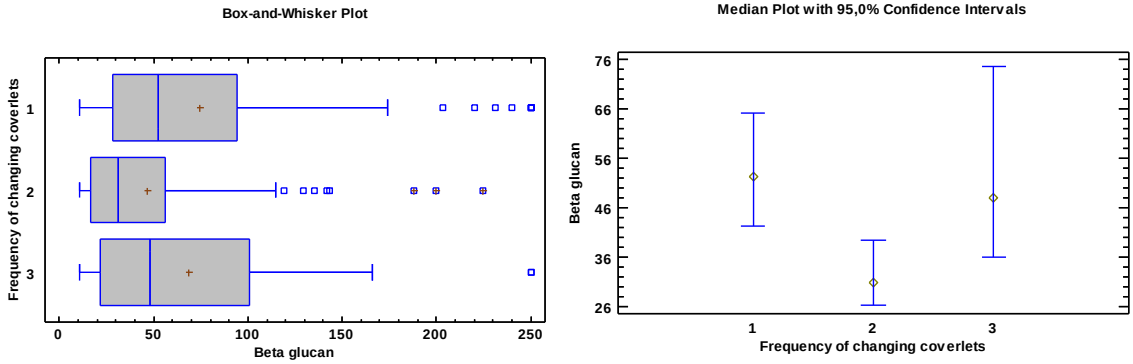


Figure 3. 39. Distribution plots for the association of β-(1→3) -D-glucan and frequency of changing coverlets

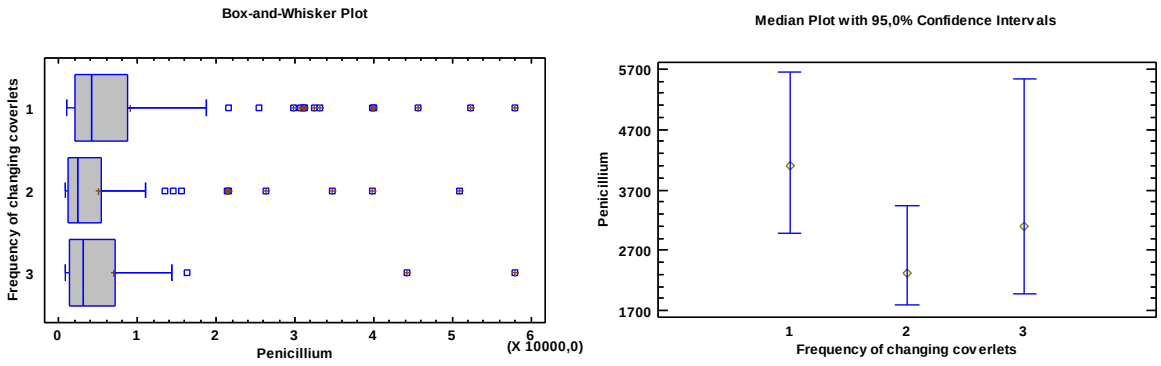


Figure 3. 40. Distribution plots for the association of *Penicillium* and frequency of changing coverlets

3.4.5. Multivariate Logistic Regression Analyses for Potential Determinants Associations with High Floor Dust and Biological Markers

Table 3.20 depicts the results of bivariate and multivariate logistic regression analyses. In the ANOVA test analyses, twelve determinants had at least one significant association with floor dust and β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations. These determinants include house floor, residential area, heating system, water damage at home within last year, dampness at home, visible mold spots at home, houseplants, having new furniture, type of rug, frying frequency, where to dry clothes, and the frequency of changing coverlets and bedsheets. Of these, water damage at home, dampness, and visible mold spots have a moderate correlation. Therefore, in the multivariate logistic regression models, visible mold spot was chosen because it had more significant associations with β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations than water damage and dampness in the non-parametric test. Heating systems are another factor that is not included in multivariate logistic models because the number of houses that use coal or wood for heating systems is only four when compared to other groups with different heating systems. In this case, the multivariate models had nine determinants. There was a low or slight correlation between these factors.

Table 3. 20. Multivariate logistic regression analyses for potential determinants associations with high floor dust, endotoxin, β-(1→3) -D- glucan, *Aspergillus* and *Penicillium*

	Floor Dust (mg/m²)		**Endotoxin (EU/g)**		**β-(1→3)-D glucan (µg/g)**		***Aspergillus* (CFU/g)**		***Penicillium* (CFU/g)**	
	OR, 95 % CI (> median vs ≤ median; reference: ≤ median)									
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
(1) Level of house floor (ref: ≥2)										
≤1	**1.99(1.24-3.40) ***	**2.17(1.17-4) ***	**1.64(0.97-2.80) ***	**2.31(1.25-4.29)****	1.45(0.87-2.42)	1.29(1.04-2.29)	1.35(0.80-2.28)	1.20(1.52-2.22)	1.03(0.62-1.71)	1.39(0.76-2.54)
(2) Residential area (ref:>100 m²)										
≤100 m²	**1.82(1.08-3.29) ***	1.29(1.04-2.56)	0.93(0.53-1.64)	1.77(0.90-3.48)	**1.59(0.91-2.79) ***	1.38(1.26-2.62)	**1.62(0.91-2.88) ***	1.25(1.56-2.43)	**2.15(1.21-3.80)****	**1.95(1.01-3.78)***
(3) Mold spores (ref: No)										
Yes	1.02(0.55-1.89)	1.63(0.98-1.87)	1.15(0.62-2.15)	1.20(0.59-2.44)	1.42(0.77-2.61)	1.33(0.68-2.61)	**2.77(1.4-5.50) ****	**3.02(1.42-6.44)****	**2.73(1.43-5.22)****	**3.02(1.46-6.24)****
(4) Houseplants (ref: No)										
Yes	1.33(0.96-1.86)	1.24(0.25-6.05)	**2.52(0.67-9.47) ***	**3.71(1.40-10) ****	**1.47(1.05-2.05) ***	**4.23(1.51-11.9)****	1.14(1.01-1.44)	**1.77(1.11-3.38) ***	**1.41(1.01-1.96)***	1.36(0.72-2.57)
(5) New Furniture (ref: Yes)										
No	**2.21(1.24-3.91) ***	**2.04(1.1-3.84) ***	**1.67(0.94-2.95) ***	1.78(1.07-3.36)	1.10(0.62-1.93)	1.15(0.61-2.16)	0.805(0.45-1.42)	0.74(0.39-1.43)	0.90(0.51-1.58)	1.03(0.54-1.96)
(6) Type of rug (ref: wool)										
Synthetic rug	0.70(0.26-1.89)	0.54(0.18-1.62)	0.88(0.33-2.30)	0.74(0.24-2.25)	**4.1(1.42-11.58)****	2.76(0.87-8.72)	**5.2(1.82-14.87)****	**4.64(1.35-15.9)***	**7.67(2.18-26.7)****	**5.99(1.49-24.08)***
(7) Frying Frequency (ref: Once / month)										
Once or more / Week	1.04(0.49-2.02)	1.05(0.47-2.33)	1.32(0.67-2.62)	1.50(0.69-3.24)	1.79(0.91-3.53)	1.59(0.75-3.38)	**2.01(1.02-3.97) ***	1.95(0.89-4.29)	1.84(0.93-3.64)	1.62(0.73-3.60)
Once / 2 Weeks	0.72(0.31-1.62)	0.88(0.35-2.21)	1.69(0.74-3.86)	2.83(1.10-7.24)	2.08(0.92-4.67)	1.94(0.80-4.70)	1.88(0.84-4.22)	1.83(0.73-4.54)	1.65(0.74-3.68)	1.45(0.58-3.64)
(8) Where to dry clothes (ref: Dryer machine)										
Outdoors	**3.99(1.32-12. 2)***	**3.7(1.2-11.27) ***	2.43(0.93-6.35)	2.76(0.93-8.23)	0.99 (0.35-2.79)	0.74(0.51-1.04)	0.65(0.22-1.91)	0.56(0.36-0.89)	0.67(0.27-1.65)	0.47(0.26-0.91)
Indoors	**5.32(2.3-14.1)****	**4.7(1.6-13.4)****	**3.78(1.27-11.2)***	**5.16(1.52-17.4)****	1.28(0.49-3.32)	1.22(0.68-2.19)	1.15(0.42-3.16)	2.04(1.10-3.77)	1.33(0.50-3.48)	2.17(1.17-3.99)
(9) Frequency of changing coverlets (ref: Once / Week)										
Once / 2 Weeks	1.02(0.57-1-80)	0.99(0.52-1.87)	**1.84(1.03-3.27) ***	**2.26(1.19-4.29) ***	**1.25(0.54-2.85) ***	**2.17(1.19-4) ***	**1.78(1.01-3.15) ***	**1.87(1.04-3.54)***	**2.08(1.18-3.64) ***	**2.03(1.08-3.81) ***
Once / month	1.01(0.45-2.26)	0.92(0.37-2.27)	1.21(0.55-2.67)	1.19(0.50-2.85)	2.24(0.82-3.27)	1.36(0.55-3.38)	1.04(0.93-2.16)	1.24(1.09-3.08)	1.11(0.87-1.98)	1.36(0.72-2.36)

