PUBLICATIONS OF
THE UNIVERSITY OF EASTERN FINLAND

Dissertations in Forestry and Natural Sciences

HANNA LEPPÄNEN

ASSESSING MICROBIAL EXPOSURE IN INDOOR ENVIRONMENTS BY USING HOUSE DUST SAMPLES

HANNA LEPPÄNEN

Assessing microbial exposure in indoor environments by using house dust samples

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences
No 276

Academic Dissertation
To be presented by permission of the Faculty of Science and Forestry for public examination in the Auditorium SN201 in Snellmania Building at the University of Eastern Finland, Kuopio, on August, 25, 2017, at 12 o'clock noon.
Department of Environmental and Biological Sciences

Grano oy
Jyväskylä, 2017
Editor: Research Dir. Pertti Pasanen

Distribution:
University of Eastern Finland Library / Sales of publications
P.O.Box 107, FI-80101 Joensuu, Finland
tel. +358-50-3058396
www.uef.fi/kirjasto

ISBN: 978-952-61-2564-0 (printed.)
ISSNL: 1798-5668
ISSN: 1798-5668, Publications of the University of Eastern Finland

ISBN: 978-952-61-2565-7 (online, pdf)
ISSN: 1798-5676, Publications of the University of Eastern Finland

Author's address: National Institute for Health and Welfare
Department of Health Security
P.O.Box 95
70701 KUOPIO
FINLAND
email: hanna.leppanen@thl.fi

Supervisors: Research Professor Anne Hyvärinen, Ph.D.
National Institute for Health and Welfare
Department of Health Security
P.O.Box 95
70701 KUOPIO
FINLAND
email: anne.hyvarinen@thl.fi

Research Director Pertti Pasanen, Ph.D.
University of Eastern Finland
Department of Environmental and Biological Sciences
P.O.Box 1627
70211 KUOPIO
FINLAND
email: pertti.pasanen@uef.fi

Research Professor Emerita Aino Nevalainen, Ph.D.
National Institute for Health and Welfare
Department of Health Security
P.O.Box 95
70701 KUOPIO
FINLAND
email: aino.nevalainen@thl.fi

Senior researcher Martin Täubel, Ph.D.
National Institute for Health and Welfare
Department of Health Security
P.O.Box 95
70701 KUOPIO
FINLAND
email: martin.täubel@thl.fi

Reviewers: Professor Joanne Sordillo, Sc.D
Harvard Medical School
Department of Medicine
181 Longwood Ave
Boston, MA 02115
email: rejoa@channing.harvard.edu

Professor Rafal Górny, Ph.D
Laboratory of Biohazards, Department of Chemical, Aerosol and Biological Hazards
Central Institute for Labour Protection
National Research Institute
Czerniakowska 16, 00-701
WARSAW
POLAND
email: ragor@ciop.pl

Opponent:

Professor Heidi Salonen Ph.D.
Aalto University
Department of Civil Engineering
P.O. Box 11000
00076 AALTO
FINLAND
email: heidi.salonen@aalto.fi

Dedicated to Juha, Konsta and Väinö

ABSTRACT

Understanding the role of microbes in the protective as well as harmful health effects represents major challenges to microbiological exposure research: profound knowledge is needed on composition, sources and determinants of human exposure to environmental microbes. The aims of this thesis were: to evaluate the major determinants affecting human exposure to environmental microbes indoors, to clarify the reproducibility of the different microbial determinations, and to compare different sample types in order to define feasible and informative way to assess microbial exposure in indoor environments. Four studies building on thorough microbial exposure assessments were conducted in different indoor environments, including rural and urban residential homes and schools.

In the home environments, concentrations of the bacterial cell wall agents were commonly related to the type of the dwelling and to farming. Common determinants for the fungal marker ergosterol and also for viable fungi in dust bag dust samples were observations of visible mold and using a wood operated fireplace. Otherwise the determinants varied, depending on which sample type and which microbial agent were considered. Up to 48% of the variation in microbial levels could be explained with questionnaires and therefore, environmental samples are still necessary if one seeks to adequately describe overall microbial levels and exposure in a home.

Microbial levels in school buildings varied widely between countries, as well as between repeated measurements over one year. This variation was only for some microbial markers explainable by seasonal variation. Differences between countries with respect to how moisture and dampness conditions in school buildings affect the microbial levels, both qualitatively and quantitatively, were observed. The impact of moisture and dampness on microbial exposures was the most pronounced in Dutch schools, visible for some markers in

Spanish schools and not visible in the Finnish study schools in a wide variety of DNA based markers assessed with quantitative PCR.

The repeatability of determinations of fungal and bacterial chemical markers in parallel subsamples was strong, indicating the reliability of the method for assessing microbial levels in house dust. Due to the large temporal variation in microbial levels, however, more than one sample is needed to describe the overall microbial exposure in house dust and indoor air in the indoor environments.

Settled dust reflected well the microbial composition and seasonal variation of indoor air. Microbial determinations from reservoir dusts, such as floor and mattress dust, showed better reproducibility in repeated sampling campaigns over time than samples of indoor air. Significant differences in microbial levels between rural and urban homes were observed, most pronounced for personal air samples, likely referring to activities in microbe-rich micro-environments, such as cowsheds.

In summary, this last study showed that determinants of microbial agents in house dust vary depending on which sample type and which microbial agent is considered. Microbial levels seem to vary greatly, both spatially – in buildings between countries, between buildings within a country and even within a building - and temporally, following long-term outdoor environmental trends (i.e. seasonal variation) and indoor activity patterns. Repeated settled dust and floor dust sampling is recommended to adequately assess the overall microbial exposure in indoor environments. In farming environments, additional sampling from microbe-rich micro-environments, where the study person spends time, should be performed.

Universal Decimal Classification: 544.772.43, 551.584.6, 579.63, 628.8

National Library of Medicine Classification: QW 82, WA 754

CAB Thesaurus: indoor air pollution; exposure; microorganisms; bacteria; fungi; buildings; dwellings; homes; schools; house dust; endotoxins; chemical analysis; polymerase chain reaction; biochemical markers; ergosterol; beta-glucan; hydroxy fatty acids; moisture; cell wall components; seasonal variation; representative sampling; reliability

TIIVISTELMÄ

Mikrobien roolin ymmärtäminen sekä suojaavissa että haitallisissa terveysvaikutuksissa asettaa suuren haasteen mikrobiologisen altistumisen tutkimukselle: perusteellista tietoa tarvitaan niin mikrobiston koostumuksesta, lähteistä kuin mikrobialtistukseen vaikuttavista tekijöistä. Tämän väitöstutkimuksen tavoitteena oli määrittää merkittävimmät sisäympäristön mikrobeille altistumiseen vaikuttavat tekijät. Lisäksi tavoitteena oli selvittää erilaisten näytetyyppien ja mikrobiologisten määritysmenetelmien soveltuvuus mikrobialtistumisen kuvaajina. Työ käsittää neljä tutkimusta, jotka perustuivat perusteelliseen mikrobialtistuksen määrittämiseen. Tutkimukset suoritettiin erilaisissa sisäympäristöissä: maatila- ja ei-maatilakodeissa sekä kouluissa.

Kotiympäristöissä bakteerien soluseinämää kuvaavien markkereiden pitoisuus oli yhteydessä rakennustyyppiin ja maanviljelyyn. Näkyvä home ja puilla lämmitettävän tulisijan käyttö selittivät puolestaan sienipitoisuutta kuvaavan ergosterolin ja elinkykyisten sienten pitoisuutta pölypussipölyssä. Muut mikrobipitoisuuksia selittävät tekijät vaihtelivat riippuen näytetyypistä sekä mikrobimarkkerista. Altistumisympäristöä käsittävien kyselyiden perusteella enintään 48 % mikrobipitoisuuksien vaihtelusta pystyttiin selittämään kyselyiden perusteella, minkä vuoksi ympäristönäytteitä tarvitaan edelleen kuvaamaan kotien mikrobialtistumista kattavasti.

Koulurakennusten mikrobipitoisuudet vaihtelivat suuresti kolmen tutkimusmaan välillä, kuten myös eri näytteenottokertojen välillä vuoden aikana. Tämä vaihtelu pystyttiin selittämään ainoastaan osalle mikrobimarkkereista vuodenaikaisvaihtelulla. Tutkimusmaiden välillä havaittiin myös eroja siinä, kuinka kosteusvauriot ja kosteusolosuhteet koulurakennuksissa vaikuttivat mitattuihin mikrobipitoisuuksiin, sekä laadullisesti että määrällisesti. Kosteusvaurioiden vaikutus mikrobialtistumiseen oli suurinta hollantilaisissa kouluissa, joidenkin mikrobimarkkereiden osalta

Espanjassa. Suomalaisissa tutkimuskouluissa tätä vaikutusta ei havaittu määritettäessä laajaa kirjoa DNA-pohjaisia markkereita kvantitatiivista PCR-menetelmää käyttämällä.

Sieni- ja bakteeripitoisuuksia kuvaavien kemiallisten markkereiden määritysten toistettavuus oli hyvä rinnakkaisissa näytteissä. Tämä viittaa siihen, että menetelmä on luotettava mikrobipitoisuuksien määrittämiseen huonepölystä. Mikrobipitoisuudet vaihtelivat kuitenkin ajallisesti, minkä vuoksi tarvitaan useampi kuin yksi näyte kuvaamaan huonepölyn ja sisäilman mikrobipitoisuuksia sisäympäristöissä.

Ilmasta laskeutunut pöly kuvasi hyvin sisäilman mikrobijakaumaa ja vuodenaikaisvaihtelua. Pidempiaikaiset pölykertymät kuten matto- ja sänkypöly antoivat toistettavampia tuloksia vuoden aikana tehdyissä toistomittauksissa. Mikrobipitoisuuksissa oli selviä eroja maatalous- ja kaupunkikotien välillä. Ero oli suurin henkilökohtaisissa ilmanäytteissä, mikä viittaa toimintaan mikrobirikkaissa ympäristöissä kuten karjasuojissa.

Yhteenvetona voidaan todeta, että mikrobimarkkereiden pitoisuuksiin vaikuttavat tekijät vaihtelevat riippuen sekä näytetyypistä että mikrobimarkkerista. Mikrobipitoisuuksissa on selvää paikallista ja ajallista vaihtelua. Tämän tutkimuksen tulosten perusteella ilmasta laskeutuneen pölyn ja mattopölyn näytteitä suositellaan otettavaksi toistetusti, jotta mikrobialtistusta voidaan arvioida kokonaisvaltaisesti sisäympäristöissä. Maatilaympäristöissä tulisi ottaa lisäksi näytteitä tiloista, joissa on suuret mikrobipitoisuudet ja joissa tutkimushenkilö viettää aikaansa.

Yleinen suomalainen asiasanasto: sisäympäristö; sisäilma; altistuminen; mikrobit; bakteerit; sienet; rakennukset; asunnot; koulurakennukset; pöly; kemiallinen analyysi; polymeraasiketjureaktio; markkerit; kosteus; vaihtelu; vuodenajat; toistettavuus; luotettavuus

Preface

This thesis was carried out at the Department of Health Security of the National Institute for Health and Welfare. I wish to thank the former and current department heads for providing the facilities for this study. Sincere thanks also go to the Finnish Work Environment Fund (grant 115243) and Juho Vainio Foundation (grant 201310220) for funding this thesis.

I wish to express my deepest gratitude to my principle supervisor, Research Professor Anne Hyvärinen, for her excellent scientific support and guidance. Her support carried me through the process, including when I was faced with health problems. I am also extremely grateful to my supervisor and "big brother" Dr. Martin Täubel. He was the driving force, pushing me to do my best. He supported me enormously, and I learned a lot about writing from him. I would also like to express my warmest gratitude to my supervisors Research Professor Emerita Aino Nevalainen and Research Director Pertti Pasanen, for their guidance, commitment and encouragement throughout the PhD project.

I sincerely thank the official reviewers, Professor Joanne Sordillo ScD of Harvard Medical School and Professor Rafal Górny PhD of the Central Institute for Labour Protection, Warsaw, Poland for their careful review and helpful comments. I would like to thank all my co-authors: Juha Pekkanen, Asko Vepsäläinen, Balamuralikrishna Jayaprakash, Marjut Roponen, Panu Rantakokko, Olli Laine, Helena Rintala, Jóse Jacobs, Alicia Borras-Santos, Esmeralda Krop, Jan-Paul Zock, Dick Heederik and Erica von Mutius, as well as the study groups PASTURE and HITEA for sharing their expertise during this thesis.

I would also like to express my warm thanks to statistician Asko Vepsäläinen and researcher Anne Karvonen for their guidance and support in statistical analyses, laboratory assistants Heli Martikainen, Katja Saarnio and Pirkko Karakorpi

for their friendship and laboratory analyses, measurement technician Tuomo Korhonen for chemical marker analyses and civil engineers Juho Halla-aho and Jari Koivisto for their building inspections. Researchers Eeva Kauhanen and Ulla Lignell are sincerely acknowledged for their fungal identifications, and research assistant Santtu Turunen for our unforgettable field trips during the HITEA study. Assistant Kirsi Korhonen is warmly acknowledged for all her help in practical matters and for her warm heart.

Special thanks to all of my present and former colleagues and friends in our group: Kati, Terhi, Päivi, Mari, Maria, Kaisa, Heidi, Katja, Heli, Mervi, Ritva, Bala and Martin, as well as Anna and Anna-Maria from the neighbor group for encouragement and laughs during lunch and coffee breaks. Without you guys my days would have been drab and lonely.

I would like to express my deepest and warmest thanks to my foster-mum Raili, grandma Milka, mum Hilkka, sister Eveliina and aunt Aune for their love and outstanding support throughout my life. You have really taught me to see what is important in life. I want to thank all of my friends for listening to me, going for walks with me and filling my life with happiness during this challenging period.

I wish to express my warmest and heartfelt thanks to my husband Juha and our lovely sons Konsta and Väinö. I love you so much. You give me reason to smile every day. Thank you for loving and supporting me always.

Finally, but most importantly, I want to thank my God for all the friends and loved-ones He has given me during this journey, as well as the strength and guidance to be able to finish this thesis.

Hanna Leppänen
Kuopio, August 2017

LIST OF ABBREVIATIONS

3-OHFAs	3-hydroxy fatty acids
CFU	Colony forming unit
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
EDC	Electrostatic dustfall collector
EPS	Extracellular polysaccharides
EU	Endotoxin unit
GM	Geometric mean
GC-MS	Gas chromatography-mass spectrometry
GC-MS-MS	Gas chromatography-tandem mass spectrometry
GLC-MS	Gas liquid chromatography- mass spectrometry
HPLC	High-performance liquid chromatography
ICC	Intraclass correlation
LAL	Limulus amebocyte lysate
LPS	Lipopolysaccharide
MD	Median
NGS	Next generation sequencing
NO	Nitric oxide
ODTS	Organic dust toxic syndrome
qPCR	Quantitative polymerase chain reaction
RCS	Reuter Centrifugal sampler
rDNA	Ribosomal Deoxyribonucleic acid
rFC	Recombinant Factor C
SAS	Surface-air-sampler
SDB	Settled dust box
SSCP	Single-strand conformation polymorphism
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I–IV.

I Leppänen HK, Nevalainen A, Vepsäläinen A, Roponen M, Täubel M, Laine O, Rantakokko P, von Mutius E, Pekkanen J and Hyvärinen A. Determinants, reproducibility and seasonal variation of ergosterol levels in house dust. *Indoor Air 24: 248-259, 2014*.

II Leppänen HK, Täubel M, Roponen M, Vepsäläinen A, Rantakokko P, Pekkanen J, Nevalainen A, von Mutius E and Hyvärinen A. Determinants, reproducibility, and seasonal variation of bacterial cell wall components and viable counts in house dust. *Indoor* Air *25:260-272, 2015.*

III Täubel M, Leppänen HK, Jacobs J, Borras-Santos A, Krop E, Rintala H, Pekkanen J, Zock J-P, Heederik D, Hyvärinen A, HITEA study group. A longitudinal assessment of microbial exposures in European schools in relation to moisture damage and dampness.

IV Leppänen HK, Täubel M, Jayaprakash B, Vepsäläinen A, Pasanen P and Hyvärinen A. Quantitative assessment of microbes from samples of indoor air and dust.

The above publications have been included at the end of this thesis with their copyright holders' permission.

AUTHOR'S CONTRIBUTION

Paper I and II: The author contributed to the sample preparation and laboratory analyses. The author was responsible for data checking and statistical analyses for univariate associations and multiple linear regression models. The covariance parameter estimates and intra class correlations were made by statistician Asko Vepsäläinen. Interpreting the results and writing of the paper were done by the author with the support of co-authors.

Paper III: The author contributed to the planning and implementation, including application of the study for ethical approval, organizing and carrying out fieldwork to collect data and samples. The author did the statistical analyses for univariate associations, contributed to a review of the literature and supported writing the paper as a second author.

Paper IV: The author contributed to the sample processing. She did the statistical analyses together with statistician Asko Vepsäläinen. Interpretation of the results and writing the paper were done by the author with supportive co-operation of the co-authors.

Contents

1 Introduction

Farmers' children have smaller risk for developing allergic diseases than children growing up in non-farm, urban environments (Braun-Fahrlander, 1999; Ege, 2011; Pekkanen, 2001). This protective effect is thought to result from more frequent contact to environmental microbes and animal allergens during early childhood when the immune system is developing (Braun-Fahrlander, 1999; Bufford, 2008; Ege, 2011; Genuneit, 2012; Platts-Mills, 2001; von Mutius & Vercelli, 2010). On the other hand, exposure to environmental microbes is also linked to respiratory diseases (Bolte, 2003; Celedon, 2007). Some studies have shown that timing and persistence of the exposure to certain microbial groups is relevant in producing positive or negative health outcomes later in life (Sordillo, 2010). Among adverse outcomes, specifically respiratory symptoms, infections and the exacerbation and development of asthma have been consistently linked with occupying moisture damaged buildings (Kanchongkittiphon, 2015; Karvonen, 2009; Mendell, 2011; Pekkanen, 2007; WHO, 2009). In Finland, the prevalence of significant moisture and mold damage has been estimated for care institutions (20-26%), schools and day care centers (12-18%), homes (6-10%) and offices (2.5-5%) (Reijula, 2012).

The respiratory diseases aggravated by moisture and mold damage have high public health importance, and their etiology needs to be better understood as well as the role of possible protective microbial exposures. This implies great challenges to microbiological exposure research: profound knowledge is needed on composition, sources and determinants of human exposure to environmental microbes in particular indoors, where we spend most of our lifetime.

Microbial exposure in most indoor environments is notoriously diverse (Pitkäranta, 2008; Rintala, 2008; Täubel, 2009) and approaches for both quantitative and qualitative

assessments of the microbial exposures are needed. It appears that to this date, there are no perfect methods that would allow assessing microbial exposure at high resolution and with good quantitation, but compromises on one or the other end need to be made. Determination of microbial biomass by using their cell wall agents is one type of quantitative approach, but these methods have no resolution in terms of microbial species. Cultivation technique combines both absolute counts and specification usually up to genus level for fungi; however, it reflects only the viable portion (0.03-10%) of the microbes in indoor environments, and thus a major part of the microbes can stay undetected (Golofit-Szymczak & Gorny, 2010; Pasanen, 1989; Toivola, 2002). DNA based methods overcome this limitation, as they are not restricted by viability. Quantitative PCR is truly quantitative if performed well, allowing targeting different microbial species, genera or groups of microbes. However, the method is limited by only enumerating a microbial group that is also targeted in the specific assay. DNA fingerprinting methods are non-targeted, but they are restricted to the more abundant microbial groups in a sample and this method is at best semi-quantitative. The current technology available, next generation sequencing (NGS) approach has revolutionized the study of genomics and molecular biology meaning that with NGS the microbiome of indoor environments can be studied. NGS allows virtually complete characterization of complex microbial communities and individual genomes.

The ideal way to assess the indoor airborne microbial exposure in theory would be active air sampling, since inhalation is the main route for microbial exposure from indoor air. The airborne microbial levels, however, vary both temporally and spatially, and therefore multiple repeated sampling campaigns are needed to describe the microbial status of a building (Hyvärinen, 2001). In addition, air sampling is equipment intensive and typically not easy to perform by study subjects themselves, so that sampling campaigns usually need to be carried out by field workers. This is resource-intensive work and means high costs in studies with a large number of

buildings to be studied. Another route for exposure to indoor microbes is ingestion. This may be a major route for microbial exposure during infancy, when the infants crawl on floors and ingest dust of typically 100 mg/day (U.S. EPA, 2008). Considering this exposure route, floor dust could be the best sample type to assess the microbial exposure from. Reservoir dust samples, especially floor and mattress dust, are often used since they integrate better long-term microbial exposure than individual short time air samples (Hyvärinen, 2006a), and are easy and inexpensive approaches to implement in large epidemiological studies. These sample types lack, however, a good representation of the actual airborne exposure, and samples of settled dust (i.e. dust that has settled and is collected from surfaces above floor level) have been proposed to better represent the particles that have been airborne (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004).