Bold indicates significance. * $p < 0.05$, ** $p < 0.01$. OR: Odd Ratio.

Families were divided into two subgroups (≤ 50 percentile (median) vs.> 50 percentile) based on the amount of floor dust and concentrations of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium*, and then used bivariate and multivariate logistic regressions to look for relationships between higher (> median) concentrations for biological markers and household determinants. First, we used bivariate logistic regression models to investigate crude associations. After that, in the bivariate logistic regression analysis, those determinants that had at least one significant relationship with higher floor dust or concentration of biological markers in the non-parametric tests were selected for multivariate modeling. In the multivariate logistic models, the selected determinants were mutually adjusted. Multivariate results were expressed as add ratios (OR) and adjusted odds ratios (aOR) with a 95% confidence interval (Cl).

Multivariate logistic regression analyzes presented that high amounts of floor dust were associated with the level of house floor (≤1) (OR = 2.17, 95% CI: 1.17–4), having old furniture at home (OR = 2.04, 95% CI: 1.1–3.84) and drying clothes at home (indoors) (OR = 4.7, 95% CI: 1.6–13.4) and house balcony (outdoors) (OR = 3.7, 95% CI: 1.2–11.27) compared to a dryer machine. Multivariate logistic modeling indicated that high endotoxin concentration was associated with the level of house floor (≤1) (OR = 2.31, 95% CI: 1.25–4.29), having houseplants at home (OR = 3.71, 95% CI: 1.4–10), drying clothes at home (indoors) (OR = 5.16, 95% CI: 1.52–17.4) and frequency of changing coverlets and bed sheets (once / 2 weeks) (OR = 2.26, 95% CI: 1.19–4.29) compared to changing coverlets and bedsheets once per week. Higher β-(1→3)-D-glucan concentration was associated with having houseplants at home (OR = 4.23, 95% CI: 1.51–11.9) and the frequency of changing coverlets and bed sheets (once / 2 weeks) (OR = 2.17, 95% CI: 1–19.4) compared to changing coverlets and bedsheets once per week in multivariate logistic modeling. According to the results of multivariate logistic regression analyzes the higher level of *Aspergillus* was associated with visible mold spots (OR = 3.02, 95% CI: 1.42–6.44), having houseplants at home (OR = 1.77, 95% CI: 0.93–3.38), synthetic rug (OR = 6.64, 95% CI: 1.35–15.9) compared to wool rug, and the frequency of changing coverlets and bed sheets (once / 2 weeks) (OR = 1.87, 95% CI: 1.04–3.54) compared to changing coverlets and bedsheets once per week. Multivariate logistic regression analyzes indicated that a high level of *Penicillium* was associated with the residential area (≤100 m^2) (OR = 1.95, 95% CI: 1.01–3.78), visible mold spots (OR = 3.02, 95% CI: 1.46–15.9), synthetic rug (OR = 5.99, 95% CI: 1.49–24.08) compared to wool rug, and frequency of changing coverlets

and bedsheets (once / 2 weeks) (OR = 2.03, 95% CI: 1.08–3.81) compared to changing coverlets and bedsheets once per week. Among the potential determinants, having houseplants at home and the frequency of changing coverlets and bedsheets were the factors that were significantly associated with most of the biological markers. Having houseplants at home was the determinant that positively associated with the concentration of endotoxin, β-(1→3)-D-glucan, and *Aspergillus*. Furthermore, the frequency of changing coverlets and bed sheets (once / 2 weeks) presented a significant association with a higher risk of having high endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations.

Based on our information, this is the first study that measures concentrations of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* in household dust and reports their potential determinants in Turkish children's homes. Therefore, we compared our study with previous European and other studies. Table 3.21 illustrates the summary of some European studies which were investigated the concentrations of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* and their determinants in household dust. Only a few studies investigated β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium*; most studies focused on endotoxin. Comparing two European multicenter studies revealed significant variations in the concentrations of endotoxin and β-(1→3)-D-glucan [178][179]. Endotoxin levels were found to be low in Scandinavia, higher in Southern, Middle, and Alpine Europe, and reported highest in the Mediterranean regions. In contrast, as compared to mid-European areas, BDG concentrations were high in both Sweden and Finland [179][158][172]. A study conducted in Denmark reported high endotoxin levels and low BDG concentration that appeared to contradict the multicenter studies' proposed findings [40]. Similar endotoxin and β-(1→3)-D-glucan levels were found in the United States and New Zealand. Among these studies, differences in concentrations of endotoxin and β-(1→3)-D-glucan is large. The endotoxin concentration in this study was reported to be close to other studies, but we detected lower β-(1→3)-D-glucan concentration compared to previous European and other studies. However, it is better to note that Ankara has a cold semi-arid climate and has cold winters and dry summers because of its location. Moreover, variation in the level of biomarkers can be attributed to climate and geographical conditions, methodological differences and the location of sampling, home characteristics, and cultural differences. Overall, levels of biomarkers were impacted by the sampling location at home. The lowest concentrations detected in mattress dust, higher concentrations detected in bedroom floor dust,

and the highest concentrations detected in the living room floor dust. Extraction methods such as sonication (increase) and freezing the dust extract (decrease) can significantly influence the concentrations of biomarkers in settled house dust.

Previous studies reported some significant factors for concentrations of endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* (Table 3.21). These factors included season, flooring type (carpeting), farming, pet ownership, crowding index, frequency of cleaning, moisture, and dampness. Furthermore, most of these determinants are the same as observed in the studies conducted outside Europe [196][197][198]. Endotoxin concentrations were found to be higher in rural regions, particularly farms, than in urban areas [158][86]. In a study conducted in Denmark, dust samples were collected from 330 children's homes. Flooring type and type of dwelling (1 floor, 2-3 floor, apartment) are the factors associated with endotoxin level. In the same study, the significant determinants associated with β-(1→3)-D-glucan concentration are pet keeping and location of dwelling (near farm areas or not) [40]. House dust samples were collected from 25 German homes to measure endotoxin and β-(1→3)-D-glucan levels. Building age and heating system are determinants significantly associated with floor dust, endotoxin, and β-(1→3)-D-glucan levels [174]. We found the heating system as a significant determinant for the amount of floor dust and endotoxin concentration in this study, too. In a multicenter study, dust samples were collected from 10 European countries (974 homes) and, pet-keeping, crowding index, and dampness were the factors associated with endotoxin concentration [178]. This study found no association between endotoxin concentration and the number of occupants and dampness, like a study conducted in Colorado [199]. In a study conducted in Germany, dampness and water moisture were found significant factors for *Aspergillus* and *penicillium* concentrations [168]. Also, we found the same results in this study. Dampness, water moisture, and visible mold are significant determinants for *Aspergillus* and *penicillium* concentrations. β-(1→3)-D-glucan levels were measured in 395 German homes, and carpeting (type of rug), dog keeping, and occupants are the factors associated with BDG [173]. The type of rug is a significant association for β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* in this study, too. Wool rugs are natural, while synthetic rugs are made from nylon, polypropylene, and other chemicals.

Furthermore, when comparing results from different studies on determinants of biological markers, it is better to remember that each study employed its unique set of determinants, so the comparison between studies cannot be completely comprehensive. The key strength of this work is that it is one of the first to determine endotoxin β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations, and determinants in Turkish homes. This research used a diverse set of determinants, including home characteristics and the lifestyle of families. Table 3.21 shows a literature summary of different studies on concentrations and determinants of biological markers.

Table 3. 21. Different studies on concentrations and determinants of biological markers

References	Country	Population	Agents	Levels	Determinants
Holst et al. (2015)	Denmark	330 homes	Endotoxin	31.1×10^3 EU/g	Flooring type, type of dwelling
Holst et al. (2015)	Denmark	330 homes	β-(1→3) -D-glucan	0.71×10^3 µg/g	Pet keeping, location of the dwelling
Chen et al. (2012)	10 European Union countries	974 homes	Endotoxin	$0.82–4.81\times10^3$ EU/g	Pet keeping, crowding index, dampness
Cases et al. (2013)	Germany, Netherlands, Spain, Finland	1572 homes	Endotoxin	$3.20–23.0\times10^3$ EU/g	Seasons, dog keeping
Cases et al. (2013)	Germany, Netherlands, Spain, Finland	1572 homes	β-(1→3) -D-glucan	$0.90\text{-}2.40\times10^3$ µg/g	Dampness, occupants
Gehring et al. (2001)	Germany	395 homes	β-(1→3) -D-glucan	1.71×10^3 µg/g	Flooring(carpeting), dog keeping, occupants
Barnig et al. (2012)	France	150 homes	Endotoxin	$3.75–5.00\times10^3$ ng/g	Farming
Moniruzzan et al. (2012)	Sweden	383 homes	Endotoxin	6.22×10^3 EU/g	Pet keeping, agricultural activities
Jacob et al. (2002)	Germany	272 homes	*Aspergillus* *Penicillium*	5000 CFU/g 15000CFU/g	Moisture, dampness
Jose E Greda et al. (2001)	Colorado, US	86 homes	Endotoxin	891 EU/ml	Animals at home, central air conditioning
This study	Turkey	239 homes	Endotoxin β-(1→3)-D-glucan *Aspergillus* *Penicillium*		Houseplant, Frequency of changing coverlets and bedsheets

3.4.6. Correlations of Floor Dust, Endotoxin, B-(1→3) -D-Glucan, *Aspergillus* and *Penicillium*

Pearson's correlation coefficients described the associations between the amount of floor dust, endotoxin, β-(1→3) -D-glucan, Aspergillus, and Penicillium concentrations. Table 3.22 shows the results of correlations for floor dust and biological markers. The concentration of endotoxin was moderately correlated with dust loads (r=0.59). Correlation between β-(1→3)-D-glucan concentrations and *Aspergillus* and *Penicillium* were moderate (r=0.67, r=0.69), respectively. A strong positive correlation was found between *Aspergillus* and *Penicillium* and was also significantly correlated (r=0.94, $P < 0.001$). Generally, no correlation was found between endotoxin and β-(1→3)-D-glucan concentrations. This study supports previous findings that the amount of dust is moderately correlated with endotoxin [173][174]. Nevertheless, we do not find any significant correlation between floor dust and β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* concentrations. Due to various populations and sizes of them and also methodological differences in dust sampling and analyzing the biological markers, comparisons between studies might be problematic.

Endotoxin, β-(1→3)-D-glucan, *Aspergillus*, and *Penicillium* had no significant correlation with either indoor relative humidity or indoor temperature.

Table 3. 22. Correlation among floor dust, endotoxin, β-(1→3)-D-glucan, *Aspergillus* and *Penicillium*

	Endotoxin	**β-(1→3) -D-glucan**	***Aspergillus***	***Penicillium***
Floor Dust	**0.5934****	0.0898	0.0624	0.0350
Endotoxin		0.0528	0.0890	0.0834
β-(1→3) -D-glucan			**0.6721****	**0.6943****
Aspergillus				**0.9410****

Bold indicates significance ** P <0.001

3.5. DIFFERENT PARAMETERS INCLUDING ENVIRONMENTAL POLLUTANTS AS A RISK FACTOR FOR THE PRESENCE OF ASTHMA

Figure 3.41 shows concentrations of biological markers by asthma vs. control groups, uncontrolled vs-controlled asthma, and mild asthma vs. moderate/severe asthma. Figures 3.41 (A) and 3.41(C) show results for endotoxin and *Aspergillus*, where geometric mean (95% CI) endotoxin and *Aspergillus* concentrations were significantly higher in the control group compared to the asthma group. There is no difference between uncontrolled vs-controlled asthma and asthma severity for endotoxin and *Aspergillus* concentrations. β-(1→3)-D-glucan and *Penicillium* levels are presented in Figures 3.41(B) and 3.41 (D), respectively. For all comparisons by asthma vs. control groups, uncontrolled vs. controlled asthma and mild asthma vs. moderate/severe asthma were not significantly different between groups ($P > 0.05$).

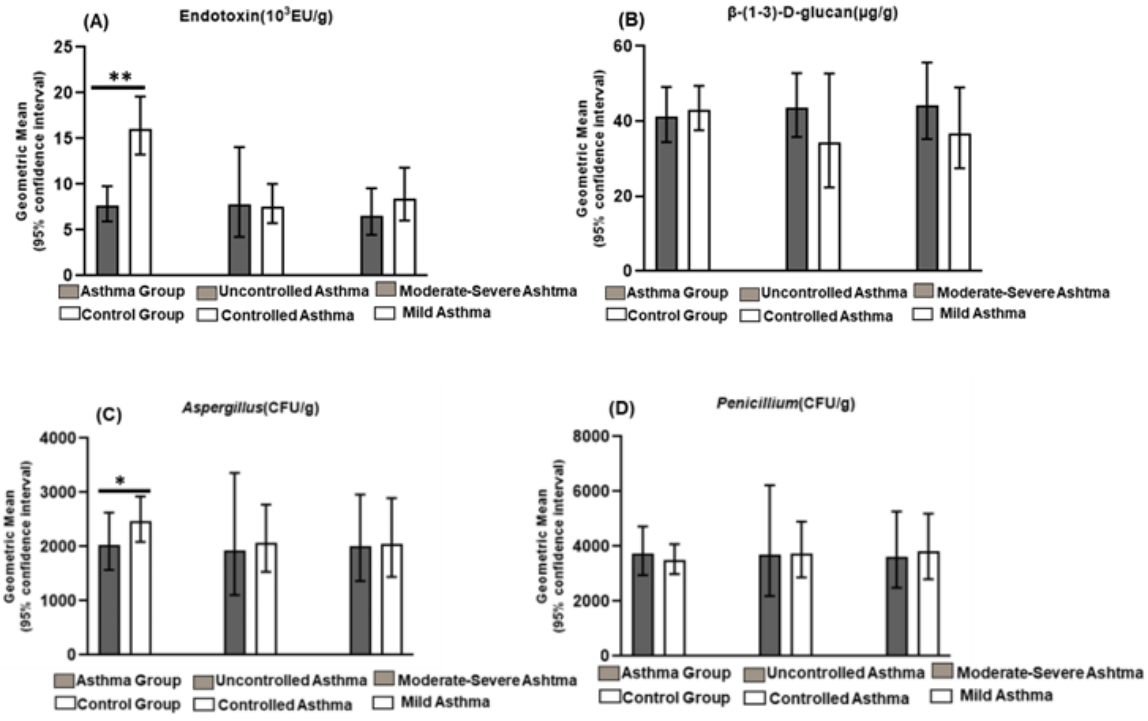


Figure 3. 41. Geometric mean concentrations for A: endotoxin, B: β-(1→3)-D-glucan, C: *Aspergillus* and D: *Penicillium*, respectively. The histograms present geometric means, and the error bars present 95% confidence intervals. * $P<.05$, ** $P<.01$.