To reliably assess the exposure, the method for measuring the exposure should be reproducible. In any determination of environmental agents relevant to human health, it is also essential to have knowledge on parameters causing variation in levels of the targeted compound. Therefore, this thesis aimed to define the major determinants of human exposure to environmental microbes indoors, to clarify the reproducibility of the different methods, and also to compare different sample types for proposing good ways to assess the microbial exposure in indoor environments.

2 Literature review

2.1 HEALTH EFFECTS OF MICROBES IN INDOOR ENVIRONMENTS

2.1.1 Microbial exposures in farming environments

Beneficial and harmful effects of environmental microbes on human health are linked with the source, timing, duration, amount and diversity of the microbial exposure. According to the so called hygiene hypothesis, hygienic living conditions can lead to the development of allergic diseases (Strachan, 1989). This is due to decreased exposure to microbes which are involved in maturation of the immune system, especially during early childhood (von Mutius & Vercelli, 2010). In farming environments, the microbial exposure is more extensive and diverse than in non-farming environments, and thereby challenges the developing of the immune system of infants. The microbial levels, determined as different cell wall agents or as fungal and bacterial taxa have been significantly higher in farming homes than in non-farming homes in a number of studies (Ege, 2011; Hyvärinen, 2006b; Kärkkäinen, 2010; Moniruzzaman, 2012; Schram, 2005). It has been shown that farmers' children suffer less from allergies than children living in urban homes. This effect has been linked to differences in exposure to microbial agents, such as bacterial and fungal taxa, endotoxin, muramic acid and allergens (Braun-Fahrlander, 1999; Ege, 2011; Karvonen, 2012; Pekkanen, 2001; Roponen, 2005; van Strien, 2004; von Mutius & Vercelli, 2010). A meta-analysis estimated that the prevalence of asthma among children living in traditional farms was approximately 25% lower than that of children living in non-farming environments (Genuneit, 2012). The protective effect of farming environment has also been observed for young adults as decreased risk of atopy when assessed by the levels of allergens and dust mite (Pekkanen,

2001). The links between health outcomes and microbial exposures are not, however, straightforward. A recent study shows that when studying associations between single microbial agents and health outcomes, only few associations are found. Instead, a score for the total quantity of microbial exposure, that is, the sum of indicators for fungi (ergosterol), Gram-positive (muramic acid) bacteria, and Gram-negative (endotoxin) bacteria associates significantly (inverted U-shape) with asthma incidence. When using microbial diversity score, that is, sum of detected qPCRs, it is inversely associated with the risk of wheezing and significantly associated with inhalant allergens (Karvonen, 2014).

In contrast to beneficial health effects of farming and microbial exposures, farmers may suffer from occupational diseases, such as allergic alveolitis or hypersensitivity pneumonitis (farmer's lung), occupational asthma, and organic dust toxic syndrome (ODTS). These diseases are all associated with agricultural work with massive microbial exposure (doPico, 1986; Kotimaa, 1984; Lacey & Crook, 1988; Malmberg, 1993).

2.1.2 Health effects associated with moisture damage

The health effects associated with moisture, dampness and mold in buildings have been evaluated by working groups of World Health Organization (WHO, 2009) and Institute of Medicine (2004), and more recently reviewed by Mendell et al. (2011) and Kanchongkittiphon et al. (2015). These reports have concluded that the moisture, dampness and mold problems are associated with different respiratory symptoms, such as irritant effects on the respiratory system and mucous membranes, respiratory infections, asthma exacerbation and development of asthma. However, the causative agents and mechanisms behind these effects are still unknown.

The location, severity and extent of the moisture or mold damage may be crucial in causing adverse health effects (Karvonen, 2009; Pekkanen, 2007; Verhoeff, 1995). This has been recognized as a public health issue in Finland, and according to the Degree of Housing and Health (2015), when assessing the

health effect, the likelihood, frequency and duration of the exposure should be paid attention to, as well as possibility to avoid the exposure or remove the damage. Risk of asthma in early childhood seems to increase with the severity of moisture damage and when visible mold is detected in the main living areas (Pekkanen, 2007). In another study, severity of moisture damage in the kitchen and visible mold in the main living areas were associated with doctor diagnosed wheezing of the child (Karvonen, 2009), while moisture damage in bathrooms or other interior spaces did not have such an effect. In school studies, moisture damages have been associated with several different adverse health outcomes. A recent study in three European countries (Jacobs, 2014b) found higher prevalence of respiratory symptoms in moisture damaged schools than in schools without moisture damages. Haverinen et al. (1999) showed that several irritative and recurrent symptoms, and also asthma incidence were common in a moisture damaged school center. Along with higher symptom prevalences, significantly higher levels of various inflammatory markers in nasal lavage fluid of exposed workers have been observed in a moisture damaged school (Hirvonen, 1999; Purokivi, 2001).

Generally, many studies link moisture damage in buildings to an increase in microbial exposure levels in the indoor environment, and in some cases, also changes in the microbiota are reported. However, analyzing health effects of mold repairs has produced very contradicting information as shown in a Cochrane review of 12 studies conducted in homes and schools (Sauni, 2013). The review concluded that there was only low to moderate evidence for a beneficial effect on asthma and respiratory infections, when repairing the homes and schools compared to situation with no intervention. In a study of Meklin et al. (2005), remediation of the moisture and mold damage in the school showed an improvement in symptom prevalence of respiratory and other symptoms as compared to the pre-remediation situation.

2.1.3 Health effects of different microbial agents

Microbial agents are associated with divergent effects on health also in homes and schools without any specific source of microbes such as farming or moisture damage. Endotoxin seems to have a protective effect on asthma, wheezing and atopy in most cross-sectional studies, whereas in longitudinal studies the protective effect is not consistent, when studied among children up to school age (Douwes, 2006b; Gehring, 2007; Gillespie, 2006; Perzanowski, 2006; Tischer, 2011; Tischer, 2015a). Other agents for bacteria, 3-hydroxy fatty acids (3-OHFAs) and muramic acid have shown preliminary evidence on association with decreased asthma and wheezing among children (Hyvärinen, 2006b; Sordillo, 2010). One fungal agent, extracellular polysaccharides (EPS) has shown mostly protective health effects of asthma, wheezing and atopy, whereas another fungal agent, ergosterol has been associated with increased risk of asthma and wheezing for both children and adults (Dharmage, 2001; Hyvärinen, 2006b). As for (1→3)-β-D-glucans, the effects have been contradictory, from no associations to positive health effects (Casas, 2013c; Gehring, 2007; Tischer, 2015a). Iossifova et al. (2007) showed association with increased risk of recurrent wheezing at low levels, but decreased risk at high levels of (1→3)-β-D-glucans during first year of life.

In school studies, both protective and adverse associations with health have been observed when studying different microbial agents. The SICAS cohort in the US found that endotoxin exposure in schools was associated with increased reports of asthma symptoms in non-atopic asthmatic children (Lai, 2015). Based on the studies conducted so far there is a notion that bacterial exposure may also link to beneficial effects (Norback, 2014; Zhang, 2011), whereas fungal exposures are more often linked to adverse health effects (Cai, 2011a; Chen, 2014; Smedje, 1997; Zhang, 2011). In some cases, opposite associations are reported for the same microbial agent or group in different studies.

The causal connections between microbial exposure and health effects are not well established yet. This is likely due to

the complexity of both exposure and the effects, and hence, more studies thoroughly assessing the microbial exposure are needed.

2.2 FACTORS AFFECTING MICROBIAL LEVELS INDOORS

In the following chapters, the main microbial sources and determinants affecting the exposure in homes and schools are presented. In indoor environments, several so called normal sources and activities exist as well as unwanted sources, such as moisture and mold damage.

2.2.1 Outdoor sources

Soil and vegetation are the main microbial sources for outdoor air and they cause variation in composition of outdoor air in diverse climates and geographical regions. Outdoor air acts as one of the main sources for microbial content of indoor environments and hence, geographical and climatic factors have a great influence on the microbial concentrations and communities indoors (Adams, 2013; Amend, 2010; Burge, 2000; Dekoster & Thorne, 1995; Jacobs, 2014a; Li & Kuo, 1994; Reponen, 1992; Rintala, 2012; Yamamoto, 2015). Seasonal effects have been reported in many studies, but the results are conflicting. It is evident that the effects vary in different regions and depending on the agents measured. Often the microbial levels indoors are much higher during summer than in the colder seasons (Abraham, 2005; Casas, 2013a; Frankel, 2012; Koch, 2000; Wu, 2007). On the other hand, some studies show higher microbial levels during fall or winter (Baxi, 2013; Holst, 2015; Johansson, 2011; Mentese, 2012; Park, 2004). There are also studies that show different seasonal effect for different microbial agents (Dharmage, 2002; Heinrich, 2003; Kaarakainen, 2009; Sordillo, 2011; Wady, 2004). It is evident that for recognizing a seasonal effect, the type of microbial species/groups has to be considered in addition to the geographical location, as the

different microbial agents have different seasonal effects even in the same geographical area (Rintala, 2012).

Outdoor air affects the indoor microbial content through ventilation, open windows and doors and air leakages in the building envelope. The microbial content of indoor air closely follows that of outdoor air in buildings (Meadow, 2014; Miletto & Lindow, 2015; Shelton, 2002). This is true especially in locations with warm and mild climates throughout the year. In cold climates with snow-covered ground for a large part of the winter, the microbial levels outdoors during these periods are extremely low and hence have hardly any effect on microbial levels indoors (Reponen, 1992). Also the type of ventilation has an important role. Mechanical ventilation with filtration removes microbes from the intake air more or less efficiently (Dekoster & Thorne, 1995; Reponen, 1992). Naturally ventilated schools generally have been shown to contain higher microbial levels than schools with mechanical ventilation (Bartlett, 2004a; Bartlett, 2004b). The ventilation method can affect also the microbiome of indoor air. Kembel et al. (2014) observed with sequencing of the bacterial 16S gene that taxa associated closely with mechanically ventilated offices were different from those associated with window ventilated offices.

2.2.2 Occupants, occupant behavior and indoor environmental characteristics

Humans are one of the main sources of microbes in buildings, specifically of bacteria (Casas, 2013b; Dannemiller, 2016; Gehring, 2001a; Giovannangelo, 2007c; Hospodsky, 2012; Meadow, 2014; Täubel, 2009). The human presence is a major determinant of the bacterial content in house dust. It has been shown that 69% to 88% bacterial sequences of mattress dust have human origin. The corresponding value for floor dust is 45% to 55% (Täubel, 2009). In schools, on average, 81% of allergenic fungi from indoor sources have been observed to originate from occupant-generated emissions (Yamamoto, 2015). For bacteria, Qian et al. (2012) observed about 18% of the bacterial emissions originated from taxa that were closely associated with the

human skin microbiome with a rDNA gene sequencing method. In addition to human occupancy, occupant behavior and activities are also significant sources of microbes. For example, handling firewood and other organic materials, having organic waste bins inside the home and low bin-emptying frequency increase the microbial levels in the indoor environments (Dekoster & Thorne, 1995; Gehring, 2001a; Lehtonen, 1993; Wouters, 2000). Cleaning is also a significant factor with diverging effects: lower frequency of cleaning increases the microbial levels (Bischof, 2002; Gehring, 2001a; Giovannangelo, 2007c; Hyvärinen, 2006b; van Strien, 2004; Waser, 2004), while regular cleaning can decrease the levels remarkably (Sordillo, 2011; Wu, 2012). On the other hand, cleaning can increase the levels temporarily, through the effect of resuspension. Resuspension of dust is more pronounced in places where the human activity occurs, such as floors and other low levels (Rintala, 2012). Resuspension concerns mainly larger particles; particles < 1 μm do not resuspend. This is probably because of small particles do not deposit in first place, but stay airborne (Thatcher & Layton, 1995).

Having pets, potted plants or carpets affects the microbial levels indoors (Casas, 2013a; Douwes, 2000; Gehring, 2001a; Johansson, 2011; Loo, 2010; Moniruzzaman, 2012; Pessi, 2002; Ren, 2001; Sordillo, 2011). The age of the building has also been found to affect indoor microbes: microbial levels have been shown to be significantly higher in old buildings (Bartlett, 2004a; Bischof, 2002). For multilevel buildings, floor level has been found to be a determinant. Samples from lower level of the apartment increase the bacterial content in house dust (Bischof, 2002; Heinrich, 2001). This is probably due to more direct location of the lowest apartment levels to the outdoor environment, from where the microbes are carried into the dwelling through entrance halls, hallways and stairways. Similarly, lower fungal levels have been observed in apartments compared to terraced houses or single family houses (Chew, 2003; Sordillo, 2011).

2.2.3 Farming environment

In many studies, farming environments have been shown to be strong sources for indoor microbes. This has been shown using, different markers of microbial biomass, such as (1→3)-β-D-glucans and EPS (Schram, 2005), 3-OHFAs (Hyvärinen, 2006b; Kärkkäinen, 2010), muramic acid (Kärkkäinen, 2010; van Strien, 2004), endotoxin (Moniruzzaman, 2012; Schram, 2005; von Mutius, 2000; Waser, 2004), ergosterol (Hyvärinen, 2006b) and fungal and bacterial taxa (Ege, 2011; Kärkkäinen, 2010). In addition to airborne route, microbes are transferred into the buildings on clothes and shoes that have been used in microbe-rich environments, for example barns and stables (Korthals, 2008; Krop, 2014; Normand, 2011; Pasanen, 1989; Rintala, 2012). Farming activities such as manure fertilization and proximity to agricultural land increase the microbial concentration in the indoor environments (Kaarakainen, 2011; Tager, 2010).

On the other hand, it has been observed that endotoxin levels in stables do not necessarily correlate well with levels found in floor and bed dust samples in the respective farming homes (Waser, 2004). However, a dose-dependent association between the child's activity on a farm and indoor home endotoxin levels has been observed for both farming and non-farming children (Waser, 2004). Even for schools, Wady et al. (2004) found that ergosterol and 3-OHFAs levels were higher in rural schools than in schools in the urban environments.

2.2.4 Mold and moisture damage

Microbial growth on surfaces and in building structures is considered an abnormal condition. The main reason behind microbial growth is basically the wetting of materials that provides favorable conditions for metabolic activity, germination and proliferation of microbes. When distributed into indoor spaces, spores, cells and cell fragments, and microbial metabolites (Nevalainen, 2015) and also other organisms like amoebae and house dust mites (Verhoeff, 1995; Yli-Pirilä, 2004) may contribute to biological indoor pollution. Indoor dampness and moisture damage can be caused by

failures in structures such as leakage on roofs or water pipes; by condensation due to poor ventilation; as a consequence of inadequate insulation or occupant behavior such as careless use of water. Moisture damage may also result from floods, heavy rains or rising water from the soil by capillary force (Haverinen, 1999; Nevalainen, 1998; Nevalainen & Seuri, 2005). The type of building material seems to have an effect on how microbes behave, when moisture is available. Every material serves as a substrate and surface more or less favorable to support proliferation and growth of microorganisms. This depends on the composition of the material, water activity and nutrient content (Hoang, 2010; Pasanen, 2000). These properties determine the diversity and extent of growth of the microbes (Andersen, 2011; Hyvärinen, 2002). The most vulnerable materials for microbial growth are those with a natural organic composition such as wood and paper. Hyvärinen et al. (2002) studied different moisture damaged building material types and found highest median concentrations of fungi in wooden and paper materials, and lowest in samples of mineral insulation, ceramic products, and paints and glues. Meklin et al. (2002) saw clear effect of moisture damage on levels of airborne viable fungi in schools with concrete/brick construction, but not in wooden schools.

Visible observation of moisture damage, dampness and/or mold, as well as indicators of hidden mold problems, such as the odor of mold, have been used as surrogates of microbial exposure. These observations have been linked to elevated levels of fungal and bacterial markers in residential homes (Chen, 2012a; Dales, 1997; Douwes, 2006; Gehring, 2001a; Hyvärinen, 1993; Hyvärinen, 2006b; Kettleson, 2013; Klanova, 2000; Reponen, 2010; Rintala, 2012; Sordillo, 2011). Similarly, higher microbial levels in air and dust samples from damaged schools and classrooms have been reported than in non-damaged ones (Cai, 2009; Cho, 2016; Jacobs, 2014b; Krop, 2014; Lignell, 2005; Purokivi, 2001; Simoni, 2011). Some of these studies show that microbial biota in such buildings may contain genera indicative for moisture and mold problems. Occurrence

of e.g. *Aspergillus versicolor*, *Eurotium*, *Stachybotrys* and actinobacteria have been shown to associate with moisture damage, when viable microbes have been assessed from air samples (Haverinen, 1999; Meklin, 2002). When determining the effect of repairs on airborne viable microbes in Finnish school buildings, a clear effect has been seen on concentrations as well as on composition of the indoor mycobiota (Lignell, 2007; Meklin, 2005; Roponen, 2013). Such moisture damage interventions are highly informative, but they are very difficult to perform, because the renovations are not done respecting the study schedule and other demands. Therefore the literature is clearly limited with respect to reports of thoroughly conducted studies with good sample numbers.

2.3 MICROBIAL AGENTS TARGETED IN HYGIENIC QUALITY ASSESSMENT

Different microbial agents mostly used in microbial determinations and their applications in indoor environmental research are presented in Table 1.

2.3.1 Ergosterol

Ergosterol is a primary sterol found in the cell membrane of filamentous fungi. It is also a minor component in some higher plants and present in the yeast cell wall membranes and mitochondria (Axelsson, 1995). Ergosterol is widely used as a marker of fungal biomass in the analysis of samples from different sources e.g. soil, aquatic systems, building materials and house dust (Gessner & Chauvet, 1993; Grant & West, 1986; Pasanen, 1999; Saraf, 1997). The ergosterol content per cell is to some extent dependent on the type of the fungal species as well as on growth conditions (Axelsson, 1995; Dales, 1997; Pasanen, 1999). Ergosterol is photodegradable and strict care has to be taken in the sample processing and storage by using actinic glassware and limit exposure to light (Mille-Lindblom, 2004). Ergosterol is analyzed using gas chromatography-mass

spectrometry (GC-MS) (Axelsson, 1995) or with combination of high-pressure liquid chromatography (HPLC) and GC-MS (Dales, 1997) or gas chromatography-tandem mass spectrometry (GC-MS-MS) (Nielsen & Madsen, 2000).

Ergosterol has been used as a marker of fungal biomass in several studies in homes. In settled dust samples taken from homes, the levels of ergosterol have ranged between GM 0.6 – 0.7 ng/mg in urban homes (Sebastian & Larsson, 2003; Sebastian, 2005) and 2.1 ng/mg in farming homes (Szponar & Larsson, 2001). For floor dust, the variation has been between GM 2.3 – 18.0 ng/mg in urban homes (Dharmage, 2002; Saraf, 1999; Sebastian & Larsson, 2003) and 8.5 ng/mg in farming homes (Lappalainen, 2008). As for dust bag dust the levels have been GM 3.4 in urban homes (Hyvärinen, 2006b) and 12.5 ng/mg in farming homes (Lappalainen, 2008). For bed dust samples the ergosterol levels have varied from GM 2.7 ng/mg (non-farming homes) to GM 3.8 ng/mg (farming homes) (Lappalainen, 2008).

There are also some studies assessing ergosterol exposure in school environments. Wady et al. (2004) observed ergosterol levels of 0.4 - 6.9 ng/mg from settled dust samples. When using samples collected from floors, chairs and desks, the concentration has varied between GM 0.6 – 2.7 ng/mg (Cho, 2016; Norback, 2016b; Zhang, 2011; Zhao, 2008).

The ergosterol levels have been observed to be affected by the farming and livestock, cleaning (Hyvärinen, 2006b; Wady, 2004), presence of pets, visible mold or dampness and old carpets, and season (Dharmage, 1999; Dharmage, 2002; Jacobs, 2014b; Sordillo, 2011; Wady, 2004). On the other hand, the levels are reduced by central air conditioning and living in an apartment (Sordillo, 2011).

There are only a few studies that have assessed the reproducibility of ergosterol determinations. Moderate within-home variation was observed in floor dust samples in a study where the samples were collected twice, two years apart (Matheson, 2003). In another study, the concentrations of ergosterol in six replicate house dust samples correlated highly (0.91) (Saraf, 1997). According to these results, both the

repeatability of determination of replicate samples as well as the reproducibility of ergosterol determination over time are good.