Figure 3.42 shows the associations of microbial markers and home characteristics with asthma. Surface dust loading is inversely associated with asthma in univariate logistic regression (OR = 0.47, 95% CI: 0.253–0.873, *P*=0.017). One of the reasons we found low dust loading in asthmatic children's homes is doctors' advice to parents of asthmatic children to keep their children away from house dust. Moreover, the endotoxin level correlated with floor dust is inversely associated with asthma. β-(1→3)-D-glucan presented a positive association with asthma (OR = 1.384, 95% CI: 1.070–2.488, *P*=0.098). Univariate logistic regression analyzes presented that high levels of *Aspergillus* were inversely associated with asthma (OR = 0.612, 95% CI: 0.336–0.957, *P*=0.10). Due to the doctor's recommendation, parents of asthmatic children take care of their homes and avoid the factors that can exacerbate asthma. As a result, dampness, water moisture, and visible mold were not common in the homes of asthmatic children. According to univariate logistic regression analysis, having a separate kitchen at home is a protective factor for asthma (OR = 0.19, 95% CI: 0.052–0.695). Having a separate kitchen reduces exposure to gases and pollutants released during cooking. Frying once a week or more than once a week can be a risk factor for asthma (OR = 2.214, 95% CI: 1.309–3.744, *P*=0.012). Smoke exposure at home, even at the home balcony, is positively associated with asthma in children (OR = 1.559, 95% CI: 1.134–2.603, *P*=0.089). Living on the first floor or bottom floors (house floor (≤1) in an apartment is positively associated with asthma (OR = 3.042, 95% CI: 1.071–3.144, *P* <0.0001). Dwelling age (≥ 20) is another factor that is positively associated with asthma as a result of univariate logistic regression analysis (OR = 1.835, 95% CI: 1.134–2.603, *P*=0.027). Univariate logistic regression analysis presented that cleaning frequency at home (one or less week) is a risk factor for presence of asthma (OR = 1.701, 95% CI: 1.185–2.940, *P*=0.057). Damp smell is inversely associated with asthma (OR = 0.612, 95% CI: 0.336–0.987, *P*=0.10). Surprisingly, we found an inverse association between pet ownership and asthma. Because parents of asthmatic children prefer not to keep pets at home to avoid asthma triggers. Occupants (> 4) and repair and paint at home within the last year are inversely associated with asthma. Univariate logistic regression analysis shows that having new furniture at home is a risk factor for presence of asthma (OR = 2.019, 95% CI: 1.144–3.562, *P*=0.015). Using a wool mattress for children is inversely associated with asthma (OR = 0.374, 95% CI: 0.178–0.788, *P*=0.010). Because parents of asthmatic children prefer not to use wool mattress according to children’s doctor recommendation.

Univariate logistic regression analyses presented high endotoxin concentrations inversely associated with asthma (OR = 0.336, 95% CI: 0.177–0.640, *P*=0.001) (Figure 3.42). In the same way, multiple logistic regression analysis presented statistically significant inverse associations with asthma in the high endotoxin levels (OR = 0.324, 95% CI: 0.155–0.677, *P*=0.003). On the other hand, high β-(1→3)-D-glucan level tended to be positively associated with asthma in univariate logistic regression analysis . Also, in logistic multivariate modeling high concentration of β-(1→3)-D-glucan is positively associated with asthma (OR = 3.162, 95% CI: 1.101–9.028, *P*=0.032).

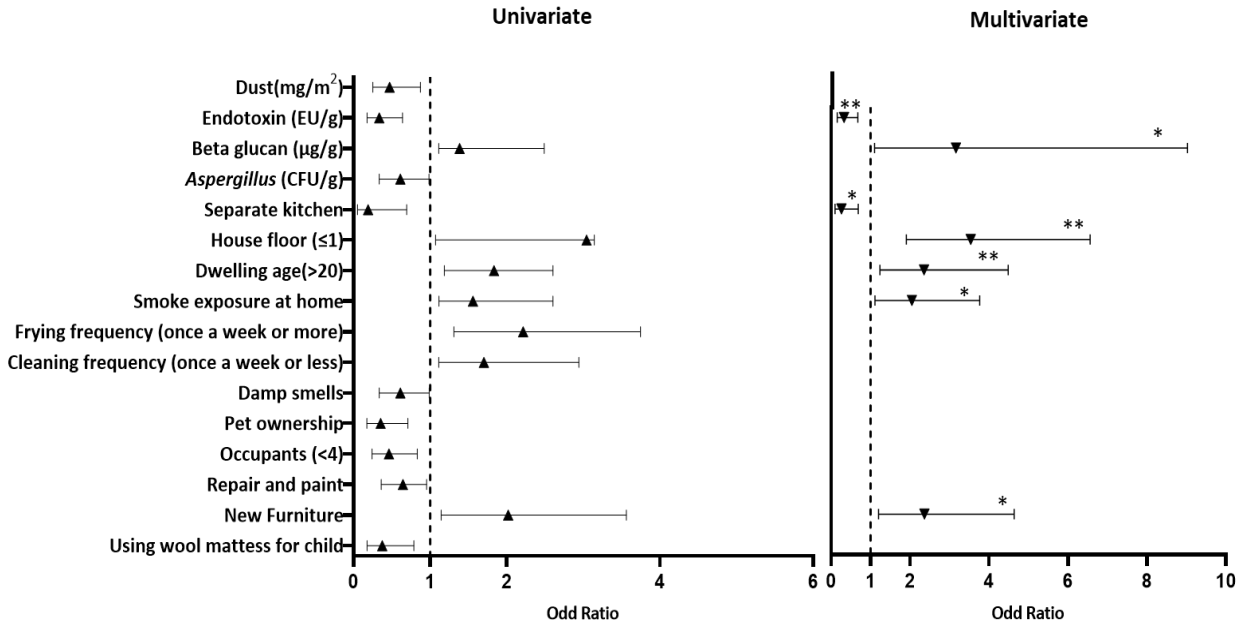


Figure 3. 42. Multivariate logistic regression model for asthma risk. * *P*<.05, ** *P*<.01.

Multivariate logistic modeling showed that house floor (≤1) (OR = 3.535, 95% CI: 1.904–6.563, *P*<0.0001), dwelling age (≥20) (OR = 2.354, 95% CI: 1.235–4.487, *P*=0.009), having new furniture at home (OR = 2.361, 95% CI: 1.202-4.634, *P*=0.013) and smoke exposure at home (OR = 2.044, 95% CI: 1.110–3.761, *P*=0.022) were significantly associated with asthma. Separate kitchen at home had protective effect on asthma (OR = 0.158, 95% CI: 0.036–0.684, *P*=0.014).