2.3.2 Extracellular polysaccharides

Fungi produce immunologically active extracellular polysaccharides (EPS). Extracellular polysaccharides are heat-stable and water-soluble non-branched carbohydrate polymers secreted or shed during fungal growth and can be measured by a sandwich enzyme immunoassay from house dust samples. EPS of the fungal genera *Penicillium* and *Aspergillus* is suggested to be a quantitative marker for fungal biomass (Douwes, 1999).

There are many studies assessing EPS levels of homes without a specific microbial source from different house dust samples. EPS levels in floor dust samples have ranged between GM 4000 – 41000 EPSU/g (Chew, 2001; Douwes, 2006a; Wouters, 2000). It seems that the levels are higher in samples that are collected from rugs compared to samples taken from smooth floors (Douwes, 2006b; Karvonen, 2012). In mattress dust samples EPS concentrations have varied GM 78000 – 89000 EPSU/g between different sampling methods (Schram-Bijkerk, 2006).

The determinants that increase EPS levels in house dust are farming, pets, organic waste bins, low bin-emptying and cleaning frequency, higher number of persons living in the home and self-reported mold spots or dampness in the home. Also ventilation, age of the house and cooking with gas affect the EPS levels (Casas, 2013a; Douwes, 1999; Douwes, 2006; Giovannangelo, 2007c; Schram, 2005; Wouters, 2000).

The reproducibility of EPS determination has been assessed in two studies. Smaller within-home variation than between-home variation was observed in both studies from samples of floor and mattress dust, showing good reproducibility (Chew, 2001; Giovannangelo, 2007c).

2.3.3 (1→3)-β-D-glucans

(1→3)-β-D-glucans are glucose polymers, originating from several sources, predominantly from most fungi, but also some

bacteria and plants. The content of (1→3)-β-D-glucan in a sample has been used as a proxy of fungal biomass. Douwes et al. (1997) developed an inhibition enzyme immunoassay that specifically recognizes (1→3)-β-D-glucans. Also a glucan-reactive preparation of Limulus amebocyte lysate (LAL) method can be used to determine (1→3)-β-D-glucans from indoor environments (Rylander, 1992). In addition, a specific enzyme immunoassay have been developed to quantify (1-6) branched, (1→3)-β-D-glucans in environmental samples (Milton, 2001).

The majority of studies have determined (1→3)-β-D-glucan levels in homes without a specific microbial source from floor dust. The variation has been GM 2.1 – 2800 μg/g (Casas, 2013a; Chew, 2001; Douwes, 1997; Douwes, 2000; Douwes, 2006a; Fahlbusch, 2003; Gehring, 2001a; Gehring, 2007; Giovannangelo, 2007b; Holst, 2015; Iossifova, 2007; Wouters, 2000). In a study performed in primary, middle, and high schools in USA, the (1→3)-β-D-glucan levels in floor dust varied between 39.0 – 90.5 μg/g (Cho, 2016). The levels in homes are also determined from dust bag dust samples, resulting MD 2553 (with dogs) and MD 2795 μg/g (without a dog) (Bertelsen, 2010). When studying a group of homes, from which a part was mold and moisture damaged, the settled dust from child´s bedroom contained (1→3)-β-D-glucan level of GM 170 μg/g (Berghout, 2005). Correspondingly, the level in child's bedroom without a specific microbial source has been MD 140 μg/m^2 (Miller, 2007). While in mattress dust the levels have varied between 680 – 2800 μg/g (Douwes, 1997; Douwes, 2000; Douwes, 2006a; Gehring, 2007; Giovannangelo, 2007b; Schram-Bijkerk, 2006; Wu, 2012).

(1→3)-β-D-glucan levels have been observed to be affected by textile floor covering or rugs (Douwes, 2000; Gehring, 2001a; Holst, 2015; Wouters, 2000), with organic waste bin and low bin-emptying frequency (Wouters, 2000), having pets (Blanc, 2005; Gehring, 2001a), farming (Krop, 2014; Schram, 2005), human occupancy (Gehring, 2001a; Giovannangelo, 2007c), cleaning (Gehring, 2001a; Giovannangelo, 2007c; Wu, 2012), visible mold and water damage (Douwes, 2006; Gehring, 2001a) and season (Casas, 2013b; Holst, 2015).

The knowledge about the reproducibility of (1→3)-β-D-glucan determination is rather limited. Chew et al. (2001) found the within-home variation to be similar than between-home variation, when samples were collected six times, twice in three seasons. Giovannangelo et al. (2007b) observed that the reproducibility was better when (1→3)-β-D-glucan levels were presented as load, that is per sampling area, taking dust amount into account, as compared to concentration (per mg of dust).

2.3.4 Endotoxin

Lipopolysaccharides (LPS), referred to as endotoxins, are large, biologically active molecules that form the outer membrane of Gram-negative bacteria. Endotoxin is typically being assessed with the Limulus amebocyte assay (LAL) (Douwes, 1995), but some studies have also used the Recombinant Factor C (rFC) for determining endotoxin from house dust samples (Alwis & Milton, 2006; McKenzie, 2011; Thorne, 2010). Both methods measure the biological activity of LPS of Gram-negative bacteria.

Endotoxins have been widely studied in different indoor environments. The review article of Salonen et al. (2016) shows that in residential, school and office environments the mean endotoxin loads in floor dust vary between 660 – 107000 EU/m^2, 2180 – 48000 EU/m^2, and 2700 – 12890 EU/m^2, respectively. The mean concentrations of air samples are varying between 0.04 - 1610 EU/m^3 in homes, and 0.07 and 9.30 EU/m^3 in schools and offices, respectively.

The factors that affect endotoxin levels have been determined in many studies, pointing out higher levels in old versus newer buildings, at lower floor level in apartment buildings (Bischof, 2002; Heinrich, 2001), in farming homes (Schram, 2005; von Mutius, 2000), with presence of children, with increasing occupancy (Bischof, 2002; Casas, 2013a; Giovannangelo, 2007a; Johansson, 2011; Rennie, 2008; Thorne, 2009; Wickens, 2003). The endotoxin levels in house dust are also affected by carpets, pets, organic waste bins, tobacco smoke, frequency of cleaning and water damage (Bischof, 2002; Blanc, 2005; Campo, 2006; Casas, 2013a; Douwes, 2000; Douwes, 2006;

Gehring, 2001b; Gereda, 2001; Giovannangelo, 2007a; Heinrich, 2001; Jacobs, 2014b; Johansson, 2011; Perzanowski, 2006; Rennie, 2008; Sordillo, 2011; Waser, 2004; Wickens, 2003; Wouters, 2000). Lower endotoxin levels are associated with wet mop cleaning and central air conditioning (Gereda, 2001; Perzanowski, 2006). Seasonal effect has been found with contradictory results in different studies and climates (Abraham, 2005; Jacobs, 2013; Park, 2000; Su, 2001).

The conclusions concerning the reproducibility of endotoxin determinations vary in different studies, and appears to depend on which sample type was considered. Greater within-home than between-home variation in endotoxin levels has been found for bedroom floor dust and kitchen floor dust, when samples have been collected once a month during 14 months (Park, 2000). Also Topp et al. (2003) found that in examining floor dust samples from low endotoxin exposure environments, the reproducibility is low, and that a single measurement does not accurately reflect the true long-term exposure. On the other hand, a study with five sampling campaigns during one year in high and low endotoxin exposure environments using mattress dust found higher between-home variance than within-home variance referring to strong reproducibility (Hyvärinen, 2006a). In a study of Heinrich et al. (2003), the endotoxin levels in two repeated measures of floor dust were highly correlated. Giovannangelo et al. (2007b) showed that duplicate sample of floor and mattress dust collected at same time point had smaller within-home than between-home variation indicating good reproducibility for parallel samples.

2.3.5 3-hydroxy fatty acids

Measures of 3-hydroxy fatty acids (3-OHFAs) with 10- to 18-carbon chain lengths in a sample can be used to indicate the amount of lipopolysaccharide (LPS) and hence of mostly Gram-negative bacteria. In one LPS molecule, the toxic component lipid A backbone carries typically four molecules of 3-OHFAs of varying chain lengths. Sebastian & Larsson (2003) have

developed a method to characterize 3-OHFAs from environmental samples with GC-MS-MS. They have found that 3-OHFAs with 10-14 carbon chain lengths correlate well with endotoxin concentrations measured with the limulus assay. They also suggested that longer chain 3-OHFAs with 16 carbon atoms or more links to the presence of actinobacteria in a sample (Sebastian, 2005). Before the analysis, samples must be hydrolyzed because the marker compounds are linked covalently to various compounds in cell membranes. They are also purified by extractions and further derivatized.

The levels of 3-OHFAs in homes without a specific microbial source have varied between GM 20 – 130 pmol/mg for floor dust (Park, 2004; Täubel, 2009) and GM 26 – 30 pmol/mg for settled dust (Sebastian, 2005). The 3-OHFAs levels in bed dust samples have ranged between GM 31 – 155 pmol/mg (Park, 2004; Täubel, 2009). As for dust bag dust samples, the variation has been GM 29.8 – 30.2 pmol/mg (Hyvärinen, 2006b). The levels in schools have varied GM 17.6 – 30 pmol/mg in samples taken from floors, desks and chairs (Norbäck, 2016b; Zhao, 2008). Slightly higher levels 13.1 – 57.6 pmol/mg have been observed from settled dust collected from classrooms (Wady, 2004).

The levels of 3-OHFAs are increased by various factors, such as moisture damage, having livestock or pets, using fireplace, lower level of cleanliness in homes and with higher human occupancy in schools (Fox, 2003; Hyvärinen, 2006b; Kärkkäinen, 2010; Sordillo, 2011). The effect of season has been assessed in two studies performed in USA, with highest levels in house dust during summer (Sordillo, 2011) or fall (Park, 2004). Wady et al. (2004) reported on the geographical variation of 3-OHFAs levels in schools.

2.3.6 Muramic acid

Muramic acid (N-acetyl muramic acid (2-acetamido-3-O-[(R)-1 carboxyethyl]-2-deoxy-d-glucose) is an amino sugar and a constituent of the peptidoglycan layer of the cell walls of bacteria. The amount of peptidoglycan is much higher in Gram-positive bacteria (30 – 70% of the cell wall) than in Gram-

negative bacteria (< 10% of the cell wall) (Schleifer & Kandler, 1972). Hence, muramic acid is used as a chemical marker of mainly Gram-positive bacteria in the biomass of environmental samples, by using gas chromatography–tandem mass spectrometry (GC-MS-MS) (Sebastian, 2004), gas liquid chromatography mass spectrometry (GLC-MS) (Findlay, 1983) or high-pressure liquid chromatography (HPLC) (Mimura & Romano, 1985).

In settled dust samples collected from homes without a specific microbial source, mean muramic acid levels have ranged between 11 - 13 ng/mg (Sebastian, 2005). The levels in floor dust have varied GM 7.0 – 18 ng/mg (Adhikari, 2014; Lappalainen, 2008; Täubel, 2009). In two studies performed in Finnish farming and non-farming homes, the muramic acid levels in dust bag dust samples have been MD 26 ng/mg and GM 26 ng/mg, respectively (Kärkkäinen, 2010; Lappalainen, 2008). In bed dust samples, higher levels have been observed in farming homes compared to non-farming homes. Levels have varied between GM 16 – 160 ng/mg (Eder, 2006; Lappalainen, 2008; Täubel, 2009; van Strien, 2004).

In school study, Wady et al. (2004) found mean muramic acid levels varying 8.1 – 31 ng/mg in settled dust samples collected from urban and rural schools in three different countries. While in other schools studies, using dust samples collected from floors, desks and chairs, the levels have ranged GM 8.6 – 23 ng/mg (Cho, 2016; Norbäck, 2014; Norbäck, 2016b; Zhao, 2008), whereas in air samples the concentrations have varied between 1.44 – 2.84 ng/m^3 in two elementary schools (Liu, 2000).

In addition to farming, the factors that increase muramic acid levels in homes are heating with wood or coal, lower cleaning frequency, smoking and water damage and not having central air conditioning (Adhikari, 2014; Cho, 2016; Kärkkäinen, 2010; Lappalainen, 2008; Sordillo, 2011; Tischer, 2015; van Strien, 2004). It is known that occupancy is a determinant for bacterial levels in homes. It has been shown to greatly increase the muramic acid levels also in schools (Fox, 2003). Effect of season

varied in two different studies using different sample types and indoor environments, i.e. homes and schools (Sordillo, 2011; Wady, 2004).

2.3.7 Viable microbes

Traditionally, fungal and bacterial exposure has been assessed by cultivation technique. Cultivation is used to determine the number of viable microbes (colony forming units (CFU)) and to identify fungal colonies to genus and even species level. Indoor bacteria have seldom been identified. Airborne microbes are either collected directly on the growth media (impactors) or into a liquid medium (impingers). Samples collected on filters are suspended into a liquid and used for culturing (Nevalainen, 2015). The levels in house dust are usually analyzed with dilution method (Dales, 1997; Verhoeff, 1994a), but also direct spread of the dust on the growth medium can be used (Verhoeff, 1994a; Wickman, 1992). The total number of microbes can be determined with microscopical counting of spores and cells with light microscopy, epifluorescence microscopy or scanning electron microscopy (Blomquist, 1994; Eduard, 1990; Palmgren, 1986). Culture based methods have a disadvantage, since only a part (0.03-10%) of the microbes in indoor environmental samples are viable, that is alive and culturable (Golofit-Szymczak & Gorny, 2010; Pasanen, 1989; Toivola, 2002) and thus a large fraction of the microbes can stay undetected. Hence, it does not reveal the non-viable microbes contributing to the total microbial biomass, which may also have health effects (Hirvonen, 1997). There is also information about the immunological reactivity of fungal fragments extracts estimated by their cytotoxicity and the production of proinflammatory mediators (Gorny, 2004). Cultivation is selective because of the growth media used, since only those microbes are detected that favor or are capable to utilize the nutrients in the media. In addition, the culture method is laborious and needs a long incubation time (1 week minimum). Identification of the fungal colonies via macroscopic and microscopic evaluation requires training and a high level of expertise.

2.3.7.1 Viable fungi

Most studies on indoor viable fungi are focused on airborne samples, but exposure is estimated also with various house dust sample types, including floor dust (Chew, 2001; Dales, 1997; Jacob, 2002; Verhoeff, 1994b), dust bag dust (Miller, 1988), mattress dust (Jovanovic, 2004) and settled dust (Frankel, 2012). The most common genera or groups detected in different house dust sample types are *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* (Dallongeville, 2015; Jacob, 2002; Miller, 1988; Niemeier, 2006; Wickman, 1992).

In schools, airborne viable fungal concentrations have been observed to be lower than those in homes (Madureira, 2015; Toivola, 2004). This difference may be due to e.g. higher ventilation in schools, and lower impacts of other indoor sources e.g. pets, carpets, and plants in schools. In non-damaged schools in Finland, the airborne fungal concentrations have been very low varying during wintertime between 10^0 – 10^1 CFU/m^3 (Meklin, 2003; Meklin, 2005). According to a review of Salonen et al. (2015) the concentration of airborne viable fungi in different school studies varies to a large extent (10^0 - 10^3 cfu/m^3). Typical fungal genera or groups detected in school buildings have been *Penicillium*, *Cladosporium*, *Aspergillus*, yeasts and *Mucor* (Dotterud, 1995; Foarde & Berry, 2004; Madureira, 2014; Meklin, 2005; Orman, 2006; Salonen, 2015).

Mold, dampness and moldy odor have been observed to increase levels of viable fungi both in homes and schools (Dales, 1997; Dallongeville, 2015; Lignell, 2007; Meklin, 2005; Purokivi, 2001; Wickman, 1992). Also the age of the dwelling and type of housing has been shown to affect viable fungal levels (Chew, 2003; Dallongeville, 2015). Levels have also been associated with carpeted floors, presence of pets, smoking, aeration habits, temperature and relative humidity (Chew, 2003; Dallongeville, 2015; Verhoeff, 1994b). In schools, the flooring and building materials have been found to affect the fungal levels. The airborne levels have been higher on tiled floors compared to carpeted floors, suggesting that carpet flooring is not the major contributor to airborne levels of biocontaminants (Foarde &

Berry, 2004). In addition, Meklin et al. (2003) found significantly higher concentrations of viable airborne fungi in wooden schools than in concrete schools. The levels were shown to decrease with the use of mechanical ventilation (Bartlett, 2004a). Seasonal effects on indoor fungal exposure have been evaluated in various studies with contradicting results (Dallongeville, 2015; Horner, 2004; Koch, 2000).

In schools, fungal levels have been noticed to have diurnal and daily variation (Chen, 2012b; Mentese, 2012). Also Normand et al. (2016) found poor reliability of a single air-impaction measurement. Hyvärinen et al. (2001) observed that airborne fungal levels have great temporal and spatial variation, and hence, even 11 samples are needed to describe the overall fungal levels in indoor environments. Verhoeff et al. (1994a) found better reproducibility of the fungal levels in mattress dust than in floor dust. However, the predictive value of a single measurement was low. Also Chew et al. (2003) observed that variation of total culturable fungal levels in air and floor dust samples was similar within and between homes, showing that a single measurement of fungal propagules does not describe overall fungal levels and exposure in a home.

The correlations between different sample types have been studied in a study of Wurtz et al. (2005). The study showed good correlation between culturable fungi from air samples and from settled dust. However, the fungal levels between floor dust and settled dust did not correlate.

2.3.7.2 Viable bacteria

Similarly to fungi, exposure to viable bacteria indoors has been mainly determined from air samples. The culturable bacteria in house dust have only been analyzed in a few studies. The viable bacterial biota in dust seems to be dominated by Gram-positive genera, such as *Staplylococcus*, *Corynebacterium*, and *Lactococcus* (Rintala, 2012). Vogel et al. (2008) studied bacterial levels from mattress dust in farming homes and found *Bacillus* spp. and coliform bacteria being the most abundant groups of bacteria. Concentrations of viable mesophilic bacteria were 130×10^4 –

140 x 10^4 CFU/g when assessed from dust bag dust samples in the study of Hyvärinen et al. (2006b). In the same study, the levels of mesophilic actinomycetes were 6.5 x 10^3 – 10 x 10^3 CFU/g. In the study of Frankel et al. (2012) concentration of viable bacteria was 3.3 x 10^6 CFU/g in vacuumed dust from surfaces above floor level, while that of actinomycetes was 2.3 x 10^5 CFU/g. In another study conducted in farming and non-farming homes, bacterial levels ranged from 10 to over 6.5 x 10^7 CFU/g in dust bag dust samples (Kärkkäinen, 2010).

The levels of viable airborne bacteria in homes have varied between 10^0 – 10^4 CFU/m^3 (Dekoster & Thorne, 1995; Pessi, 2002; Reponen, 1992). The main genera found in the indoor air belong to *Micrococcus* and *Staphylococcus* groups (Gorny, 1999). In schools and day care centers, viable airborne bacterial concentrations have ranged between 7 x 10^0 – 2.0 x 10^4 CFU/m^3 according to a review of Daisey et al. (2003). The review states that the most commonly observed bacteria in the schools are *Micrococcus* and *Bacillus* species, pigmented Gram-negative rods, coryneforms and *Staphylococcus*.

Determinants affecting the viable bacterial levels have been the maintenance of the building, building frame material and farming (Hyvärinen, 2006b; Kärkkäinen, 2010). In school studies, the human occupancy has been shown to be one of the most important determinants affecting bacterial levels (Bartlett, 2004b; Liu, 2000). The occupancy in the building causes the elevation of especially Gram-positive bacteria (Fox, 2005). Naturally ventilated classrooms have been observed to contain higher bacterial levels than mechanically ventilated rooms (Bartlett, 2004b). Smedje et al. (2001) reported that classrooms cleaned with wet mopping showed higher airborne bacterial levels, but less settled dust than classrooms with dry mopping. Bacterial concentrations have also been higher in schools with moisture damage (Cho, 2016; Lignell, 2007; Meklin, 2002). The study of Lignell et al. (2007) showed that after the moisture damage renovation the airborne bacterial concentrations decreased to the level of a reference school. Bacterial levels have

been found higher in winter than in summer and have also shown remarkable daily and diurnal variations (Mentese, 2012).

2.3.8 Microbial DNA

Targeting microbial DNA allows the description of microbial exposure quantitatively or qualitatively, depending on the methods applied. In general, DNA based methods provide several advantages - determinations are typically quick, accurate, and have a high level of analytical sensitivity of detection. They also allow detection of dead or dormant organisms. This is important, since health effects of indoor microbial exposure are not considered to be solely or always dependent on viability (Gorny, 2004; Hirvonen, 1997). The first step in all DNA-based methods is the extraction of DNA from a sample. This happens by suspending microbial cells from the sample matrix, and the cell envelopes are disrupted either mechanically, enzymatically, and/or chemically to free the intracellular DNA, which is then purified for following analysis steps (Nevalainen, 2015).