Table 3.23 presented the univariate and multivariate modeling for asthma severity. In univariate logistic analysis, the amount of dust is inversely associated with asthma severity (OR = 0.335,

95% CI: 0.098–0.911, *P*=0.069). In univariate logistic regression, high endotoxin level was inversely associated with asthma severity (OR = 0.325, 95% CI: 0.086–0.923, *P*=0.098). Residential area (≤100) is a risk factor for asthma severity according to univariate analysis (OR = 2.114, 95% CI: 1.113–4.975, *P*=0.086). Univariate logistic regression analysis presented that synthetic rugs were positively associated with asthma severity (OR = 2.932, 95% CI: 1.693–4.492, *P*=0.088). Frying more than once a week can be a risk factor for asthma severity (OR = 2.03, 95% CI: 1.126–4.450, *P*=0.077). Repair and painting within the last year were inversely associated with asthma severity (OR = 0.384, 95% CI: 0.139–0.861, *P*=0.065). Visible mold at home is a risk factor for asthma severity (OR = 2.326, 95% CI: 1.065–5.780, *P*=0.069). In multivariate logistic modeling, no associations were found between any microbial exposures, covariates, and asthma severity. However, visible mold approach to significance (OR = 2.888, 95% CI: 1.289–8.438, *P*=0.053).

Table 3. 23. Logistic regression for asthma severity

Parameters	**Univariate**			**Multivariate**		
	OR	**95% Cl**	***P* VALUE**	**OR**	**95% Cl**	***P* VALUE**
Dust (mg/m²)	**0.335**	**0.098-0.911**	**0.069**	-	-	-
Endotoxin (EU/g)	**0.325**	**0.086-0.923**	**0.098**	-	-	-
Residential area (≤100)	**2.114**	**1.113-4.975**	**0.086**	-	-	-
Type of rug (synthetic)	**2.932**	**1.693- 4.492**	**0.088**	-	-	-
Frying frequency (more than once /week)	**2.030**	**1.126-4.450**	**0.077**	-	-	-
Repair and paint	**0.384**	**0.139-0.861**	**0.065**	-	-	-
Visible mold	**2.326**	**1.065-5.780**	**0.069**	2.888	1.289-8.438	0.053

Figure 3.43 presents risk factors for uncontrolled asthma. Univariate logistic regression analysis showed that uncontrolled asthma is more common among boys (OR = 2.518, 95% CI: 1.112–6.396, *P*=0.050). Having house dust allergy among asthmatic children is a risk factor for uncontrolled asthma (OR = 2.468, 95% CI: 1.087–7.356, *P*=0.098). Residential area (≤100) is a risk factor for uncontrolled asthma according to univariate analysis (OR = 2.404, 95% CI: 1.185–6.154, *P*=0.067). Living on the first floor or bottom floors (house floor ≤1) in an apartment is positively associated with uncontrolled asthma (OR = 3.325, 95% CI: 1.320–8.378, *P*=0.011). Dwelling age (≥ 20) is another factor that is positively associated with uncontrolled

asthma as a result of univariate logistic regression analysis (OR = 2.648, 95% CI: 1.061–6.610, *P*=0.037).

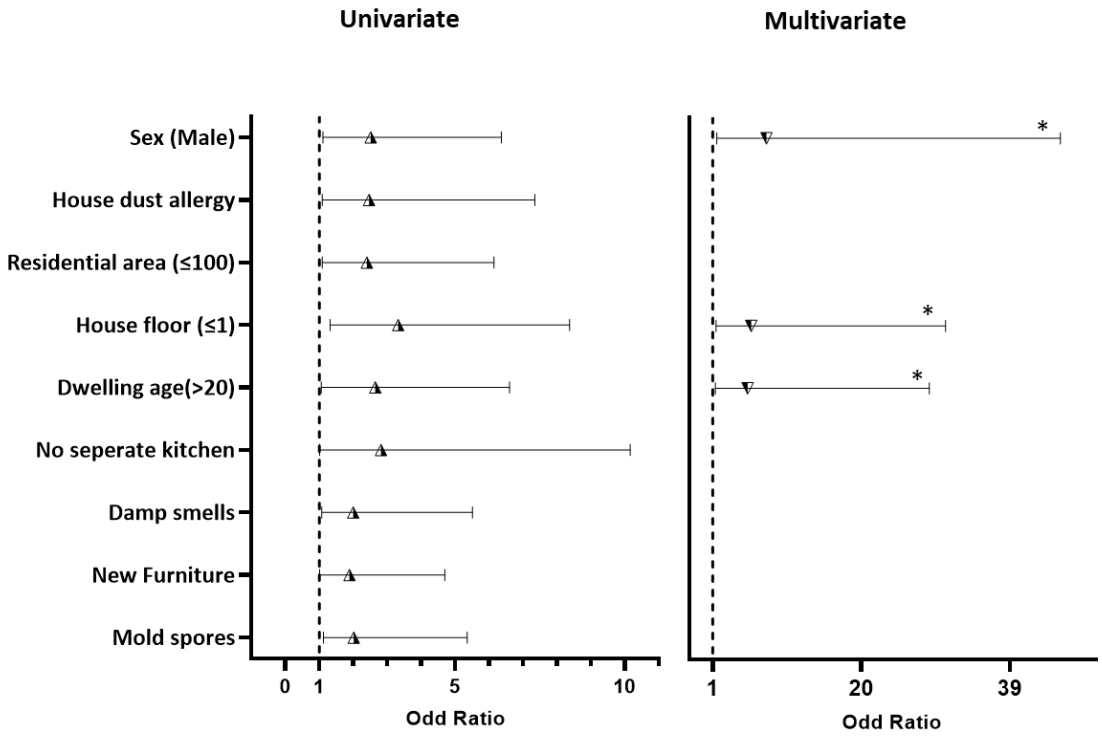


Figure 3. 43. Risk factors for uncontrolled asthma. * *P*<0.05.

Living in a home without a separate kitchen is a risk factor for uncontrolled asthma (OR = 2.817, 95% CI: 1.014–10.162, *P*=0.098). Dampness and damp smell at home were positively associated with uncontrolled asthma (OR = 2, 95% CI: 1.075–5.515, *P*=0.094). Univariate logistic regression analysis shows that having new furniture at home is a risk factor for uncontrolled asthma (OR = 1.893, 95% CI: 1.012–4.7, *P*=0.095). Visible mold at home is a risk factor for uncontrolled asthma (OR = 2.018, 95% CI: 1.125–5.365, *P*=0.096). Multivariate logistic regression analysis showed a significant positive association with uncontrolled asthma in dwelling age (≤20), (OR = 5.478, 95% CI: 1.046–28.70, *P*=0.044). Furthermore, the house floor (≤1) is one of the risk factors for uncontrolled asthma (OR = 5.917, 95% CI: 1.138–30.774, *P*=0.035). In the same way, multiple logistic regression analysis presented statistically significant positive associations with uncontrolled asthma in asthmatic boys (OR = 7.874, 95% CI: 1.322–45.454, *P*=0.023). Previous studies have shown that in childhood, males have a more

significant frequency of asthma than girls, but this trend changes in adolescence, with adult women having a higher prevalence of asthma than men [200].

This study represented different associations between biological marker levels, home characteristics, the lifestyle of families, and asthma risk as a case-control study. The present study demonstrates that high endotoxin levels were inversely associated with asthma while high β-(1→3) -D-glucan concentrations were positively associated with asthma in school-age children. No significant associations were found for high concentrations of *Aspergillus* and *Penicillium* with asthma. Furthermore, our results indicate that high endotoxin concentrations are inversely associated with asthma severity. None of the biological markers measured in this study are risk factors for uncontrolled asthma.

Some studies in the literature have shown the association of various biological markers with respiratory diseases. The results of these studies are conflicted. In a study conducted in Germany, Austria, and Switzerland, researchers discovered an inverse relationship between endotoxin load and atopic asthma in school-age children (6 to 13 years) [170]. The endotoxin level from living room dust was a risk factor for asthma in a case-control study including 4-17-year-olds in England [165]. Case-control research including 6–12-year-olds in Saskatchewan found no association between play area floor endotoxin or mattress endotoxin with asthma, but an association was found between endotoxin and school absenteeism for children with allergies [201]. Another study conducted in Saskatchewan, Canada, which included schoolchildren (7-17 years), demonstrated that exposure to high endotoxin levels is a risk factor for asthma severity, but β -(1→3)-D-glucan had the opposite effect. Moreover, endotoxin concentrations had an association with lower lung function, but no such association was seen for β-(1→3)-D-glucan concentrations [163]. High β-(1→3)-D-glucan levels were positively associated with asthma severity among 6-14-year-olds children in a study conducted in Puerto Rico [202]. In a study conducted in the Netherlands, high BDG levels in the play area of children (aged 7-11 years) were positively associated with increasing PEF (peak expiratory flow) variability [29]. Another study in the United States reported an inverse association between high β-(1→3)-D-glucan level and frequency of recurrent wheeze in newborns [203]. Five countries (Albania, Italy, New Zealand, Sweden, and the United Kingdom) and 840 children (9-12 years) contributed to a study to determine the association between endotoxin and asthma. They

reported an inverse association between endotoxin levels in house dust and asthma in children [160]. A case-control study conducted in Germany reported that a high level of *Aspergillus* (> 90th percentile) is a risk factor for allergic sensitization in children. In the same study, no association was found between *Penicillium* and allergic sensitization [168]. Another case-control study represented exposure to higher levels of *Aspergillus* and *Penicillium spp*. from extracellular polysaccharides (EPS) from mattress dust was associated with a decreased frequency of allergic sensitization in children (2-4 years) [162]. Some studies have demonstrated an inverse association between *Aspergillus* and *Penicillium* from EPS with asthma and wheezing in children [169][204][205]. However, the factors causing these various effects remain unknown. Different methods of assessing mold exposure could explain the contradictory results. In a study conducted in Germany (358 children) and the Netherlands (338 children), a negative association was found between endotoxin and *Aspergillus* and *Penicillium* EPS with asthma in children (6 years old). No association was reported between exposure to biological markers and asthma for the Dutch people [159]. Rylander et al. reported high β-(1→3)-D-glucan levels in the school were positively associated with respiratory disorders in school-aged children [191]. Furthermore, elevated β-(1→3)-D-glucan loading has been related to increased peak expiratory flow variability in asthmatic children [29], as well as persistent atopic asthma and new-onset bronchial hyper-responsiveness [171]. Additionally, endotoxin has been suggested to increase the incidence of wheeze in younger children while acting as a protective agent for asthma in older children[206][207]. Endotoxin also helps to prevent atopic sensitization, hay fever, and atopic asthma[85][208][160][189].

Moreover, this study revealed that elevated endotoxin levels in children's homes were inversely associated with the risk of asthma in Turkish children. Previous investigations have found a decreased prevalence of allergic sensitization and doctor-diagnosed asthma in children who were exposed to elevated amounts of endotoxin at home [162][205], supporting the "hygiene hypothesis" [209][210]. It was hypothesized that biological markers like endotoxin could develop children's immune systems in early life and play a key role in the development of tolerance to allergens found in the environment [199][189]. To support the "hygiene hypothesis", which assumes that household size and siblings have an inverse effect on hay fever risk, there have been a significant number of epidemiological studies in the past investigating the impact of living on a farm and the risk of allergic diseases [211][209][210]. Endotoxin is

thought to have robust immune-stimulatory characteristics. In neonates and infants, it may thus have the capacity to increase the Th1-dominated immune response while suppressing the Th2-dominated allergic response [29][212]. Hay fever and allergy sensitization in childhood have not been seen frequently among children who are born and grow up on a farm, and some previous research suggests that these protective effects last into adulthood [211][213]. Endotoxin exposure in nonfarming indoors was also found to protect children from respiratory and atopic illnesses. Exposure to a high endotoxin concentration during the first years of life is attributed to a lower prevalence of asthma and allergic sensitization in children [205][214][162][189]. Exposure to the high level of endotoxin was found to be negatively related to asthma and allergy sensitization at school age in a recent study of a US birth cohort [214]. Additionally, a German investigation observed that exposure to the bacterial endotoxin at home reduced the risk of asthma in children [159].

Early-life exposure to mold components had a distinct effect on allergy health outcomes than later-life exposure [215]. T-helper (Th)2-cells dominate the neonate's immune response, and a transition to a Th1-mediated immune response occurs during early childhood. It is thought that exposure to mold components such as *Aspergillus* and *Penicillium* EPS has a similar effect on the immune system development of newborns in early life as endotoxin exposure[158][216]. A positive association was found between high levels of β-(1→3)-D-glucan and asthma in childhood. However, it is better to take into account that β-(1→3)-D-glucan is not only derived from mold; it has another source like pollen or plants [159]. Maybe it could be an explanation for our finding. It is not easy to attribute an apparent reason for the reported health impacts because the indoor environment includes several indoor and outdoor sources, not only the ones measured. For instance, a birth cohort study demonstrated that an indicator of the overall quantity of ambient microbial exposure could identify respiratory disorders better than single biological agents [217].

The results of univariate analysis showed that a high amount of dust is associated with house dust allergy (OR = 3.364, 95% CI: 1.047–10.803) (*P* value= 0.042), atopic dermatitis (OR = 3.524, 95% CI: 1.154–10.766) (*P* value= 0.027) and pet dander allergy (OR = 7.810, 95% CI: 1.576–38.706) (*P* value= 0.012). Household dust contains many typical allergens such as pollen spore, pet hair and dander, mold, dust mites, Etc. When we keep in touch with these allergens

or inhale them, we may experience an allergic reaction [218]. The Univariate logistic regression analysis showed that high endotoxin concentrations were positively associated with house dust allergy in asthmatic children (OR = 3.565, 95% CI: 1.020–12.456) (*P* value= 0.046). In addition, univariate logistic regression analysis shows that high endotoxin levels are a risk factor for atopic dermatitis (OR = 4.018, 95% CI: 1.207–13.373) (*P* value= 0.023). High β -(1→3)-D-glucan level is a risk factor for asthma exacerbation (OR = 2.563, 95% CI: 1.076–6.106) (*P* value= 0.034). Asthmatic children who had high β -(1→3)-D-glucan levels detected in their homes had more than twice as many asthma attacks within the last year. A study conducted in Amsterdam reported that peak expiratory flow (PEF) variability was found to be highly related with β -(1→3)-D-glucan levels in living room house dust in children with respiratory disorders [29]. Additionally, the proinflammatory effects of BDG have been demonstrated in animal and in vitro investigations, including activation of neutrophils, macrophages, complement, and maybe eosinophils [219]. The Univariate logistic regression analysis demonstrated that high *Aspergillus* concentrations were associated with fungal allergy (OR = 6.667, 95% CI: 1.037–42.860) (*P* value= 0.046). Fungus allergies are common in developed countries (almost 2-6% of the population). *Aspergillus* is among the common genera that cause allergies [220].

The present study represented that house floor (≤1), dwelling age (>20), smoke exposure at home, and having new furniture at home are risk factors for asthma. Further, homes with separate kitchens have a protective effect on asthma. Some previous studies investigated the home environment in the development of asthma [147]. Still, less is known about the effect of the home condition and lifestyle of families on asthma morbidity and exacerbations. One of the strong points of this study is including several determinants about the home characteristics and lifestyles of families to determine triggers of asthma in the indoor environment.