Quantitative Polymerase Chain Reaction (qPCR) provides quantitative information on the amount of a specific taxonomic group targeted. Using qPCR with 'universal' fungal or bacterial primers gives a general estimate of total fungal or bacterial spore equivalents in a sample (Haugland, 2004; Rintala & Nevalainen, 2006). Primers and probes are designed for the detection of a taxonomic group, genus or a single species of interest. Fungal 18S ribosomal and internal transcribed spacer (ITS) regions or bacterial 16S ribosomal RNA genes are targeted, since they contain sequence regions that are highly conserved between members of the same genus or species, but the sequences are variable among different genera (Stetzenbach, 2004).

In studies of the indoor environments, qPCR method has been mostly used to target various fungal (Kaarakainen, 2009; Meklin, 2004; Pitkaranta, 2011; Yamamoto, 2011), but also bacterial groups (Adhikari, 2014; Kärkkäinen, 2010; Torvinen, 2010) from indoor dust samples. Moisture damage, observations of dampness and mold have been shown to increase levels of

several fungal groups, such as *Wallemia sebi*, *Trichoderma viride*, *Cladosporium sphaerospermum*, *Eurotium amstelodami*, and of streptomycetes (Kettleson, 2013; Lignell, 2008; Pitkaranta, 2011). In school studies associations of mold, dampness and moldy odor with fungal and bacterial DNA has also been observed for several PCR-markers and total fungal DNA (Cai, 2009; Jacobs, 2014b; Simoni, 2011). Other factors affecting the DNA markers in schools have been building constructions, and flooring (Cai, 2009; Cai, 2011).

Genetic profiling methods, such as denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism (SSCP) are used to visualize the diversity and changes in diversity of amplified genes from environmental DNA (Muyzer, 1993; Zumstein, 2000). However, the reproducibility of these is limited (Hong, 2007; Nunan, 2005), and so are sample throughput and resolution.

More recently, the microbiome of indoor environments has been studied with next generation (NGS) approaches, which allow virtually complete characterization of complex microbial communities. Microbial communities are primarily assessed with bacterial 16S and fungal ITS amplicon sequencing techniques. These new methods allow for high sample throughputs and enable describing the overall microbial diversity in hitherto unknown depth. The promise is – just as for the human microbiome – that applying these new techniques for the characterization of the indoor microbiome will provide answers on its role in human health and disease. Thus far, these methods have offered a view on the hitherto not fully appreciated vast diversity of bacteria and fungi, and improved our understanding of the microbial ecology in buildings (Stephens, 2016). Truly novel findings, in particular with respect to health effects in response to the indoor microbiome, have however yet to be presented.

Studies using NGS methods have found that typical bacterial phyla in dust samples are Firmicutes and *Actinobacteria*, while *Staphylococcus*, *Corynebacterium*, and *Lactococcus* are typical genera. Common fungal genera are *Cladosporium*, *Penicillium*,

Aspergillus and yeasts (Barberan, 2015; Dannemiller, 2014; Flannigan, 2011; Pitkäranta, 2008; Prussin & Marr, 2015). As observed also with other, earlier methods, the indoor microbiome is affected by outdoor air and by the building characteristics, such as the type of ventilation (Leung & Lee, 2016). NGS-results have also confirmed that human occupancy is one of the main sources for microbes in indoor environments, which is to a good part due to the fact that humans are shedding microbes from their bodies (Qian, 2012; Rintala, 2008; Ross & Neufeld, 2015; Täubel, 2009; Yamamoto, 2015). The human presence affects the indoor microbiome also due to resuspension of settled particles (Adams, 2015).

The microbiome is divergent in different sample types. Studies of the bacterial diversity in house dust by sequencing of 16S rRNA clone libraries have revealed that floor dust has a more diverse bacterial biota than mattress dust. Different determinants have been shown to affect bacterial and fungal microbiota, such as sample type (Rintala, 2008; Täubel, 2009), different outdoor environments such as urban versus suburban area and seasonal variation (Dannemiller, 2016; Pitkäranta, 2008).

***Table 1**. Microbial agents and applications in indoor environmental research*

Microbial agent	Short description	Applications in indoor environment research	References e.g.
Ergosterol	Primary sterol in cell membranes of filamentous fungi. Minor component in higher plants and present in the yeast cell wall membranes and mitochondria.	Ergosterol content is used as a marker of fungal biomass.	Axelsson et al. (1995) Dales et al. (1997) Saraf et al. (1997) Pasanen et al. (1999) Nielsen and Madsen (2000) Szponar and Larsson (2001) Sebastian and Larsson (2003)
Extracellural polysaccharides (EPS)	Stable, non-branched carbohydrate polymers, produced during fungal growth.	EPS content is used as a marker of fungal biomass (mainly species *Penicillium* and *Aspergillus*).	Douwes et al. (1999) Wouters et al. (2000) Chew et al. (2001)
(1→3)-β-D-glucans	Glucose polymers, originating from many sources, e.g. most fungi, some bacteria and plants.	The content of (1→3)-β-D-glucan is used as a proxy of bioactive portion of fungal biomass.	Rylander et al. (1992) Douwes et al. (1997) Wouters et al. (2000) Chew et al. (2001) Gehring et al. (2001a)
Endotoxin	Lipopolysaccharides (LPS), biologically active molecules that form the outer membrane of Gram-negative bacteria.	Endotoxin content describes the biological activity of LPS of Gram-negative bacteria.	Douwes et al. (1995) Alwis and Milton (2006) McKenzie et al. (2011) Thorne et al. (2010)
3-hydroxy fatty acids (3-OHFAs)	In one LPS molecule, the toxic component lipid A backbone, carries typically four molecules of 3-OHFAs.	3-OHFAs with 10- to 18-carbon chain lengths are indicating the amount of lipopolysaccharide (LPS) and hence gram-negative bacteria.	Sebastian and Larsson (2003) Sebastian et al. (2005)
Muramic acid	Amino sugar and a constituent of the peptidoglycan layer of the cell walls of bacteria. The amount of peptidoglycan is much higher in Gram-positive bacteria.	Muramic acid is used as a chemical marker of the biomass of Gram-positive bacteria.	Findlay et al. (1983) Mimura and Romano (1985) Sebastian et al. (2004)
Viable bacteria and fungi	Captures the alive fraction of the microbes in a sample.	Are determined by cultivation technique, which assesses the number of colony forming units (CFU). For fungi, species and genus can be determined via microscopy. Also direct spread of the sample on the growth medium can be used.	Dales et al. (1997) Verhoeff et al. (1994a) Wickmann et al. (1992)
Microbial nucleic acids	Contains the genetic information of a fungal or bacterial cell (DNA) or participates in converting of the genetic information (RNA). Present in the different cellular organelles and cytoplasm.	Are used in DNA-based methods (including quantitative PCR, denaturing gradient gel electrophoresis, single-strand conformation polymorphism and sequencing applications).	Muyzer et al. (1993) Zumstein et al. (2000) Haugland et al. (2004) Rintala and Nevalainen (2006) Stetzenbach et al. (2004) Pitkäranta et al. (2008) Adams et al. (2014) Dannemiller et al. (2016)

2.4 SAMPLING METHODS

2.4.1 Air samples

Bioaerosols can be collected with several sampling methods. The most commonly used methods are presented in Table 2. The selection of the method depends on the purpose of the microbial measurement or the study question, the environment to be monitored and on the resources available. Air sampling approaches are designed to capture culturable and/or non-culturable microbes. There are several physical principles behind the active air sampling methods: inertial impaction, centrifugal impaction, liquid impingement and filtration (Reponen, 2001; Willeke & Macher, 1999). Inertial impactors (with different number of stages), Burkard spore trap and surface-air-sampler (SAS) as well as different slit samplers are based on inertial impaction. Impaction directly on growth media in microbe-rich environments may allow only for very short sampling times to secure that the agar plates can be analyzed properly and are not overgrown. In Reuter Centrifugal samplers (RCS) and cyclone samplers centrifugal impaction is used (An, 2004; Willeke & Macher, 1999). Cyclone personal air samplers can be used to enable particle size fractioning by collecting bioaerosols into microcentrifuge tubes (Lindsley, 2006). The function of liquid impingers is based on inertial impaction and also diffusion within bubbles (Reponen, 2001). The method is convenient for higher expected microbial concentrations and it allows longer sampling time. Filtration is used for example in IOM (Kenny, 1999) and Button Inhalable Aerosol samplers (Kalatoor, 1995) (SKC Inc., Eighty Four, PA, USA). These samplers can be used both in stationary and personal sampling. In filtration, air is passing through filter medium and particles are collected to the filter (Willeke & Macher, 1999). All of the above sampling methods require active sampling using stationary or personal pumps. The samplers differ in their cut-off sizes (d50), which means the particle size above which 50% of particles or more are collected. The sampler should be selected with the cut-off size being below the particle size of

interest. For air sampling, which is mostly strictly limited with respect to the sampling collection duration, it should be noticed that a single measurement does not give a reliable picture of the microbial exposure in indoor environments, since airborne microbial concentrations vary both temporally and spatially, and a large number of air samples is needed to describe the microbial status of a building (Hyvärinen, 2001).

2.4.2 Dust samples

Due to the limitations of active air sampling, such as short sampling times and demands of human resources and equipment, different types of house dust samples have been used to represent a long-term, integrated exposure. They describe the airborne microbial exposure through mechanisms of deposition and resuspension of dust. House dust samples make large sample collections in epidemiological studies feasible, since they typically can be collected by the study participants themselves. However, there are limitations as to how well these surrogates reflect the actual relevant exposure.

Reservoir dusts such as floor dust, dust bag dust and mattress dust have been used in many studies as they are thought to integrate the microbial content of the indoor environment over time. However, these samples may contain material tracked indoors on shoes, clothes, paws, shedded from humans and pets etc., material which in part may never or only partly contribute to resuspension. Hence, reservoir dusts may not represent well the indoor *airborne* exposure. In mattress dust, the occupant itself is the major source of microbes, contributing skin and other human microbiota to the sample (Täubel, 2009). Also the time window of sample accumulation is variable, difficult to control and typically not precisely known for reservoir dusts. These facts can affect the disconnection of reservoir dust from airborne particulate matter (Adams, 2015). Studies have shown that different house dust sample types may not closely represent the airborne microbial inhalation exposure (Frankel, 2012; Noss, 2008). In addition, comparing the results of different studies can be challenging, since the protocols are often

similar but differing for sampling times, sampling areas as well as sample processing and storage. Reservoir dusts are usually collected by vacuuming into a sampling sock, onto a filter cassette or a nozzle (Hyvärinen, 2006b; Schram-Bijkerk, 2006; van Strien, 2004). House dust can also be obtained by collecting the homeowners' vacuum bags (Hyvärinen, 2006a). Dust can be sieved to achieve more homogenous subsamples and remove larger particles such as hair or stones that could interfere with downstream analyses, but analytical results may differ and are not fully comparable to analysis without homogenization.

Samples of settled dust, i.e. dust that has settled and is collected from surfaces above floor level, is considered to better represent the particles that have been airborne than for example dust bag dust (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004). Settled dust samples can be collected passively with simple and inexpensive sampling devices, such as cardboard boxes (dustfall collector; (Wurtz, 2005), petri dishes (Adams, 2013), glass plates (Wady, 2004), or electrostatic wipes (electrostatic dust fall collector (EDC) (Noss, 2008)). Settled dust can actively be collected by surface wiping (Niemeier, 2006; Thorne, 2005) or vacuuming into a sampling sock (Frankel, 2012).

Table 3 presents commonly used sampling methods for assessing microbial exposure from house dust.

***Table 2**. Sampling methods for assessing airborne microbial exposure*

Sampling principle	Example of device	Short description	References e.g.
Inertial impaction	Multistage impactor (single stage, 2-stage, 6-stage) e.g. Andersen impactor Surface-air-sampler (SAS)	Direct impaction on culture plates specifically applied for culturable microbes. Only for short sampling times.	Verhoeff et al. 1992 McLarnon et al. 2006
	Burkard spore trap impactor Slit samplers e.g. Mattson-Garvin Slit impact onto coated slide e.g. Allergenco	Determination for total counts. For larger sample volumes.	Jensen et al. 1992 Mehta et al. 1996 Levetin 2004 Macher et al. 2008
Centrifugal impaction	Reuter Centrifugal sampler (RCS) Cyclone samplers	Determination of culturable microbes. Only for short sampling times.	An et al. 2004 Lindsley et al. 2006
Impingement	AGI-30 BioSampler	Impaction into liquid.The method is convenient for higher microbial concentrations and it allows longer sampling time. For determination of e.g. chemical markers and qPCR.	Lin et al. 1999 Reponen et al. 2001
Filtration	IOM sampler Button sampler	Air is passing through filter medium and particles are collected to the filter. For determination of e.g. chemical markers and qPCR.	Kenny et al. 1999 Aizenberg et al. 2000 Burton et al. 2007

Table 3. *Sampling methods for assessing microbial exposure from house dust*

Sampling principle	Example of device	Short description	References e.g.
Floor dust	Vacuuming into a nylon sampling sock or ALK nozzle	Sample is vacuumed with a vacuum cleaner from a defined area of a carpet or a bare floor during defined time. Sampling time and area may vary in different studies.	Douwes et al. 1997 Wouters et al. 2000 Schram-Bijkerk et al. 2006
Mattress dust	Vacuuming into a nylon sampling sock or ALK nozzle	Sample is vacuumed with a vacuum cleaner from a defined area of a mattress during defined time. The sampling time and area may vary in different studies.	van Strien et al. 2004 Hyvärinen et al. 2006b Schram-Bijkerk et al. 2006
Dust bag dust	Collecting the study participant' s vacuum bag or the content of a central vacuum cleaner	The sample is collected by the study participant by vacuuming the home normally during a defined period of time. The sampling period varies in different studies.	Hyvärinen et al. 2006b Vesper et al. 2007
Settled dust	Electrostatic dust fall collector (EDC) Dustfall collector Petri dish Glass plate Surface wipe Vacuuming into a nylon sampling sock	Airborne dust is passively settling onto a sampling device or a surface, typically over a defined period of time. Sampling happens above floor level at a defined height. Sample accumulation time and height may vary in different studies.	Wady et al. 2004 Thorne et al. 2005 Wurtz et al. 2005 Niemeier et al. 2006 Noss et al. 2008 Frankel et al. 2012 Adams et al. 2013

2.4.3 Comparability of different sampling methods

A variety of sampling methods for assessing the microbial exposure in indoor environments, as shown in the previous chapter, are in use. However, the lack of standardized sampling methodology makes the comparison of the results from different studies difficult, and investigating the dose-response relationships between exposure and health effects is challenging (Frankel, 2012). The level of comparability between methods is briefly discussed here.

There are studies which have shown that microbial levels in passively settled dust strongly correlate with those in active air samples. Frankel et al. (2012) found that settled dust sample with the electrostatic dust collector (EDC) was the most representative sample type for the airborne microbial content. Culturable microbes and endotoxin were determined from different indoor samples including of indoor air, settled dust and reservoir dusts. In another study, endotoxin was determined from dust collected with EDCs and from indoor air samples collected with Button inhalable aerosol samplers. A strong and significant correlation was found between these two sample types (Kilburg-Basnyat, 2015). Similarly, Noss et al. (2008) found a strong correlation between endotoxin levels from samples collected with EDCs and Harvard impactors. However, also results with no significant correlation between airborne and settled dust have been reported (Hyvärinen, 2006a).

When studying the correlation of reservoir dusts and actively collected air samples, no or poor correlations have been found. In two studies assessing endotoxin levels from reservoir dusts (bed and floor dust) and air samples collected with filter samplers, no correlations between air and reservoir dust samples were found (Barnig, 2013; Park, 2000). Singh et al. (2011) found poor correlation for endotoxin and (1→3)-β-D-glucan levels between floor dust and active air samples collected by bioaerosol cyclone sampler and Button sampler. Also Hyvärinen et al. (2006a) observed poor correlation of endotoxin levels between reservoir dusts (floor, bed and dust bag dust) and air

samples collected with IOM sampler. In some other studies, levels of culturable fungi in house dust have not been found to be representative of those from indoor air (Chew, 2003; Miller, 1988; Ren, 1999).

Somewhat better correlations have been reported when comparing different *reservoir* sample types to one another. Hyvärinen et al. (2006a) found endotoxin concentrations correlating moderately between floor dust and bed dust samples, floor dust and dust bag dust samples, and bed dust and dust bag dust samples. Kaarakainen et al. (2009) studied microbial concentrations using qPCR and found that the correlation between floor dust and dust bag dust samples varied for different microbial species and groups from poor to moderate. Park et al. (2000) did not find correlation between endotoxin levels in bed dust samples and floor dust samples, while floor dust samples collected from kitchens and bedrooms correlated well.

In summary it appears that the knowledge on sampling that would represent the relevant indoor microbial exposure is still limited and more research is needed to better describe the actual microbial exposure. The breakthroughs of assessing the health effects caused by the exposure will rely – among other things - on proper sampling methods the quantitatively and qualitatively represent the relevant microbial exposures.

2.4.4 Reproducibility of microbial levels using different sampling methods

To adequately assess the microbial exposure, the method for measuring the exposure should be well reproducible. In order to characterize the method to be reproducible, repeated samples taken over time should be highly correlated i.e. the variation of microbial levels should be smaller within study buildings than between study buildings, in the repeated samples. In the study of Kaarakainen et al. (2009), the microbial concentrations in study homes determined by qPCR had good or moderate repeatability in the parallel subsamples of floor dust and dust bag dust samples. The reproducibility was weak, as within-

home variation was greater than between home variation in the repeated samples. Hyvärinen et al. (2006a) studied the reproducibility of endotoxin levels in different sample types collected repeatedly over one year. Bed dust samples showed the best reproducibility. The reproducibility of settled dust and floor dust samples was moderate, while the reproducibility of dust bag dust samples was poor. Also Park et al. (2000) found lower within-home variation than between-home variation for endotoxin concentrations in bed dust samples, showing good reproducibility. For floor dust and airborne samples, however, the within-home variation was greater than between-home variation. Topp et al. (2003) reported poor reproducibility for endotoxin levels in repeated floor dust samples collected over a time period of six years. The group concluded, that a single floor dust measurement does not accurately reflect the true long-term exposure. For ergosterol concentrations, Matheson et al. (2003) found poor within-home variation for floor dust samples, when the samples were collected twice in two years interval. The reproducibility of floor dust samples has also been poor for determinations of viable fungi (Verhoeff, 1994a).

3 Aims of the study

This thesis combines the results of the original publications I-IV. The overall aim of the current thesis was to assess the microbial exposure in different indoor environments by using house dust samples and samples of indoor air.

The specific aims were:

1. To assess the determinants - including mold and moisture damage, housing, living and environmental characteristics - that affect the levels of different microbial agents in residential homes and schools (publications I, II and III).
2. To determine the reproducibility of microbial levels in repeated sampling campaigns over time in indoor dust and air samples (publications I–IV).
3. To investigate how well different house dust sample types reflect the fungal and bacterial content determined from indoor air; and to assess how stationary indoor sampling in homes compares to personal air sampling in terms of the daily microbial exposure in rural and urban sites (publication IV).

4 Material and methods

4.1 STUDY CHARACTERISTICS

This thesis is based on four studies: the Finnish birth cohort study LUKAS1 (childhood living environment and allergy) study; a dust validation study; HITEA (Health Effects of Indoor Pollutants: Integrating microbial, toxicological and epidemiological approaches) study and HNO (Comparison of sampling methods) study. The publications I and II include LUKAS1 and the dust validation study; publication III is based on HITEA study; and publication IV is based on the HNO study. The characteristics of the studies are presented in Table 4.

4.1.1 Study I: the LUKAS1 study (publications I and II)

The LUKAS1 study is the Finnish arm of the international birth cohort PASTURE study (von Mutius, 2006). The study population consisted of 112 farming and 102 non-farming Finnish homes with a newborn child. The children were recruited between September 2002 and May 2004. All pregnant women living on farms and an equal number of non-farming women living in rural areas were invited to the study at 20-34 weeks of pregnancy in the areas of four central hospitals in Eastern and Middle Finland (Kuopio, Jyväskylä, Joensuu and Iisalmi). The recruitment of the study population is presented in Figure 1.