Exposure to environmental tobacco smoke (ETS) increased asthma prevalence and lung cancer because it is a complex compound with more than 4000 chemicals [130]. Eisner et al. reported that elevated levels of secondhand smoke exposure were linked to increasing asthma severity [221]. The association of ETS with asthma prevalence has been studied in several publications, and the data shows that people who were exposed to ETS are more likely to develop asthma [135][222][223]. These studies are in agreement with findings in this thesis for exposure to indoor smoking (OR = 2.044, 95% CI: 1.110–3.761). A study conducted in Denmark reported

that exposure to ETS for more than five hours per day is a risk factor for wheeze, chronic cough, and decreased FEV_1[135]. In a study conducted in India, an asthma attack risk was higher in persons who were exposed to environmental tobacco smoke [133].

The present study found that having a separate kitchen at home had a protective effect on asthma (OR = 0.158, 95% CI: 0.036–0.684). Because carbon monoxide, formaldehyde, and other hazardous chemicals released by natural gas and during cooking in the kitchen can be toxic to human health. Furthermore, using a wood stove or fireplace can cause a lot of indoor air pollution because of the wood smoke. Hazardous air pollutants can generate during cooking by food ingredients, heating oil, and fat, particularly at high temperatures. Exposure to pollutants can induce or exacerbate a variety of diseases, especially in people with asthma, heart, or lung illness. Some previous studies carried out the relation between respiratory symptoms and exposure to cooking smoke [224] [225] [226]. A study in India reported that exposure to cooking smoke is a risk factor for asthma (OR, 1.59; 95% CI, 1.30-1.94) [225]. Having a separate kitchen and reducing the frying frequency, especially at a high temperature, can reduce the asthma risk. Also, ventilation is the best way to improve indoor air quality during cooking.

This study found that living on the first floor or lower floors (house floor (≤1) in an apartment is positively associated with asthma (OR = 3.042, 95% CI: 1.071–3.144). Further, the house floor (≤1) is one of the risk factors for uncontrolled asthma (OR = 5.917, 95% CI: 1.138–30.774). Our findings reveal that living upstairs is better for reducing respiratory disorders because air quality is better upstairs in typical apartments. In agreement with our study, according to the WSP Parsons Brinckerhoff report (2017) [227], the findings demonstrate that, in typical buildings, especially near the roadside, up to the fourth floor, air quality improves with height. Beyond this, any more decrease is minimal. In other circumstances, commonly in background areas, air pollution levels remained broadly stable as height increased. This study was conducted in 26 various areas across London and Cardiff [227].

Age of the house is one of the factors associated with asthma. Additionally, we found that dwelling age (≥20) is a risk factor for uncontrolled asthma. Even though both new and old houses are prone to indoor pollution, some contaminants are more prevalent in older homes. Some of these pollutants are lead, asbestos, mold, radon, phthalates, and formaldehyde [228]. In addition, many older houses were constructed before central air conditioning systems became

widely available. Therefore, older homes are a risk factor for their occupants because of building materials and poor indoor air quality [228].

Having new furnishings within the last year is one of the risk factors for asthma in this study (OR = 2.361, 95% CI: 1.202-4.634). In a study conducted in China, 5922 children (1-8 years old) participated. This study investigates the associations between asthma and allergies in children with new home furnishings [229]. This study reported that having new decorations and furnishings at home one year before pregnancy was positively associated with asthma and allergies in children. Furthermore, homes with new decorations were positively associated with wheezing and rhinitis, and also, having new furnishings during pregnancy was a risk factor for eczema [229]. The majority of modern home decorations are comprised of synthetic board and resin, which can generate high levels of formaldehyde, benzene, and other volatile organic compounds (VOCs) [230]. Paints and glues, as well as adhesives, release a lot of VOCs and formaldehyde [231]. Childhood wheeze, asthma, and bronchitis were all related to formaldehyde exposure [232]. Exposure to VOCs and benzene indoors is a risk factor for asthma in children [233].

The key strength of this thesis is that it is one of the first in Turkey to determine endotoxin, β -(1→3)-D-glucan, *Aspergillus*, and *Penicillium spp*. concentrations and determinants in Turkish children's homes. The study population and sample size were good in this study, and a varied set of determinants such as home characteristics and the lifestyles of families were included. However, the limitations of the thesis should be considered. One limitation of this study is that one dust sample was collected from each home at a specific time. It is thought that one house-dust sample that was collected at a single time point may not indicate total exposure of biological markers because the amount of them might change over time. Nevertheless, some studies in the literature have shown that a single dust sample for biological marker measurements, especially endotoxin, shows little change over time [205][234][235]. Therefore, we can say that the present study represents at least one year of exposure to microbial components. Furthermore, as the number of atopic children in the asthmatic group is small, we cannot compare exposure to biological markers between atopic and non-atopic asthma. However, as the present study was a case-control study (asthmatic vs. non-asthmatic), it was not the study's primary aim. Table 3.24 shows some critical results of the literature studies as compared to this study.

Table 3. 24. Some significant results of the literature studies as compared to this study

References	Country	Population	Agents	Determinants
Tavernier et al. (2005)	England	90 children	Endotoxin	Endotoxin is a risk factor for asthma
Tavernier et al. (2005)	England	90 children	Fungal genus	No association was found between the fungal genus and asthma.
Lawson et al. (2012)	Canada	310 children	Endotoxin	Endotoxin has a protective effect on asthma
Gehring et al. (2008)	Italy, Albania, New Zealand, Sweden, UK	840 children	Endotoxin	Endotoxin and asthma were inversely associated
Braun et al. (2001)	Germany, Belgium, Switzerland	812 children	Endotoxin	Endotoxin and asthma were inversely associated
Tischer et al. (2011)	Germany, Netherlands	690 children	Fungal genus	Fungal genus and asthma were inversely associated
Oluwole et al. (2018)	Canada	116 homes	Endotoxin	Endotoxin and asthma are associated
Oluwole et al. (2018)	Canada	116 homes	β-(1→3) -D-glucan	High Beta Glucan level is inversely associated with asthma
Jacob et al. (2002)	Germany	272 homes	*Aspergillus, Penicillium*	High *Aspergillus* level is a risk factor for respiratory diseases
Rylander et al. (1998)	Sweden	347 children	β-(1→3) -D-glucan	High Beta Glucan level is a risk factor for asthma
Maheswaran et al. (2014)	Canada	422 children	β-(1→3) -D-glucan	High Beta Glucan level is a risk factor for persistent atopic asthma and BHR
This study	Turkey	239 children	Endotoxin, β-(1→3) -D-glucan, *Aspergillus*, *Penicillium*	High endotoxin level is an inverse risk factor for asthma; high Beta Glucan level is a risk factor for asthma, no association was found between fungal genus and asthma.

4. CONCLUSION

Exposure to indoor microbial markers in homes is thought to be associated (inverse, positive, as well as no association) with asthma and respiratory diseases. In this study, the associations of endotoxin, β-(1→3)-D-glucan, Aspergillus, and Penicillium spp. were investigated with asthma in school-aged children. Furthermore, potential determinants included home environments and behavioral factors. A total of 239 settled dust samples were analyzed for biological markers, including 109 children for the asthma group and 130 children for the control group. Floor dust loadings in the control group (644.5 mg/m^2) were significantly more than in the asthma group (464 mg/m^2). The median endotoxin concentration was lower in the asthma group (8.23×10^3 EU/g), compared to 15.1×10^3 EU/g in the control group. There were no statistically significant differences in median β-(1→3) -D-glucan and Penicillium spp. levels between asthma and control groups. The median concentration of Aspergillus (2600 CFU/g) was higher in the control group compared to the asthma group (1350 CFU/g).