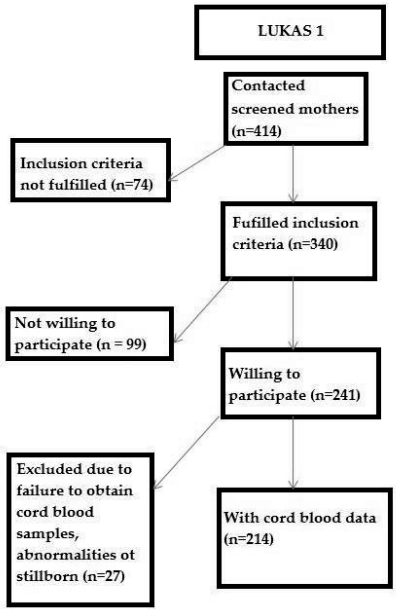

***Figure 1**. Recruitment of the study population in LUKAS1. (Modified from Karvonen et al. 2009)*

4.1.2 Study II: Dust validation study (publications I and II)

The second part of publications I and II is the dust validation study (Kaarakainen, 2009). Study sites consisted of five urban homes that had no dampness or mold problems or other obvious exceptional microbial sources, except that in two of these homes, a dog was kept as a pet. The homes were located in the city of Kuopio, Finland. The study homes were selected to represent different types of housing and family characteristics. The study sites were recruited in 2004.

4.1.3 Study III: the HITEA school study (publication III)

The HITEA school study is a multi-center European Framework Programme 7 project. A sample of 738 schools in three European countries (the Netherlands, Spain, and Finland),

representing three climatic regions, was addressed with a screening questionnaire focusing on moisture damage, dampness and mold observations in the school buildings (Haverinen-Shaughnessy, 2012). Based on this questionnaire, a minimum of 20 schools per country were selected for further studies, targeting a minimum of 10 schools with and 10 schools without self-reported moisture problems. These schools were surveyed by centrally trained personnel for their dampness, moisture and mold observations and other indicators of indoor air quality using visual observation. Categorization of the study schools was done based on moisture damage, dampness and mold observations into index and reference schools. From these inspected schools, seven schools in Spain (five index and two reference), ten schools in the Netherlands (five index and five reference) and six schools in Finland (four index and two reference) were included for the detailed, longitudinal exposure assessment, including three repeated measurement campaigns over a little more than one year; during winter/spring 2009 (Exposure assessment (EA)1), during spring/summer 2009 (EA2), and during winter/spring 2010 (EA3). Details on the final selection of schools have been described by Borrás-Santos et al. (2013).

4.1.4 Study IV: HNO study (publication IV)

The HNO study consisted of five urban and four rural homes located in Eastern Finland. The sampling campaigns – including actively collected indoor and personal air, settled dust, floor dust, mattress dust and dust bag dust – were carried out 5 repeated times over one year February 2007 – March 2008.

***Table 4**. Characteristics of the four studies included in publications I-IV*

	LUKAS 1 (publications I,II)	**Dust validation –study (publications I, II)**	**HITEA (publication III)**	**HNO (publication IV)**
Objective	To explore determinants of microbial levels in house dust	To assess reproducibility of microbial levels in house dust	Determinants of microbial levels in schools	To compare microbial content of various indoor samples with that of indoor air
Number of samples (N)	107 Finnish farming and 105 non-farming homes	20 (5 Finnish urban homes, one sample per season over one year)	Altogether 302 pooled samples from 14 index (i.e. moisture damaged) and 9 reference schools Spain, the Netherlands and Finland, collected in a longitudinal assessment three times over one year	45 x 6 (4 rural and 5 urban Finnish homes, sampled five times over one year, 6 sample types)
Sample type	Floor dust, dust bag dust	Floor dust, dust bag dust	Settled dust (EDC and SDB)	Actively collected personal and indoor air samples (Button), settled dust, floor dust, mattress dust, dust bag dust
Laboratory determinations	Ergosterol, 3-OHFAs and muramic acid (floor and dust bag dust) and viable fungi and bacteria including actinomycetes (dust bag dust)	Ergosterol, 3-OHFAs and muramic acid	qPCR, endotoxin, ergosterol and (1→3)-β-D-glucan	qPCR

4.2 BUILDING INSPECTIONS

In studies I, II and IV, visual building inspections for signs of moisture or mold damage were made by a trained civil engineer using a standardized protocol (Nevalainen, 1998). The inspection included recording of the location, severity and extent of moisture damage, and information on construction and wall coverings. The severity of the moisture damage was defined into three classes based on a six-point 'need for repair' estimation scale and the area of the damage: no damage, minor damage, and major damage. The six classes were (0) no damages with repair; (1) only cosmetic repair; (2) repair of surface materials; (3) structural components need to be repaired; (4) and (5) more extensive repair needed. From these classes, 0 and 1 belonged to class 'No damage'. In study I, 'Major damage' was formed from three different cases: (1) Repair needed class 2 with area of damage $\geqslant$1 m^2, (2) repair needed class 3 with area of damage $\geqslant$0.1 m^2, or (3) repair needed class 4 or 5. Other damages were classified as 'minor damage'. Observed mold during the inspection was categorized into three classes: no mold, spots of mold and visible mold.

In study III, centrally trained personnel performed visual school building inspection, utilizing standardized protocols for observations of moisture damage and visible mold (Haverinen-Shaughnessy, 2005), including also measurement of surface moisture. Details on this multi-phase assessment of schools in three European countries, the protocols used and information collected during the walk-through building inspections have been presented by Haverinen-Shaughnessy et al. (2012).

4.3 BUILDING CHARACTERISTICS, MAINTENANCE AND LIFESTYLE QUESTIONNAIRES, ENVIRONMENTAL MONITORING

In LUKAS1 information on housing and lifestyle characteristics was obtained via questionnaire, which was administrated by the study nurses during a home visit, when the child was two months old. The questionnaire included questions about housing characteristics (e.g. type of dwelling, type of ground slab, type of roof, ventilation system, and having a cellar), lifestyle (e.g. using a fireplace, time since last vacuum cleaning, having pets and smoking) as well as farming (e.g. number of livestock and distance to fields receiving manure fertilization). Weather and outdoor air data were produced by the Finnish Meteorological Institute and the Finnish Aerobiology Group.

In the dust validation study, following information was collected during the home visits: the housing type, construction year, type of construction, floor area, ventilation, number of persons living in the dwelling and pets.

In the HITEA school study, the janitorial service in charge of school maintenance was interviewed during the building inspections, and detailed data on building characteristics, such as building age, number of rooms, building materials, type of roof, ventilation and repairs that had been made, and building maintenance were collected. In addition, a questionnaire concerning the cleaning practices and habits was administrated to the head of cleaning personnel or principal of the schools. Moreover, environmental characteristics such as the number of heavy and light vehicles passing the nearest major road and the distance to the major road and highway, were collected by the study personnel. Weather and outdoor air data were produced by the nearby weather stations. In addition, indoor air temperature, relative humidity, and CO_2 levels were measured in the first and last sampling period with the Q-Trak Indoor Air Quality Meter (Model 7565, TSI Incorporated, St. Paul, MN, USA) every 5 min during 1 week from one classroom per school (Jacobs, 2014a). $PM_{2.5}$ was monitored from hallway

during the 8 week period with the Harvard impactor: 8 consequent hours per day for five school days.

In HNO, following housing characteristics were recorded: type of housing, number of occupants, construction year, floor area, ventilation and pets.

4.4 SAMPLING AND SAMPLE TREATMENT

4.4.1 Reservoir samples: floor dust, dust bag dust and mattress dust

In studies I, II and IV floor dust samples were collected into a nylon sampling sock by vacuuming for two minutes per one square meter of a rug, for example 8 min for 4 m^2. If there were no rugs the sample was collected by vacuuming an area of 4 m^2 of bare floor for 4 min. The vacuum cleaner dust bag dust samples were collected in studies I, II and IV by the tenants of the homes by providing a dust bag that had been used for regular vacuuming of the dwelling normally for a defined time period of two months. At the beginning of the sampling period, the occupants were asked to attach a new dust bag to their vacuum cleaner and they were instructed not to vacuum balconies, garages, or cars. After collection, samples were stored in dark at 4 °C before further processing. To obtain a homogenous sample of fine dust, large particles and fibrous material was separated from the floor dust samples by sieving through a sterile strainer (pore size 1 x 1 mm) with the resulting fine dust dried in a desiccator. Dust bag dust samples were sieved through a strainer (pore size 1 x 2 mm). All the samples were stored at -20 °C in dark prior analyses.

The mattress dust was collected in study IV into a nylon sampling sock by vacuuming two minutes the area of the whole mattress. Blankets, duvets and sheets were removed before sampling, but bed linen or mattress covers were left on the bed (Hyvärinen, 2006a). The samples were sieved through a sterile strainer (pore size 1 x 1 mm) to achieve a homogeneous sample

of fine dust. The samples were then dried in a desiccator at 4 °C for a period of 2 days and stored at -20 °C in dark prior analyses.

4.4.2 Settled dust samples

In study III, settled dust samples were collected in settled dust boxes (SDBs) and electrostatic dust fall collectors (EDCs). SDBs were polyethylene coated cardboard boxes with dimensions of 45 cm x 20 cm. EDCs contained two electrostatic cloths (Zeeman, Utrecht, the Netherlands) with a plastic frame. The SDBs and EDCs were placed in a height of 1.5 to 2.5 m; primarily in classrooms, but also in other indoor locations of the school building, where pupils and teachers spend a considerable amount of their time (eg. hallways, teachers' lounges, libraries and similar). Per school on average 15 locations were sampled. The sampling strategy also considered observations of moisture damage, dampness and visible mold made in the/during school building inspections, by sampling a similar proportion of rooms with moisture problems as determined for the whole school building in the/during the building inspections. During each sampling period, the same locations were targeted whenever possible. Samplers were placed away from windows, doors, ventilation ducts, and heating units. The settled dust was collected over a period of 8 weeks. After sampling, samplers were closed and transferred to the local study centers. In clean laboratory facilities, the settled dust was vacuumed out of the SDBs onto mixed cellulose ester (MCE)-filters, suspended in 5 ml of dilution buffer (distilled water with 43 mg/l KH_2PO_4, 250 mg/l $MgSO_4 \cdot 7\ H_2O$, 8 mg/l NaOH, and 0.02% Tween 80), sonicated for 15 min in an ultrasound bath (Bransonic B-3510) and 15 min in a shaker (IKA KS 125). The filters were removed, the suspensions were transferred into 15 ml screw cap plastic vials and stored at -20 °C until further processing. For each school, classrooms (N=2-5) closely located to each other in the school buildings were pooled into "classroom pools", typically yielding 3 to 5 such pools per school. In addition, one "other location pool" was created per school, combing settled dusts of hallways, teachers' lounges and other commonly occupied

locations in the school buildings. Altogether 302 sample pools were obtained for microbial analyses. For EDCs, one cloth per sampler was extracted as described in the study of Noss et al. (2008). The cloths were incubated for 60 min in 20 ml pyrogen-free water (B. Braun NPBI, Oss, the Netherlands) in an end-over-end roller. After centrifugation, the supernatant was stored at -20 °C (Jacobs, 2014a). While no pooling of the individual samples was performed during the laboratory analyses, we calculated the means for endotoxin and glucan concentrations of the individual sampling locations (classrooms and other locations) constituting one 'pool', as defined for the other microbial measurements.

In study IV, settled dust was collected into cardboard boxes (22 x 31 cm) over a period of two months. These SDBs were located well above floor level (typically at a height of 1.5 to 2 m) in locations away from major air flows in the main living areas, such as living rooms and bedrooms (Hyvärinen, 2006a). Two boxes were used in parallel to ensure collection of sufficient amount of dust. SDBs were stored at room temperature before vacuuming to SKC glass fiber filters, diameter of 25 mm. One to four sterile filters were used per one sample (two SDBs) depending on the amount of dust. Dust collected in the inner area of the box (22 x 31 cm) was vacuumed thoroughly to the filter using a pump with flow rate of approximately 5 l/min (Neuberger). The filter was then put into a beaker and extracted into 5 ml of dilution buffer (distilled water with 43 mg/l KH_2PO_4, 250 mg/l $MgSO_4 \cdot 7\ H_2O$, 8 mg/l NaOH, and 0.02% Tween 80). Samples were processed for 15 min in an ultrasound bath (Bransonic B-3510) and 15 min in a shaker (IKA KS 125). In cases, where there was much dust accumulated in the settled dust boxes requiring use of several filters, extractions of multiple filters (up to 4) were done in the same ratio as above (1 filter to 5 ml) and combined. The extraction liquid was stored at -20 °C in dark until the analyses.

4.4.3 Stationary and personal air samples

In study IV, air samples were collected onto polycarbonate membrane filters (Millipore, Billerica, MA, USA, pore size 0.8 μm) at a flow rate 4 l/min, over a period of 24 hours with "The Button" Personal Inhalable Aerosol Sampler (Kalatoor, 1995) (SKC Inc., Eighty Four, PA, USA). The indoor air sample was collected from the same room as the settled dust sample in a standardized height of 1.5 m. The personal air sample was collected so that the person carried the sampler and the pump everywhere she/he was going, i.e. the main indoor and outdoor locations the person spent time during the course of one day. The filters used for air sampling were equilibrated at constant temperature and relative humidity and weighed before and after sampling. After sampling and weighing, the filters were transferred to -20 °C.

4.5 CHEMICAL MARKER ANALYSIS

4.5.1 Determination of ergosterol

For samples in studies I and II, pre-treatment of ergosterol was performed as described by Sebastian and Larsson (2003). For ergosterol analyses 4-10 mg of accurately weighed dust was used in the analyses. An Agilent 6890 gas chromatograph (GC) connected to Waters AutoSpec Ultima high-resolution mass spectrometer (HRMS) equipped with a J&W Scientific DB-1MS capillary column (column length: 12 m, column diameter: 0.20 mm, film thickness: 0.33 μm) was used for the dust bag samples in LUKAS1 study. For floor dust samples in LUKAS1 study and both floor dust and dust bag dust in dust validation study, an Agilent 6890 GC with an Agilent Technologies 5973 MS detector with an Agilent capillary column (column length: 30 m, column diameter: 0.25 mm, film thickness: 0.25 μm) was used. Details of the GC-MS runs are presented in the publication I.

In study III, 2 ml of settled dust suspension (SDBs) were freeze-dried prior to sample preparation. The sample preparation of ergosterol was carried out by modifying the

method published by Axelsson et al. (1995). The samples were diluted to 100 ml of hexane before analysis. The analyses were performed with a PolarisQ ion trap mass spectrometer (MS–MS) from Thermo (Austin, TX, USA) equipped with a Trace GC-ultra gas chromatograph (GC) from (Milan, Italy) with a DB-5MS fused-silica capillary column from J&W Scientific.

4.5.2 Determination of (1→3)-β-D-glucans and endotoxin

In study III, levels of (1→3)-β-D-glucans and endotoxin were determined from EDC samples. (1→3)-β-D-glucans were analyzed with a specific sandwich enzyme immunoassay (EIA) as described in the study of Noss et al. (2010). The limit for detection of the assay was 200 ng/m^2. Endotoxin was analyzed with the Limulus amebocyte lysate (LAL) assay (Lonza Group, Basel, Switzerland) according to the manufacturer's protocol. Samples were tested in 1:25 and/or 1:50 dilutions. The resulting endotoxin units (EU) were converted to EU/m^2 (Noss, 2008). The limit of the assay was assessed at 300 EU/m^2. The analyzing of (1→3)-β-D-glucans and endotoxin is described previously in detail (Jacobs, 2014a; Jacobs, 2014b; Krop, 2014).

4.5.3 Determination of 3-OHFAs and muramic acid

In studies I and II, 3-OHFAs were analyzed using a slight modification of the method published by Sebastian and Larsson (2003); muramic acid was analyzed by modifying the method of Sebastian et al. (2004). Generally, hexane was used instead of heptane and in the first extraction of 3-OHFAs no water was used. In the analysis of muramic acid, chloroform was replaced with acetone for safety reasons. The muramic acid samples were diluted to 150 µl of acetone before analyzing them by gas chromatography tandem mass spectrometry (GC-MS-MS). The GC-MS-MS analyses parameters are reported in the study of Lappalainen et al. (2008). Limits of quantification for muramic acid and individual 3-OHFAs were 4 ng/mg and 2 pmol/mg, respectively.

4.6 ANALYSIS OF VIABLE MICROBES

Viable fungi and bacteria as well as actinomycetes were determined in study I (publications I and II) from 5 to 16 g of dust bag dust samples by cultivation as described earlier by Hyvärinen et al. (2006b). Dilution series were made for all samples and cultured on 2% malt extract agar (M2) for mesophilic fungi, dichloran glycerol agar (DG18) for xerophilic fungi and tryptone yeast extract glucose agar for viable bacteria and actinomycetes. The samples were incubated for 7 days at 25 °C in dark before enumeration of colonies and morphological identification to genus, species, or group level using an optical microscope. Actinomycetes colonies were counted after 14 days.

4.7 ANALYSIS OF MICROBIAL TAXA USING QPCR

In study III, 1.8 ml of settled dust suspensions were used for DNA extraction. The suspensions were centrifuged (10 minutes at 16.000 x g), the supernatant was reduced to 100 µl and the dust pellet resuspended and transferred into an Eppendorf tube containing glass beads. DNA extraction in study IV from mattress, floor and dust bag samples was done from 20 mg weighted dried fine dust. Settled dust samples were extracted from typically 4 ml of the settled dust extracts (prepared as described under 3.4.2). For air samples, filters were directly transferred into a glass bead tube for DNA extraction. DNA extraction including a bead-beating step for mechanical cell disruption was performed as described earlier (Kärkkäinen, 2010; Rintala & Nevalainen, 2006). In studies III and IV altogether 11 different quantitative PCR assays were performed, targeting following microbial groups or species: *Penicillium/ Aspergillus / Paecilomyces variotii* group, *Cladosporium herbarum*, *Eurotium amsteldamii*, *Penicillium chrysogenum*, *Trichoderma viride/ atroviride/ koningii*, *Stachybotrys chartarum*, *Wallemia sebi*, *Mycobacterium* spp., *Streptomyces* spp., Unifung assay and Gram-positive and Gram-negative bacteria. The qPCR primers and

probes used have been published earlier (Haugland, 2003; Haugland, 2004; Kärkkäinen, 2010; Rintala & Nevalainen, 2006; Torvinen, 2010). For study III, the qPCR laboratory analyses and calculations were performed as described by Kaarakainen et al. (2009), using the ABI Prism 7000 (Applied Biosystems) and the RotorGene 3000 (Corbett Life Science) equipment.

For study IV qPCR reactions were largely performed as described in the original publications with minor modifications (Haugland, 2003; Haugland, 2004; Kärkkäinen, 2010). In the bacterial duplex assay (Gram-positive and negative bacteria) 20 µl reaction mix included 10 µl of Environmental Master Mix (Applied Biosystems Inc., Foster City, CA), 1.5 µl Bovine serum albumin (2 mg/ml), 1 µl of forward and reverse primers, 0.4 µl of a both TaqMan probes, 3.7 µl of nuclease free water (HyClone Laboratories Inc., Utah, USA) and 2 µl of template DNA. Reactions were performed in 0.2 ml 96-Well plates (Agilent Technologies Inc., USA) in Stratagene Mx3005P qPCR System (Agilent Technologies Inc., USA). Numbers of microbial cell equivalents in the samples were calculated using standard curves and relative quantification as described earlier (Haugland, 2004).

4.8 STATISTICAL ANALYSES

4.8.1 Analyses of determinants

The concentrations of the microbial agents were not normally distributed and were hence natural log-transformed (I-III). For studying the determinants affecting the levels of different microbial agents SPSS software (version 15.0 and 21.0) (SPSS Inc., 1988) was used. For studies I and III analysis of variance for one dependent factor were tested with general linear model univariate procedure. For study I, determinants with a significance level lower than 0.2 were included into a multiple linear regression model. In addition, a limited set of variables that was commonly and uniformly tested for all fungal agents and dust sample types, a group of 12 variables that most

constantly affected on the levels of fungal agents from the final models was selected. Determinants were not dropped out from these models. All associations are presented as means ratios, that is, the ratio of the geometric mean in the respective category versus the reference category.

4.8.2 Analyses of reproducibility

For studying the reproducibility in studies II and IV, within- and between-home/school variability was estimated using random effects analysis of variance (SAS/PROC MIXED; SAS Institute, Cary, NC, USA). Intraclass correlation values (ICCs) were calculated to express the repeatability of five subsamples, analyzed from one sample, within the same home and season (Study II); reproducibility of samples taken within the same home, throughout one year (Studies II and IV); and reproducibility of samples taken within the same home (mean of five parallel subsamples) in different seasons (Study II). In study III, intraclass correlations were calculated to explore the reproducibility of exposure between and within schools. These analyses were done with SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) using log-transformed data.

4.8.3 Analyses for differences in microbial levels and correlations

In study III, non-parametric statistical methods were used, as the microbial concentrations were not normally distributed. Spearman correlation was used to test the correlation between different microbial markers in repeated assessments, as well as between air exchange rates, occupancy and cleaning frequency in classrooms and the microbial levels. Differences in microbial levels between countries and phases (over time) were tested using Wilcoxon Scores (Rank Sums) and Kruskal Wallis statistical test. Differences in the prevalence of the microbes in settled dust and correlation of microbial exposure over time was studied using Chi-square test. Differences in microbial levels between index and reference schools were tested using Wilcoxon two sample test and Kruskal Wallis test. A continuous

moisture damage severity score was created based on the area and severity of moisture damage and visible mold in classrooms and other locations in the school buildings. Microbial levels were analyzed against tertiles of moisture damage score using Kruskal Wallis test. In addition, the specific analyses on occupancy were done by calculating the average occupancy (number of students) for the classrooms in each classroom pooled sample, and related to the respective microbial measurements per pool. Two scores were developed based on a) the frequency of performing certain cleaning tasks in classrooms (dusting of surfaces, vacuuming of the floor, sweeping the floor, wet cleaning of the floor) and b) the frequency of using certain cleaning products, i.e. multi-use cleaning products, hypochlorite bleach, and disinfectants other than bleach. The statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., 1999) and with the SPSS statistical package version 21.0 (SPSS Inc., 1988).

Also in study IV, non-parametric statistical methods were used, as the microbial concentrations were not normally distributed. Differences between different sample types, seasons and rural and urban homes were analyzed using Kruskal-Wallis test and further tested using Dunn's test, when applicable. Correlations of the different microbial agents and sample types were analyzed using Spearman rank correlation. Medians of the microbial marker ratios were analyzed as ratios of Gram-positive versus Gram-negative bacteria and of *Penicillium*/*Aspergillus* spp. versus total fungal DNA. Analyses were made using IBM SPSS Statistics, version 22. The correlations figures were plotted in ggplot and corrplot packages in R programming language version 3.2.4 (The R Foundation for the Statistical Computing, 2016).

5 Results

5.1 MICROBIAL LEVELS IN DIFFERENT SAMPLE TYPES

Concentrations of the studied fungal and bacterial agents are presented in Table 5 (publications I and II), Table 6 (publication III) and in Figures 2 and 3 (publication IV). In study I (publications I and II) carried out in residential homes, the levels of ergosterol, 3-OHFAs and muramic acid were slightly higher in dust bag dust than in floor dust. Viable microbes were measured only from dust bag samples. Levels of mesophilic and xerophilic viable fungi were comparable to each other, but levels of mesophilic bacteria were slightly higher. In study IV, comparing different reservoir dust sample types, the concentrations (cells/mg) of the studied fungal and bacterial species or groups were consistently and significantly highest in dust bag dust samples, followed by floor dust and mattress dust samples (Figure 2). When expressing the microbial levels as load per sampling area (cells/m^2) for floor, mattress and settled dust samples, the highest values were obtained in floor dust samples (Figure 3). Bacterial levels were higher in mattress dust compared to settled dust; fungal levels were higher in settled dust and lowest in mattress dust (Figure 3).

The levels of all studied microbial species/groups in study IV were higher in rural than in urban homes in all sample types, with the exception of mattress dust. In mattress dust samples, while the concentrations of fungal markers were significantly higher in rural homes, the levels of bacterial markers were not. When considering rural and urban homes separately, microbial concentrations in personal air samples were 5-22 times higher than in indoor air samples in rural environments. In urban homes, these differences were only marginal (Figure 4). Also when calculating medians of the microbial marker ratios (Gram-positive vs. Gram-negative

bacteria; *Penicillium*/*Aspergillus* spp. vs. total fungi), the ratios were largely different between rural and urban environments, especially in indoor air and personal air samples. The medians of the ratios were much higher in rural homes than in urban homes. In rural homes, a close to equal contribution of Gram-positive and Gram-negative bacteria was observed in indoor air, in settled dust and dust bag dust, whereas in urban homes Gram-negative bacteria dominated these sample types.

When considering the differences in microbial levels for floor, mattress and dust bag dust samples in study IV, the highest concentrations (cells/mg) and loads (cells/m^2) were observed for Gram-positive bacteria, as compared to other sample types. In actively collected indoor air and personal air samples (cells/m^3), Gram-negative bacteria were present at the highest levels. Also when calculating medians of the microbial marker ratios (Gram-positive vs. Gram-negative bacteria; *Penicillium*/*Aspergillus* spp. vs. total fungi), reservoir dust samples exaggerated the presence of Gram-positive versus Gram-negative bacteria and underestimated the contribution of *Penicillium*/*Aspergillus* spp. to the total fungal content when compared to samples of indoor air. This was particularly true for mattress dust and floor dust.

In settled dust collected from schools (study III), bacterial levels differed significantly between the studied countries (Table 6). In Spain, the bacterial levels determined by qPCR were generally highest, while Dutch schools showed the highest levels of endotoxin and specific bacterial genera of *Streptomycetes* spp.. The bacterial levels were lowest in Finland for all the studied microbial agents. Also fungal levels assessed with qPCR and chemical markers differed significantly between countries. In Spain, the levels were generally highest, while the levels were again lowest in Finland. Levels of the more prevalent fungal markers were found highest in Spain, with the exception of (1→3)-β-D-glucans, which were highest in the Netherlands.

***Table 5**. Levels of microbial markers presented as geometric means (GM), geometric standard deviations (GSD), and interquartile range (IQR; 25th percentile – 75th percentile) in floor and dust bag dust samples in study I (Combined from publications I and II)*

	Floor dust		Dust bag dust		Publication
	GM (GSD)	**IQR**	**GM (GSD)**	**IQR**	
Ergosterol (ng/mg)	9.00 (1.99)	5.68-13.7	14.4 (2.33)	9.20-21.9	I
Mesophilic viable fungi (cfu/g)	n.a		4.55×10^5 (3.11)	2.05×10^5 – 1.09×10^6	I
Xerophilic viable fungi (cfu/g)	n.a		4.51×10^5 (2.99)	2.61×10^5 – 8.12×10^5	I
3-hydroxy fatty acids (chain length of 10–16 carbons)(pmol/mg)	24.3 (1.68)	17.8–33.6	26.5 (1.59)	20.2–35.8	II
Muramic acid (ng/mg)	17.6 (1.92)	12.7–25.5	25.9 (1.96)	17.4–40.2	II
Viable bacteria (cfu/g)	n.a		6.50×10^6 (3.27)	3.50×10^6 - 1.33×10^7	II
Actinomycetes (cfu/g)	n.a		1.96×10^3 (81.5)	5.01×10^2 - 5.00×10^4	II

n.a not analyzed

***Table 6**. Percentiles of microbial concentrations and samples < detection limit determined from settled dust in study III in Spanish, Dutch and Finnish HITEA schools via qPCR (cells/SDB/day), chemical markers (ergosterol; ng/SDB/day), endotoxin and glucans (publication III)*

	Spain					The Netherlands					Finland				
Microbial target	N	<DL (%)	25	50	75	N	<DL (%)	25	50	75	N	<DL (%)	25	50	75
qPCR markers															
*C. herbarum**	92	2 (2)	30	91	330	130	3 (2)	17	62	370	80	53 (66)	0	0	4.7
*Penicillium chrysogenum**	92	66(72)	0	0	96	130	99(76)	0	0	0	80	77(96)	0	0	0
*Eurotium amsteldamii**	92	5(5)	73	160	540	130	1(1)	52	130	320	80	37(46)	0	12	38
Stachybotrys chartarum	92	88(96)	0	0	0	130	122(94)	0	0	0	80	79(99)	0	0	0
Trichoderma viride	92	90(98)	0	0	0	130	121(93)	0	0	0	80	79(99)	0	0	0
*Wallemia sebi**	92	90(98)	0	0	0	130	96(74)	0	0	9	80	80(100)	0	0	0
*Pen./Asp./Pae. varotii**	92	0(0)	1600	4000	7600	130	0(0)	550	1500	3100	80	25 (31)	0	170	450
Mycobacterium spp.*	92	8(9)	1500	3600	8500	130	4(3)	1300	3500	14000	80	33 (41)	0	400	1300
Streptomyces spp.*	92	25(27)	0	2700	5800	130	13(10)	1600	5700	11000	80	76(95)	0	0	0
Gram positive bacteria*	92	0(0)	23000	44000	87000	130	0(0)	5800	14000	37000	80	0(0)	3700	8400	17000
Gram negative bacteria*	92	0(0)	40000	82000	150000	130	0(0)	14000	32000	76000	80	6(8)	2900	6500	17000
Cell wall markers															
Ergosterol*	92	9(10)	0.36	0.59	0.95	127	25(20)	0.27	0.46	0.97	81	65(80)	0	0	0
Glucan*	91	0(0)	5500	8300	13000	130	0(0)	7200	10000	16000	81	0(0)	1000	1500	2500
Endotoxin*	91	0(0)	14000	20000	29000	130	0(0)	21000	30000	47000	81	0(0)	1000	1800	4200

*… significant ($p<0.05$) difference in microbial levels between countries using Kruskal Wallis test

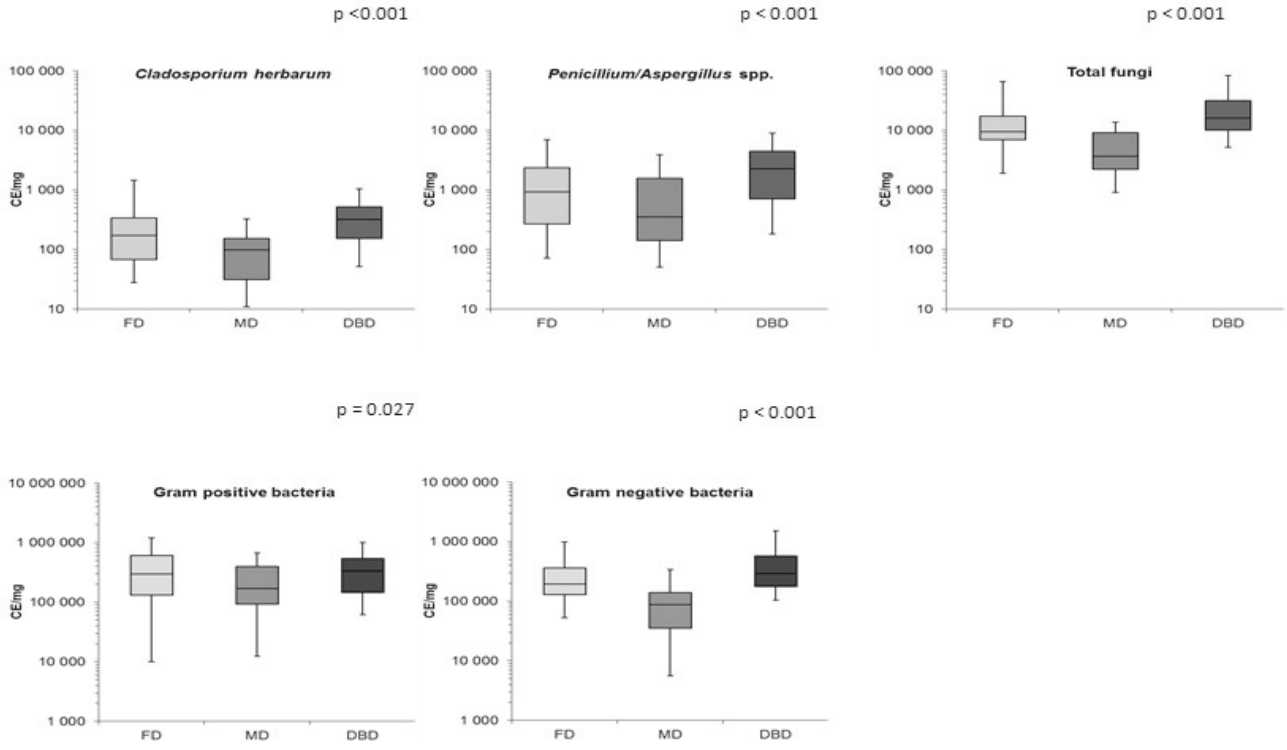

***Figure 2**. Fungal and bacterial concentrations (qPCR; cell equivalents per mg of dust (CE/mg)) in different dust sample types in study IV. Abbreviations: FD, floor dust; MD, mattress dust; DBD, dust bag dust. p-values for differences between sample types according to Kruskal-Wallis test*

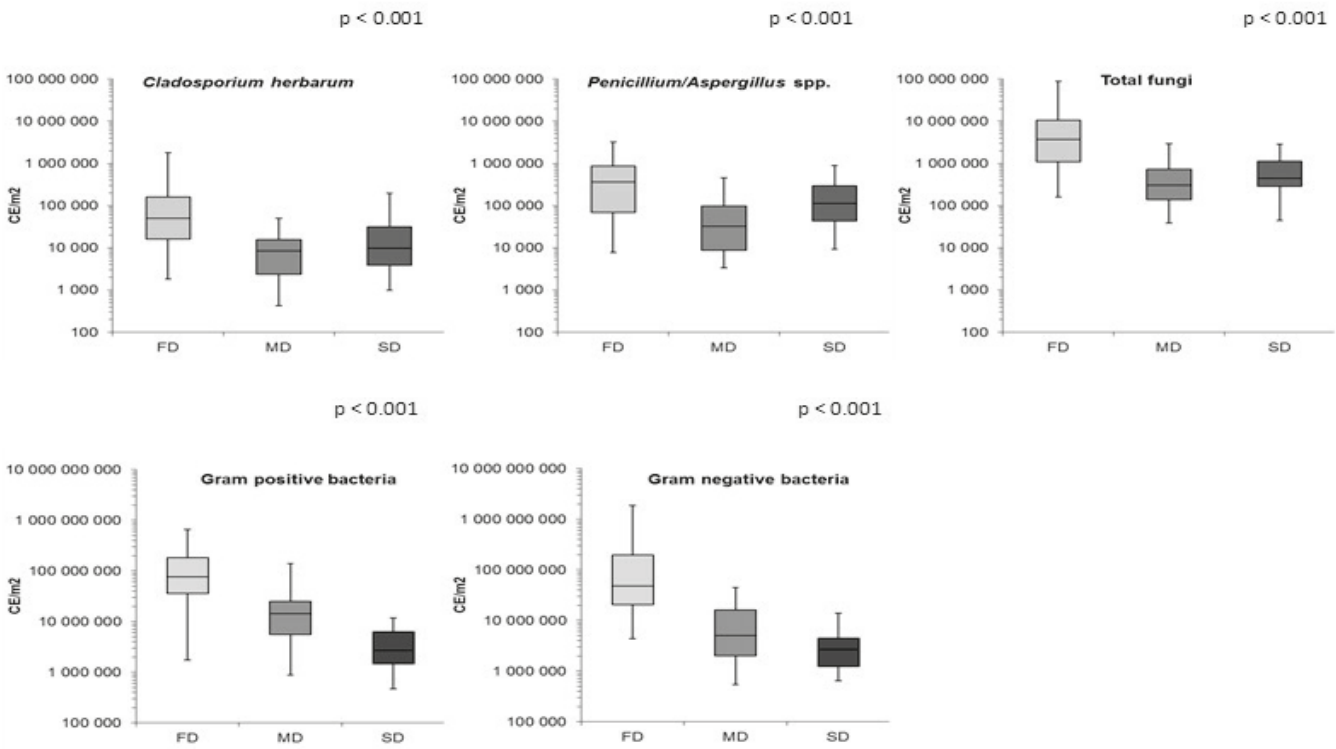

***Figure 3**. Fungal and bacterial loads (qPCR; cell equivalents per m² (CE/m²)) in different dust sample types in study IV. Abbreviations: FD, floor dust; MD, mattress dust; SD, settled dust. p-values for differences between sample types according to Kruskal-Wallis test*

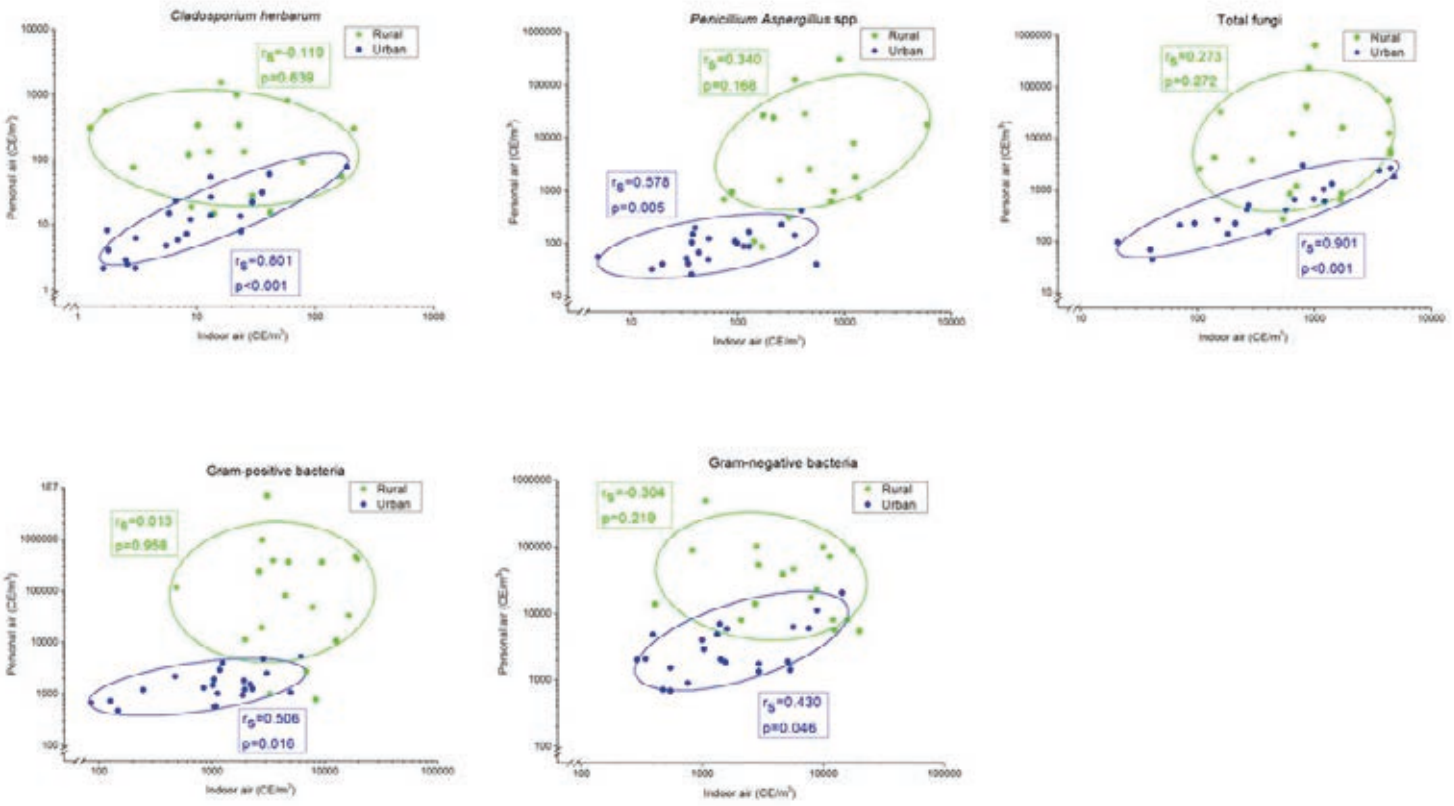

***Figure 4**. Fungal and bacterial concentrations (cells per m³) in actively collected indoor air and personal air samples in rural and urban homes of study IV*

5.2 DETERMINANTS AFFECTING MICROBIAL LEVELS

In study I (publication I), the determinants remaining in the final multiple linear regression models explained 20% and 19% of the variation of ergosterol in floor and dust bag dust, respectively, and 23% and 26% of the variation of mesophilic and xerophilic viable fungi in the dust bag dust samples. Determinants that were significant ($p < 0.05$) in one or more of these models included factors of moisture and mold observations (visible mold or moisture damage in other than living areas), housing characteristics (type of dwelling, type of ground slab, type of roof, ventilation system, and having a cellar), lifestyle (using a fireplace, time since last vacuum cleaning), farming (number of livestock, distance to fields receiving manure fertilization), and weather conditions (depth of snow on the ground, concentration of *Alternaria* and *Cladosporium* spores in outdoor air).