In summary, according to multivariate logistic regression analyzes high amounts of floor dust were associated with the level of house floor (≤1) (OR = 2.17, 95% CI: 1.17–4), having old furniture at home (OR = 2.04, 95% CI: 1.1–3.84) and drying clothes at home (indoors) (OR = 4.7, 95% CI: 1.6–13.4) and house balcony (outdoors) (OR = 3.7, 95% CI: 1.2–11.27) compared to a dryer machine. Multivariate logistic modeling indicated that high endotoxin concentration was associated with the level of house floor (≤1) (OR = 2.31, 95% CI: 1.25–4.29), having houseplants at home (OR = 3.71, 95% CI: 1.4–10), drying clothes at home (indoors) (OR = 5.16, 95% CI: 1.52–17.4) and frequency of changing coverlets and bedsheets (once / 2 weeks) (OR = 2.26, 95% CI: 1.19–4.29) compared to changing coverlets and bedsheets once per week. Higher β-(1→3) -D-glucan concentration was associated with having houseplants at home (OR = 4.23, 95% CI: 1.51–11.9) and the frequency of changing coverlets and bedsheets (once / 2 weeks) (OR = 2.17, 95% CI: 1–19.4) compared to changing coverlets and bedsheets once per week in multivariate logistic modeling. According to the results of multivariate logistic regression analyzes the higher level of *Aspergillus* was associated with visible mold spots (OR = 3.02, 95% CI: 1.42–6.44), having houseplants at home (OR = 1.77, 95% CI: 1.093–3.38), synthetic rug (OR = 6.64, 95% CI: 1.35–15.9) compared to wool rug, and the frequency of changing coverlets and bed sheets (once / 2 weeks) (OR = 1.87, 95% CI: 1.04–3.54) compared to changing coverlets and bedsheets once per week. Additionally, multivariate logistic regression analyzes indicated

that a high level of *Penicillium* was associated with the residential area (≤100 m^2) (OR = 1.95, 95% CI: 1.01–3.78), visible mold spots (OR = 3.02, 95% CI: 1.46–15.9), synthetic rug (OR = 5.99, 95% CI: 1.49–24.08) compared to wool rug, and frequency of changing coverlets and bedsheets (once / 2 weeks) (OR = 2.03, 95% CI: 1.08–3.81) compared to changing coverlets and bedsheets once per week. Among the potential determinants, having houseplants at home and the frequency of changing coverlets and bedsheets were the factors that were significantly associated with most of the biological markers. Having houseplants at home was the determinant that positively associated with the concentration of endotoxin, β-(1→3) -D-glucan, and *Aspergillus*. Furthermore, the frequency of changing coverlets and bedsheets (once / 2 weeks) presented a significant association with a higher risk of having high endotoxin, β-(1→3) -D-glucan, *Aspergillus*, and *Penicillium* concentrations.

In conclusion, the present study demonstrates that high endotoxin levels were inversely associated with asthma (OR = 0.324, 95% CI: 0.155–0.677), while high β-(1→3) -D-glucan concentrations were positively associated with asthma in school-age children (OR = 3.162, 95% CI: 1.101–9.028). No significant associations were found between high concentrations of *Aspergillus* and *Penicillium* with asthma. Furthermore, our results indicate that high endotoxin concentrations are inversely associated with asthma severity. None of the biological markers measured in this study are risk factors for uncontrolled asthma. As a result of multivariate logistic regression analysis, the present study demonstrated that house floor (≤1) (OR = 3.535, 95% CI: 1.904–6.563), dwelling age (>20) (OR = 2.354, 95% CI: 1.235–4.487), smoke exposure at home (OR = 2.044, 95% CI: 1.110–3.761), and having new furniture at home are risk factors for asthma (OR = 2.361, 95% CI: 1.202-4.634). Further, homes with separate kitchens have a protective effect against asthma (OR = 0.158, 95% CI: 0.036–0.684).

As results of this study, the amount of dust is inversely associated with asthma severity (OR = 0.335, 95% CI: 0.098–0.911). In univariate logistic regression, high endotoxin level was inversely associated with asthma severity (OR = 0.325, 95% CI: 0.086–0.923). Residential area (≤100) is a risk factor for asthma severity according to univariate analysis (OR = 2.114, 95% CI: 1.113–4.975). Furthermore, univariate logistic regression analysis presented that synthetic rugs were positively associated with asthma severity (OR = 2.932, 95% CI: 1.693–4.492). Frying more than once a week can be a risk factor for asthma severity (OR = 2.03, 95% CI:

1.126–4.450). Repair and painting within the last year were inversely associated with asthma severity (OR = 0.384, 95% CI: 0.139–0.861). Visible mold at home is a risk factor for asthma severity (OR = 2.326, 95% CI: 1.065–5.780). In multivariate logistic modeling, no associations were found between any microbial exposures, covariates, and asthma severity. However, visible mold approach to significance (OR = 2.888, 95% CI: 1.289–8.438, *P*=0.053).

Another finding of this study is about risk factors for uncontrolled asthma. Univariate logistic regression analysis showed that uncontrolled asthma is more common among boys (OR = 2.518, 95% CI: 1.112–6.396). Having house dust allergy among asthmatic children is a risk factor for uncontrolled asthma (OR = 2.468, 95% CI: 1.087–7.356). Residential area (≤100) is a risk factor for uncontrolled asthma according to univariate analysis (OR = 2.404, 95% CI: 1.185–6.154). Living on the first floor or bottom floors (house floor ≤1) in an apartment is positively associated with uncontrolled asthma (OR = 3.325, 95% CI: 1.320–8.378). Dwelling age (≥ 20) is another factor that is positively associated with uncontrolled asthma as a result of univariate logistic regression analysis (OR = 2.648, 95% CI: 1.061–6.610). Living in a home without a separate kitchen is a risk factor for uncontrolled asthma (OR = 2.817, 95% CI: 1.014–10.162). Dampness and damp smell at home were positively associated with uncontrolled asthma (OR = 2, 95% CI: 1.075–5.515). Univariate logistic regression analysis shows that having new furniture at home is a risk factor for uncontrolled asthma (OR = 1.893, 95% CI: 1.012–4.7). Visible mold at home is a risk factor for uncontrolled asthma (OR = 2.018, 95% CI: 1.125–5.365). Multivariate logistic regression analysis showed a significant positive association with uncontrolled asthma in dwelling age (≤20), (OR = 5.478, 95% CI: 1.046–28.70). Furthermore, the house floor (≤1) is one of the risk factors for uncontrolled asthma (OR = 5.917, 95% CI: 1.138–30.774). In the same way, multiple logistic regression analysis presented statistically significant positive associations with uncontrolled asthma in asthmatic boys (OR = 7.874, 95% CI: 1.322–45.454).

The results of univariate analysis showed that a high amount of dust is associated with house dust allergy (OR = 3.364, 95% CI: 1.047–10.803) (*P* value= 0.042), atopic dermatitis (OR = 3.524, 95% CI: 1.154–10.766) (*P* value= 0.027) and pet dander allergy (OR = 7.810, 95% CI: 1.576–38.706) (*P* value= 0.012). The Univariate logistic regression analysis showed that high endotoxin concentrations were positively associated with house dust allergy in asthmatic children (OR = 3.565, 95% CI: 1.020–12.456) (*P* value= 0.046). In addition, univariate logistic

regression analysis shows that high endotoxin levels are a risk factor for atopic dermatitis (OR = 4.018, 95% CI: 1.207–13.373) (*P* value= 0.023). High β -(1→3)-D-glucan level is a risk factor for asthma exacerbation (OR = 2.563, 95% CI: 1.076–6.106) (*P* value= 0.034). Asthmatic children who had high β -(1→3)-D-glucan levels detected in their homes had more than twice as many asthma attacks within the last year. According to the univariate logistic regression analysis, high *Aspergillus* concentrations were associated with fungal allergy (OR = 6.667, 95% CI: 1.037–42.860) (*P* value= 0.046).

Some recommendations for future studies in this field can be summarized in the following way:

- Select a larger study population,
- Repeat dust sampling during the year to find out total exposure,
- Consider atopic vs. non-atopic and allergic conditions of asthma and then determine the association between biological markers and asthma.

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