When selecting a common set of determinants for the multiple fungal agents and dust sample types based on the initial linear regression models, explanation rates were slightly smaller, 11–19%. In floor dust samples, ergosterol levels were significantly higher if more than 5 days had passed since last cleaning or vacuuming of the living room floor before sampling, if the number of livestock was low compared with situation with no livestock and if the distance to fields receiving manure fertilization was less than 300 meters from the home. For dust bag dust samples, visible mold and regular use of fireplace was associated with ergosterol concentration and mesophilic and xerophilic viable fungi. A similar but less significant association of visible mold with ergosterol levels in floor dust was observed. In addition, regular usage of a fireplace increased the ergosterol concentration in dust bag dust samples significantly. The concentrations of xerophilic viable fungi were significantly higher in dwellings with natural ventilation compared to fully mechanical ventilation, and in single-family houses compared to flats.

In corresponding analyses in study I (publication II), the models explained 27–29% of the variation of 3-OHFAs and 30–48% of the variation of muramic acid in floor and dust bag dust samples. In dust bag dust, 21% of the variation of total viable bacteria and 25% of the variation of actinomycetes were explained. Determinants that were significant in one or more of these models included factors relating to housing characteristics (type of dwelling, construction year, area of dwelling, number of rooms, and ventilation system), moisture and mold observations (moisture damage in living areas, moisture damage in other than living areas, visible mold in other than living areas), occupant behavior (place where the laundry is dried), farming (number of livestock, coming inside the dwelling with working clothes on, and distance to fields receiving manure fertilization) and weather conditions (temperature of outdoor air, relative humidity of outdoor air, depth of snow, and concentration of *Alternaria* spores in outdoor air).

When selecting a common set of determinants that affected concentrations of different bacterial agents in the multiple linear regression models, explanation rates were lower, reaching 0.6–37%. Farming with high number of livestock and not having fully mechanical ventilation in the home increased concentrations of 3-OHFAs in floor and dust bag dust samples. The concentrations of 3-OHFAs in floor dust samples were significantly lower when the family had lived less than 5 years in the dwelling. In dust bag dust samples, 3-OHFAs were significantly higher in single-family houses compared to flats, in homes having moisture damage in other than living areas, and in dwellings with a size of 100–200 m^2 compared to smaller dwellings. Farming and more specifically the number of livestock increased muramic acid levels in both sample types and actinomycetes in dust bag dust. In dust bag dust samples, determinants that elevated the muramic acid concentrations significantly were the type of the dwelling, mother's smoking, and moisture damage observed in other than living areas. Concentrations of viable bacteria in dust bag dust were

significantly higher in terraced houses compared to flats, and in the homes where the mother was smoking.

In study III, only univariate analyses were examined, since the number of schools (n=23) restricted the use of multiple linear regression models. Moisture damage observed by the building inspectors reflected on the microbial levels very divergently in different countries. The clearest effect was observed in the Netherlands, having higher levels of most microbial markers in moisture damaged compared to reference schools. In Spain, associations of only few microbial markers with moisture damage status were observed; no associations were found in Finland. Rather consistent associations of outdoor environmental characteristics were observed between countries: largely positive associations of microbial levels with outdoor temperature and negative associations with relative humidity were found; microbial levels were positively associated with indoor $PM_{2.5}$ levels (measured from a central hallway) in Spanish and Dutch schools.

When assessing the associations of the microbial markers with moisture damage, utilizing the categorization of school buildings into index and reference buildings, most of the significant findings were observed in the Dutch schools (Table 7). The microbial cell wall agents endotoxin, (1→3)- β -D-glucan and ergosterol were significantly higher in dust samples collected from index than from reference schools in the Netherlands. This was also true for ergosterol levels in Spain. In the Netherlands, furthermore significantly higher levels of Gram-negative bacteria, *Streptomyces* spp., *Penicillium*/*Aspergillus* spp., *Wallemia sebi* and *Penicillium chrysogenum* were observed in index compared to reference schools. In Finland, *Cladosporium herbarum* was found to be significantly higher in reference schools than in index schools; no positive association of any of the microbial markers with moisture damage was observed in Finnish schools. In addition, a three level moisture damage categorization based on tertiles a continuous moisture damage score was used in the analyses. In several cases, ´dose-response´ associations of microbial markers with the moisture damage

score were found in Dutch and – in the case of ergosterol and *Streptomyces* spp.– also in Spanish schools.

Occupancy correlated positively with microbial markers in the Finnish classrooms. This effect was mostly poor, in some cases moderate and statistically significant for *Eurotium amstelodamii* and *Mycobacterium* spp.. Both positive and negative, but in all cases poor and non-significant correlations were observed between microbial markers and occupancy in Dutch classrooms. Similar observations were made for Spanish classroom, with the exception of a statistically significant, moderate negative correlation of occupancy with ergosterol levels.

When relating air exchange rates to microbial levels in classrooms, mostly poor, but in some cases moderate or even strong and significant correlations were observed. In Dutch classrooms, correlations were all positive and mostly poor, whereas in Spanish and Finnish classrooms, both positive and negative correlations were found for different microbial markers. The direction of the correlations was in many cases opposite in Spain versus Finland.

Cleaning habits varied in schools located in the different countries. For example, frequent dusting of surfaces was common in Spain and in Finland, but not in the Netherlands, while frequent vacuuming and sweeping of the floor was more common in Dutch schools, and wet cleaning in Spanish schools. Usage of multi-use cleaning products was comparable between the countries, while hypochlorite bleach and disinfectants were mostly used in Spanish schools, less in Dutch and least in Finnish schools. Overall, cleaning frequency correlated most weakly or moderately with levels of the individual microbial markers, and especially in the Netherlands and Finland correlations were largely positive. Clearest correlations were observed in Finnish schools, strongest for Gram-negative and Gram-positive bacteria. Correlations between frequency of use of cleaning agents and microbial levels were mostly weak in Spanish and Dutch schools. In Finland, cleaning agent usage correlated consistently negative, moderate to well with

microbial levels. All of these correlation analyses were performed on school building level, and thus were heavily limited by the low number of observations, and did not reach statistical significance.

***Table 7**. Percentiles of microbial concentrations measured from settled dust in all index versus reference schools in study /// via qPCR (cells/SDB/day) and chemical markers (ergosterol; ng/SDB/day) (publication III)*

	Settled dust from index school buildings					Settled dust from reference school buildings				
Microbial target	N	<DL (%)	25	50	75	N	<DL (%)	25	50	75
qPCR markers										
C. herbarum										
Spain	69	1 (1)	30	95	330	23	1 (4)	27	88	381
The Netherlands	55	0 (0)	22	67	430	75	3 (4)	16	61	350
Finland*	**53**	**39 (74)**	**0**	**0**	**1.5**	**27**	**14 (52)**	**0**	**0**	**10**
Penicillium chrysogenum										
Spain	69	47 (68)	0	0	120	23	19 (83)	0	0	0
The Netherlands*	**55**	**35 (64)**	**0**	**0**	**82**	**75**	**64 (85)**	**0**	**0**	**0**
Finland	53	50 (94)	0	0	0	27	27 (100)	0	0	0
Eurotium amsteldamii										
Spain	69	4 (6)	66	144	580	23	1 (4)	95	200	500
The Netherlands	55	0 (0)	52	170	350	75	1 (1)	52	100	310
Finland	53	23 (43)	0	12	34	27	14 (52)	0	0	43
Stachybotrys chartarum										
Spain	69	67 (97)	0	0	0	23	21 (91)	0	0	0
The Netherlands	55	52 (95)	0	0	0	75	70 (93)	0	0	0
Finland	53	52 (98)	0	0	0	27	27 (0)	0	0	0
Trichoderma viride										
Spain	69	67 (97)	0	0	0	23	23 (100)	0	0	0
The Netherlands	55	50 (91)	0	0	0	75	71 (95)	0	0	0
Finland	53	53 (100)	0	0	0	27	26 (96)	0	0	0
Wallemia sebi										
Spain	69	67 (97)	0	0	0	23	23 (100)	0	0	0
The Netherlands*	**55**	**34 (62)**	**0**	**0**	**34**	**75**	**62 (83)**	**0**	**0**	**0**
Finland	53	53 (0)	0	0	0	27	27 (100)	0	0	0
Pen./Asp./Pae. Varotii										
Spain	69	0 (0)	1500	3500	7200	23	0 (0)	2200	5500	8400
The Netherlands*	**55**	**0 (0)**	**770**	**1900**	**5300**	**75**	**0 (0)**	**460**	**1000**	**2600**
Finland	53	14 (26)	0	160	450	27	11 (41)	0	220	430
***Mycobacterium* spp.**										
Spain	69	5 (7)	1500	3700	8400	23	3 (13)	1500	2800	10000
The Netherlands	55	0 (0)	2000	4100	15000	75	4 (5)	910	3400	12000
Finland	53	23 (43)	0	310	1000	27	10 (37)	0	650	1700
***Streptomyces* spp.**										
Spain	69	16 (23)	680	3100	6000	23	9 (39)	0	2000	5200
The Netherlands*	**55**	**3 (5)**	**3300**	**6900**	**16000**	**75**	**10 (13)**	**1200**	**3700**	**9800**
Finland	53	52 (98)	0	0	0	27	24 (89)	0	0	0
Gram positive bacteria										
Spain	69	0 (0)	22000	43000	90000	23	0 (0)	23000	45000	78000
The Netherlands	55	0 (0)	8200	17000	39000	75	0 (0)	4300	12000	31000
Finland	53	0 (0)	3500	8400	16000	27	0 (0)	4700	9600	21000
Gram negative bacteria										
Spain	69	0 (0)	40000	98000	18000	23	0 (0)	34000	79000	100000
The Netherlands*	**55**	**0 (0)**	**20000**	**38000**	**98000**	**75**	**0 (0)**	**9200**	**25000**	**60000**
Finland	53	5 (9)	2800	6500	22000	27	1 (4)	3900	6500	16000
Cell wall markers										
Ergosterol										
Spain*	**68**	**4 (6)**	**0.48**	**0.65**	**0.97**	**22**	**5 (23)**	**0.24**	**0.37**	**0.61**
The Netherlands*	**54**	**4 (7)**	**0.34**	**0.57**	**1.2**	**73**	**21 (29)**	**0**	**0.40**	**0.78**
Finland	54	41 (76)	0	0	0	27	24 (89)	0	0	0
Glucan										
Spain	68	0 (0)	5100	7700	13000	23	0 (0)	6400	12000	17000
The Netherlands*	**55**	**0 (0)**	**8700**	**14000**	**18000**	**75**	**0 (0)**	**6600**	**9700**	**13000**
Finland	54	0 (0)	1100	1700	2600	27	0 (0)	930	1300	1900
Endotoxin										
Spain	68	0 (0)	14000	19000	35000	23	0 (0)	14000	22000	27000
The Netherlands*	**55**	**0 (0)**	**26000**	**46000**	**62000**	**75**	**0 (0)**	**19000**	**25000**	**35000**
Finland	54	0 (0)	1000	1900	4300	27	0 (0)	940	1300	2900

* significant difference between index and reference samples, p-value <0.05 in Wilcoxon rank sum test

5.3 CORRELATIONS OF MICROBIAL GROUPS IN DIFFERENT SAMPLE TYPES

Correlations between different microbial groups in different sample types determined by qPCR were analyzed in study IV. When calculating the correlations of the individual microbial groups between different sample types, the highest correlations were observed for the fungal groups *Cladosporium herbarum* and *Penicillium*/*Aspergillus* spp., varying from weak to strong (Figure 5). Low correlations between sample types were observed in particular for Gram-negative, but also for Gram-positive bacteria and total fungal DNA. Considering indoor air as the point of reference, highest overall correlations were observed with personal air samples for different microbial groups (Spearman correlation 0.4-0.7). Correlations of microbial levels between indoor air and house dust samples were highest for indoor air versus settled dust, dust bag dust and floor dust for the fungal groups, and highest for indoor air versus dust bag dust and floor dust for bacteria (Figure 5).

The correlations between two different ways of presenting the microbial levels, concentrations and loads, were consistently strong for mattress dust samples (cells/mg vs. cells/m^2). Also the two different ways of presenting concentrations for actively collected indoor and personal air samples (cells/μg vs. cells/m^3) correlated strongly. This was observed for all the studied microbial species/groups. For floor dust samples, the correlations between data presented as concentrations versus loads (cells/mg vs. cells/m^2) were strong for most of the microbial species/groups, but moderate for Gram-positive bacteria.

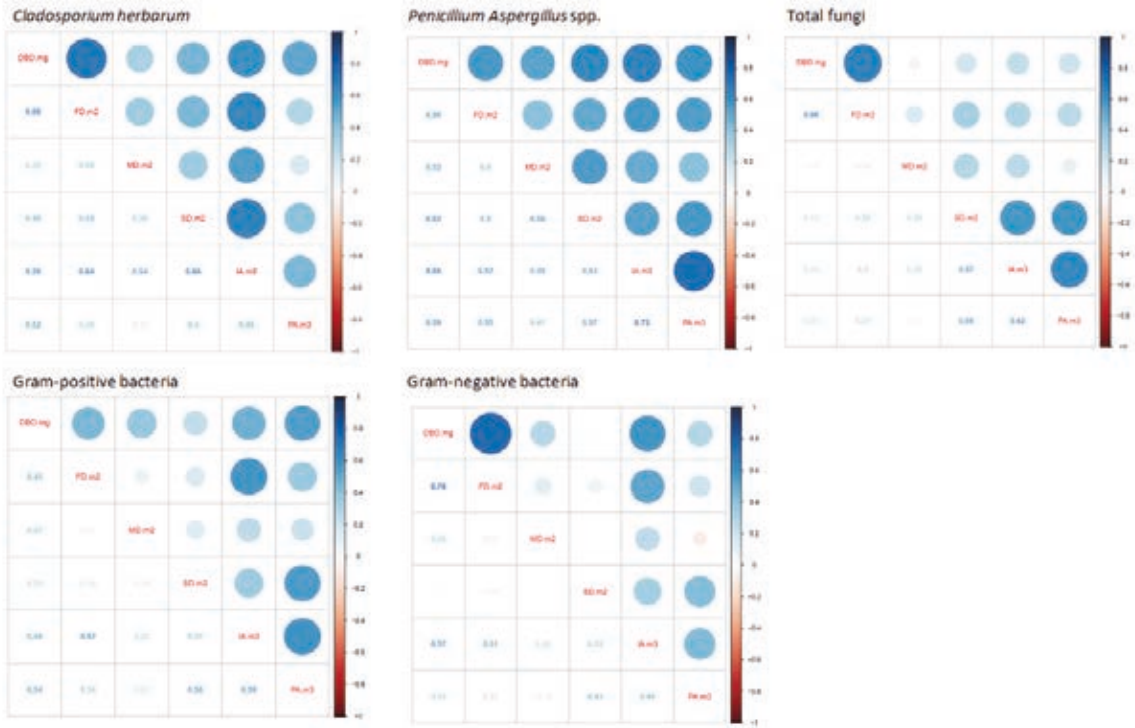

***Figure 5**. Spearman correlations between the different sample types for different fungal and bacterial qPCRs (study IV). (CE/mg for dust bag dust; CE/m² for floor, mattress and settled dust; CE/m³ for actively collected indoor air and personal air samples). Abbreviations: FD, floor dust; MD, mattress dust; DBD, dust bag dust; SD, settled dust; IA, indoor air; PA, personal air*

5.4 REPRODUCIBILITY AND SEASONAL VARIATION

For the dust validation study included in publications I and II, samples were collected from five urban homes in four different seasons. The repeatability of ergosterol determinations within repeated subsamples of a sample was excellent (ICC = 89.8) for floor dust and moderate (ICC = 63.8) for dust bag dust. Reproducibility was poor when sampling the same home throughout a year (ICC = 31.3 and 12.6, respectively). For concentrations of 3-OHFAs and muramic acid in floor dust, the repeatability of subsamples within a sample was generally high (ICC 74–84%). Temporal variation observed in repeated sampling of the same home throughout a year was more pronounced for 3-OHFAs determinations (ICC 22%) than for muramic acid (ICC 55–66%).

In study III, the variance of microbial levels was generally higher within schools than between schools. This effect was observed in all three countries, except for ergosterol levels in Dutch schools. Intraclass correlations were poor for almost all of the microbial agents, with the exception of ergosterol and *Streptomyces* spp. in Spain and the Netherlands. The reproducibility was slightly better, when the measurement period was fixed in the model, i.e. within-school versus between-school variance was considered by exposure assessment, and not including three exposure assessments over time.

The reproducibility was studied in rural and urban homes separately and in all the homes together in study IV. When considering all study homes together, within-home variation (between repeated sampling times over one year) was smaller than the between-home variation in personal air samples for all microbial species/groups resulting in strong reproducibility of these determinations with ICC-values of 64–79%. Determinations from dust bag dust samples were also mostly well reproducible (ICC 42-72%). Poor to moderate reproducibility was observed for the other sample types: floor dust, mattress dust, settled dust and indoor air samples. When

analyzing rural and urban homes separately, most of the microbial agents in different sample types had smaller between-home variation than within-home variation, especially in samples taken from rural homes. Generally, determinations from mattress dust appeared to be most reproducible. With respect to reproducibility of the individual microbial groups in the different sample types, highest values were observed for *Penicillium*/*Aspergillus* spp. determinations with ICC-values of 62–78%.

The seasonal variation of levels of indoor microbial agents was analyzed in all studies. The seasonal effect was varying for different microbial agents. The concentrations of ergosterol in floor dust and dust bag dust samples were typically highest during winter in studies I and II (publication I). Also the concentrations of viable fungi in dust bag dust samples were highest during winter. For the bacterial cell wall agents, 3-OHFAs and muramic acid, the levels were typically highest in summer for floor dust and dust bag dust samples, while the viable bacteria and actinomycetes were at their highest during winter.

In study III, *Cladosporium herbarum* representing a common outdoor air fungal species showed some seasonal variation in all three countries with highest levels during the spring/early summer assessment and lowest levels in the winter/early spring assessment. A similar seasonal effect was observed for ergosterol. For many of the other microbial markers, a significant difference was observed in the levels between repeated assessments; however, findings were typically not consistent between countries and not following a seasonal pattern as observed for *Cladosporium herbarum*. The correlations of individual microbial markers between repeated assessments showed some temporal variation, but no consistent seasonal trend.

In study IV, when assessing seasonal variation for different microbial species/groups, the concentration of *Cladosporium herbarum* was the highest during summer in all sample types, statistically significant for floor dust, settled dust,

mattress dust and indoor air samples. Actively collected indoor air and settled dust samples reflected seasonal variation of microbial groups in a similar fashion.

6 Discussion

The present thesis assesses the microbial concentrations in a variety of different indoor environments, including rural and urban homes and schools. The thesis also investigates the representativeness of different house dust samples when compared to actively collected indoor air, the reproducibility of microbial determinations and seasonal variation of microbial levels in different sample types. In addition, different housing characteristics, living and environmental factors affecting the microbial levels are assessed.

6.1 MICROBIAL CONTENT OF DIFFERENT SAMPLE TYPES

The ergosterol levels of floor dust and dust bag dust samples from homes were similar to those observed in earlier studies (Dharmage, 1999; Hyvärinen, 2006b; Sordillo, 2011), with slight differences explainable by differences in the study populations. The levels of mesophilic and xerophilic viable fungi in dust bag dust were also in line with other studies (Hyvärinen, 2006b; Miller, 1988).

Levels of bacterial agents, the concentrations of the 3-OHFAs and muramic acid, as well as of total viable bacteria and actinomycetes in house dust were similar to an earlier report from Finnish homes (Hyvärinen, 2006b). These levels, especially for bacterial cell wall agents, were somewhat lower than those reported for US homes (Park, 2004; Sordillo, 2011). The concentrations of fungal and bacterial agents tended to be higher in dust bag dust samples than in floor dust and mattress dust samples, which has earlier been observed for endotoxin (Hyvärinen, 2006a; Ownby, 2010; Park, 2000). This might be explained by the fact that dust bag dust is more of an integrated sample collecting both temporal and spatial 'exposure peaks' in

the home. Dust bag dust also includes sampling from areas that are potentially more microbe-rich (e.g. toilets or entrance areas) and capturing occasional high microbial content situations, e.g. man or pet carrying microbe-rich soil indoors. It is also possible that certain microbes proliferate on organic matter, for example, food residues or soil particles, collected in the dust bag and thus reach higher concentrations in dust bag dust.

Bacterial communities in indoor dust have been shown to be dominated by Gram-positive bacteria (Rintala, 2008; Täubel, 2009). Reservoir dusts, especially mattress dust are "sinks" of human-derived, mostly Gram-positive bacteria associated with skin, oral cavity and the human gut, among other sources. This was also seen in the current study, since reservoir dust samples, i.e. floor dust, mattress dust and dust bag dust showed a pronounced presence of Gram-positive bacteria. Instead, in settled dust as well as in actively collected indoor air and personal air samples, Gram-negative bacteria showed equally high or higher levels than those of Gram-positive bacteria. This indicates different sources for bacteria in different types of indoor samples. Reservoir dust samples seem to reflect more the human-derived microbes, whereas actively collected indoor air samples and settled dust seem to be more impacted by outdoor air, which is known to be dominated by Gram-negative bacteria (Brodie, 2007; Fierer, 2008; Hanson, 2016).

In the fungal determinations, *Penicillium*/*Aspergillus* spp. were underrepresented in reservoir dust samples when compared to indoor air, making up approximately 10% of the total fungal content in floor, dust bag and mattress dusts, while for indoor air samples, the corresponding ratio was approximately 30%. This underrepresentation may be explained by a high influx of human-associated yeasts into these sample materials, as has been shown in sequencing studies (Dannemiller, 2014; Pitkäranta, 2008). In settled dust, indoor air and personal air samples, *Penicillium*/*Aspergillus* species contributed approximately 30% to the total fungal content. Thus, the results of the study indicate that the microbial composition

of indoor air may be best reflected – in compositional terms - via samples of settled dust, if active sampling of airborne microbes is not feasible. Moreover, settled dust responded similarly to environmental determinants (discussed later), and hence, it can be used in assessing indoor airborne microbial exposure. In addition, floor dust should be taken in order to assess the microbial exposure thoroughly in qualitative and quantitative terms. This is because reservoir dusts, e.g. floor dust showed better reproducibility and hence describe better the overall microbial exposure in a home, throughout a year.

Significantly higher microbial levels were mostly observed in rural homes than in urban homes in all sample types and for all microbial groups. This is in line with many previous studies that have shown that farming homes are qualitatively and quantitatively richer in microbes than urban homes (Ege, 2011; von Mutius & Vercelli, 2010). This is likely due to microbe-rich material being transferred via outdoor air and carried from animal sheds and barns to farmers' homes on clothing and shoes (Korthals, 2008; Krop, 2014; Normand, 2011; Pasanen, 1989; Rintala, 2012).

An equal contribution of Gram-positive and Gram-negative bacteria in indoor air, as well as in settled dust and dust bag dust was observed in rural homes, whereas in urban homes, Gram-negative bacteria dominated these sample types. Moreover, dramatically higher levels of Gram-positive bacteria were observed in the personal air samples of people living in farming environments as compared to those of urban homes. These results point towards farming activities that appear to have a remarkable effect on the daily microbial exposure, dominated by Gram-positive bacteria, for people living in farming homes. In addition, clear differences in fungal quantitative composition were shown between the rural and urban homes. The effect was observed as a higher contribution of *Penicillium*/*Aspergillus* species to the total fungal content in rural homes. This likely reflects outdoor air and crop-associated fungi to be stronger sources for indoor fungal content in rural environments, while human-associated yeasts contributing to

the total fungal content indoors may be more dominant in urban indoor environments. Based on the results, samples of indoor air may be a reasonable surrogate for personal microbial exposure levels in urban environments. However, this is not the case for rural environments, where personal exposure differs quantitatively and qualitatively from samples of indoor air and dust. When microbial exposure is being assessed in rural homes, also sampling in cowsheds and other such facilities is highly recommended. Sampling should be complemented with information on frequency and duration of occupancy in such high exposure environments.

The levels of different microbial markers were also compared in school buildings across three climatic regions of Europe. The levels in settled dust varied strongly by country. In general, the levels of the studied fungal and bacterial markers were highest in Spanish schools and for some microbial markers in Dutch schools, while the levels were lowest in Finnish schools for all of the microbial agents measured. There are earlier studies that have pointed out the effect of geographical variation of microbial levels in indoor dust and air samples in school environments (Jacobs, 2014a; Wady, 2004; Yamamoto, 2015). The geographical variation of microbial levels can refer to country-specific characteristics of the school buildings and their use and maintenance. The variation can also partly be explained by differences in microbial levels of the outdoor environment – especially considering winter season and snow cover in the Nordic climate - and their contribution to the indoor air microbial content, which is discussed further below.

6.2 DETERMINANTS AFFECTING MICROBIAL LEVELS

The variation of levels of the studied microbial agents in house dust was explained by several different determinants that were only partly overlapping, indicating the different sources, characteristics and aspects of the microbial agents measured and the dust sample types used. Environmental characteristics only

partly explained the variation of microbial levels in indoor dust (19–48%) in stepwise linear regression depending on the agent measured and sample type; 0.6–37% in a set of 12 selected common variables). Sordillo et al. (2011) found slightly lower degrees of explanation for the variance of the microbial markers in house dust (4.2–19%), while Casas et al. (2013a) were able to explain 2–35% of the variability of microbial agents, with large differences between countries/cohorts. In the study of Hyvärinen et al. (2006b), home characteristics explained as much as 34–44% of the variation of different microbial groups in dust bag dust samples.

When selecting a common set of determinants for the multiple microbial agents and dust sample types based on the initial linear regression models, the determinants related to more specific characteristics of farming activities seemed to affect the microbial levels more than farming itself. Higher levels of ergosterol and muramic acid were observed in floor dust, when the home was closer to a field receiving manure fertilization. This is concordant with other studies showing that manure fertilization and proximity to agricultural land increase microbial concentration in the indoor environment (Kaarakainen, 2011; Tager, 2010). Also the number of livestock affected the levels of ergosterol, 3-OHFAs, muramic acid and actinomycetes in the present study. This is in line with previous studies reporting that farming increases microbial content in house dust (Ege, 2011; Kärkkäinen, 2010; Moniruzzaman, 2012; Schram, 2005; van Strien, 2004; von Mutius, 2000).

In terms of lifestyle and occupant behavior factors, mother's smoking was significantly associated with higher levels of viable bacteria and muramic acid in dust bag dust. Studies have shown earlier that tobacco and tobacco smoke contains significant levels of microbial compounds (Larsson, 2004; Larsson, 2008; Sebastian, 2006; Szponar, 2012). This may also be interfering with socioeconomic status of the family and level of cleanliness of homes. Handling of firewood has been shown to be an important source of indoor fungi (Hyvärinen, 2006b; Lehtonen, 1993). This was confirmed in the current study,

as regular usage of a fireplace was a significant and common determinant increasing fungal levels in floor dust.

The longer time had passed since the last cleaning or vacuuming of the home, the higher ergosterol levels in floor dust were observed, which is in line with an earlier report (Sordillo, 2011). Sample accumulation time is likely to impact both qualitative and quantitative characteristics of microbial content in house dust and in epidemiological studies using floor dust, not only the sampling itself, but also sample accumulation time should be standardized as much as possible.

Microbial levels were also associated with the type of building: concentrations in single-family houses were found to be higher than those in flats, as observed also by Sordillo et al. (2011). This can be explained by the more direct connection (i.e., direct entrance without external entrance halls, stairways, etc.) of the indoor environment of single-family houses to the outdoor environment (Bischof, 2002; Heinrich, 2001). The concentrations of both fungal and bacterial levels were significantly higher in homes with natural ventilation as compared to those with fully mechanical ventilation and filtration, which probably removes part of the microbes from the intake air and more efficiently removes indoor particles and microbes (Dekoster & Thorne, 1995; Reponen, 1992). In addition, the size of the dwelling affected 3-OHFAs levels in dust bag dust significantly, with highest concentrations observed in middle-sized dwellings, maybe due to higher occupancy levels in these homes. Typically higher bacterial levels were observed in dwellings that had been occupied by the family for a longer duration. This observation was significant for concentration of 3-OHFAs in floor dust and is similar to what Bischof et al. (2002) have reported in their study.

Factors related to mold and moisture damage were also significant factors affecting microbial levels in the current thesis. Levels of most fungal markers in dust bag dust were significantly associated with visible mold growth in the homes. Visible mold on indoor surfaces indicates active microbial growth and thereby is potentially a strong source for fungal

agents, as has been observed in earlier studies (Dales, 1997; Hyvärinen, 2006b). Moisture damages in other than living areas increased both fungal and bacterial concentrations in house dust significantly, while damage observations in living areas interestingly had a weaker effect. Both, visible observation of moisture damage, dampness and/or mold, as well as indicators of hidden mold problems, such as the odor of mold, have earlier been linked to higher levels of microbial markers in house dust (Casas, 2013a; Chen, 2012a; Hyvärinen, 2006b; Reponen, 2010; Sordillo, 2011).

In schools of three European countries, the observed mold and moisture damage affected microbial exposure in diverse ways in different countries. The clearest associations were seen in Dutch schools, where several of the fungal and bacterial biomass markers as well as more specific DNA targets were significantly higher in index than in reference schools. In the Mediterranean Spain, the effect was much less clear with a exception of increased ergosterol levels in index schools. In Finland with cold climate, no positive associations between moisture damage and microbial levels were observed. In Dutch school buildings, for some of the studied microbial markers, dose-response relationships were observed when relating to moisture damage score. In Spanish school buildings, such dose-response relationships were only observed for ergosterol and *Streptomyces* spp. levels. In Finnish school buildings, no significant increases in moisture damaged versus non-damaged buildings were found. This could be a consequence of public awareness of the dampness and mold issue being high in Finland, which frequently results in that school buildings with suspected moisture problems are being closed and remediation actions are initiated. In addition, differences in building characteristics and types of moisture problems in schools were reported between the countries (Haverinen-Shaughnessy, 2012), which may also have contributed to the microbial findings presented here. In Dutch schools, current dampness problems were commonly reported also in classrooms, the primary location of exposure assessments in the study, and this was not

the case in Spanish and Finnish schools. Spanish schools had a relatively high prevalence of current moisture and water damage, whereas in Finnish schools past moisture problems and mold odor, the latter indicative of hidden microbial growth, were more frequently reported. In earlier studies conducted in schools, the levels of different microbial markers have been higher in the damaged school buildings and classrooms than in non-damaged ones (Cai, 2009; Cho, 2016; Jacobs, 2014b; Krop, 2014; Lignell, 2005; Purokivi, 2001; Simoni, 2011).

Earlier studies show that lower frequency of cleaning increases the microbial levels in residential homes (Bischof, 2002; Gehring, 2001a; Giovannangelo, 2007c; Hyvärinen, 2006b; van Strien, 2004; Waser, 2004), whereas regular cleaning can decrease the levels remarkably (Sordillo, 2011; Wu, 2012). In the current study, cleaning habits varied between different countries. The frequency of performing cleaning tasks in classrooms correlated mostly positively with the microbial levels, especially in Finnish and to some extent in Dutch schools. Resuspension of dust during dry dusting and mopping may have resulted in higher microbial levels in settled dust samples in Finland and the Netherlands, while wet mopping – a more common practice in Spanish schools – was appeared to be more efficient in reducing microbial levels. In an earlier study, wet mopping has been noticed to decrease the amount of settled dust, but to increase levels of airborne viable bacteria in Swedish classrooms (Smedje & Nordbäck, 2001). More frequent use of cleaning products consistently reduced levels of the various microbial agents in Finnish schools, while these correlations were less clear in Spanish and Dutch schools. In Finland, the number of students in the classrooms correlated with an increase in the levels of most microbial markers, but this was not the case in Spain and the Netherlands. Qian et al. (2012) observed about 18% of the bacterial emissions in classrooms originated from taxa that were closely associated with the human skin microbiome. A recent study (Yamamoto, 2015) concluded that, on average, 81% of allergenic fungi from indoor

sources originate from occupant-generated emissions, such as desquamation.

Within the countries, air exchange rates were positively correlated with the levels of all microbial markers in Dutch schools and with the majority of markers in Spanish schools. In Finnish schools, both positive and negative correlations were observed. These relationships likely illustrate the differences in ventilations strategies between countries: in Spain and the Netherlands, higher air exchange rate in the classroom likely links to more frequent opening of the windows, supporting unfiltered outdoor air influx, whereas in Finland, higher air exchange rate links to an increase in filtered outdoor air influx and an increase in mechanically supported exhaust and by that, reducing the outdoor air microbial contribution to indoor air. Mechanical ventilation has been found to relate to lower indoor microbial levels in classrooms compared to naturally ventilated ones (Bartlett, 2004a; Bartlett, 2004b) and also to affect the bacterial composition of indoor air (Kembell, 2014).

6.3 CORRELATIONS OF DIFFERENT MICROBIAL GROUPS AND SAMPLE TYPES

Correlations between different microbial markers varied for different indoor sample types. This highlights the differences in sources and/or source strength for the microbes represented via different sampling approaches. It also points out that just as different microbial analysis approaches capture a different spectrum of the indoor microbial exposure (Frankel, 2012), similarly do different sample types. Correlations of all the microbial groups were clearly better when the results were expressed per sampling area or volume rather than per particle mass. This means that taking into account the amount of dust in the assessment improves correlations between microbial markers. The way of presenting the microbial measurements has been shown to alter conclusions also in earlier studies (Gehring, 2001a; Giovannangelo, 2007b; Heinrich, 2003). This should be

taken into account when comparing the results of different studies that use diverse metrics in the interpretation of their results.

When relating different reservoir dust samples and personal air samples to actively collected indoor air as the point of reference, the bacterial markers correlated generally weakly, moderately only for floor dust, dust bag dust and personal air samples. This result is consistent with earlier studies evaluating correlations between reservoir dusts and indoor air. Poor or no correlations have been found between endotoxin levels of indoor air and different reservoir dust samples (Barnig, 2013; Hyvärinen, 2006a; Park, 2000; Reponen, 2010). Moreover, the levels of culturable fungi or (1→3)-β-D-glucan in house dust have not been considered representative of those from indoor air (Chew, 2003; Miller, 1988; Ren, 1999). The fungal markers *Cladosporium herbarum* and *Penicillium/Aspergillus* spp. correlated mostly moderately, in some cases strongly between house dust samples and indoor air. Total fungal DNA in indoor air correlated mostly weakly with reservoir dusts and moderately with settled dust. Somewhat in contrast, levels of endotoxin in settled dust samples have been shown to correlate strongly with the levels in actively collected indoor air samples (Frankel, 2012; Kilburg-Basnyat, 2015; Noss, 2008). However, different findings with no significant correlation of endotoxin levels between these two sample types also exist (Hyvärinen, 2006a). These earlier results combined with our study findings call for more research that will clarify the relation – in qualitative and quantitative terms – of microbial content in settled dust and actively collected indoor air samples.

6.4 REPRODUCIBILITY AND SEASONAL VARIATION

The reproducibility for determinations of ergosterol, 3-OHFAs and muramic acid levels within a sample was varying from moderate to excellent, making the determinations as such a valid method for assessing overall microbial levels in house dust.

When considering reproducibility of microbial determinations over a period of one year, some of the microbial markers showed great temporal variation. This variation was clear for determinations of ergosterol and 3-OHFAs in homes, resulting in poor reproducibility, whereas the variation was less evident for muramic acid in homes. When assessing more specific microbial groups in schools, the reproducibility was poor for almost all of the microbial markers measured, with the variation within schools being greater compared to that between schools. This indicates that microbial exposure with respect to specific microbial species/genera/groups in schools is highly variable over time and space. Topp et al. (2003) reported poor reproducibility for endotoxin levels in repeated floor dust sampling in homes. The group concluded that a single measurement is not enough to represent the true long-term exposure.

Our current study highlights that the reproducibility depends on the microbial marker considered and the sample type. For example, the reproducibility for determination of muramic acid in floor dust of homes was moderate. The reproducibility for determination of different microbial species/groups with qPCR in study homes was varying from poor to strong, depending not only on the microbial agent, but also the sample type considered. Reproducibility of settled dust measurements over time was low compared to reservoir dust samples. This was true especially for bacterial levels, reflecting that bacterial cells – when getting airborne as single cells and not associated with other particles - can stay airborne for extended periods and not efficiently settle onto surfaces. Personal air samples and dust bag dust samples showed the best reproducibility, when studying low and high exposure environments i.e. both urban and rural homes together. The reproducibility was generally lower, when the rural and urban homes were analyzed separately resulting from higher within home than between home variance, especially in rural homes. Reservoir dust samples, especially mattress dust showed better reproducibility in the assessment separated for rural and urban

homes, which has been observed also earlier for endotoxin (Hyvärinen, 2006a; Park, 2000). While microbial determinations from mattress dust may be well reproducible, this could be misleading as mattress dust may not represent the environmental microbial exposure well, but rather 'benefits' – in terms of reproducibility – of the human body being a strong and constant source of its microbial content (Täubel, 2009). *Penicillium/Aspergillus* spp. was the most reproducible microbial marker in every sample type indicating a relation to constant indoor source, whereas *Cladosporium herbarum* as outdoor derived fungal species showed the weakest reproducibility, due to great seasonal variation. Also an earlier study using quantitative PCR, *Penicillium/Aspergillus* spp. showed strong reproducibility in dust bag dust, while the reproducibility of *Cladosporium herbarum* was poor (Kaarakainen, 2009).

It is worth mentioning that in our study, the reproducibility of 24 hour integrated indoor air samples was poor for most of the microbial agents measured. This strongly indicates that indoor air microbial exposure is highly variable, even when considering longer active air sampling. This finding may be of relevance as one could formulate the hypothesis that also *variations* in indoor microbial exposures may be important when exploring indoor microbe related health effects. Future studies are needed to clarify the effect of microbial variations on health.

The current study confirms findings made in other studies with respect to conflicting findings of seasonal variation of microbial levels. According to this current study, seasonal variation differs for different microbial markers, as has also been observed in earlier studies (Dharmage, 2002; Heinrich, 2003; Kaarakainen, 2009). In addition, the different sample types appear to reflect seasonal fluctuations in microbial levels differently. The seasonal variation in microbial species/groups was observed to be similar for actively collected indoor air and passively collected settled dust. When assessing microbial exposure with qPCR in indoor air and settled dust, the impact of season was clearly visible in levels of *Cladosporium herbarum*,

with highest levels in summer and lowest in winter. This is intuitive since *Cladosporium herbarum* is an outdoor related fungus with higher outdoor levels during frost free months, especially in summer (Ren, 1999). Also in an earlier study done with qPCR and conducted also in Finland, concentrations of *Cladosporium* species in house dust samples were highest during summer and fall (Kaarakainen, 2009). A similar seasonal pattern was observed for *C. herbarum* in schools in three European countries, alongside with the total fungal biomass marker ergosterol. In the school study otherwise, changes of microbial levels over time were not consistent between countries and not indicative of a clear seasonal pattern for the different microbial markers measured. Also the correlations of the individual microbial targets measured over time were poor to moderate, indicating great temporal variation in the microbial levels of the school buildings, only partly or in some cases explainable by seasonal, i.e. outdoor impact.

6.5 STRENGTHS AND WEAKNESSES OF THE STUDY

One strength of the current thesis is that the determinants affecting the fungal and bacterial agents were assessed by the multiple linear regression model (study I, publications I and II), which has been done this thoroughly only in few studies. The greatest strength of the thesis is that it furthermore adds longitudinal, quantitative microbial exposure data from school environments, comparing three different climatic regions of Europe, and relating to moisture damage and dampness observations as well as to other indoor and outdoor environmental factors (study III). Longitudinal studies using a large set of microbial markers are largely missing from the current literature on indoor microbial exposures. When studying the representativeness of microbial content of different house dust samples of indoor air, a multitude of different repeated house dust and indoor air samples, commonly used in

epidemiological indoor studies, were collected and analyzed using DNA-based methodology. (study IV).

A limitation of the current thesis is the low number of urban homes for studying the reproducibility of fungal and bacterial determinations. These homes did not have any dampness or mold problems or other obvious exceptional microbial sources, so that these analyses were restricted to a rather homogenous sample of indoor environments (study I, publications I and II). This needs to be considered when interpreting the results, especially the findings with respect to within and between-home variations. In study IV we improved our assessment by including both urban and rural homes when assessing the reproducibility of microbial levels determined by qPCR.

7 Conclusions

Based on the present thesis, the following conclusions can be drawn:

1. Determinants of microbial agents in house dust vary depending on which sample type and which microbial agent is considered.
2. In home environments, concentrations of bacterial cell wall agents are commonly related to the type of the dwelling and to farming. Visible mold and using a fireplace are common determinants for fungal markers in dust bag dust, but otherwise the determinants of fungal markers vary.
3. In schools, microbial levels have great geographical and temporal variation, which can only partly be explained by seasons. There are also differences between countries with respect to how moisture and dampness conditions in school buildings affect the microbial levels measured, both in terms of the quality and the quantity of the impact.
4. Only up to 48% of the variation in microbial levels can be explained with questionnaires, and therefore, environmental samples need to be taken in addition in order to describe the microbial condition of a building.
5. Determinations of parallel subsamples are generally highly repeatable, making the determinations of fungal and bacterial chemical markers as such a robust method for assessing microbial levels in house dust.
6. Due to temporal variation resulting in mostly low reproducibility of repeated assessments over time, more than one sampling campaign is needed in various seasons to describe the overall microbial concentrations in indoor environments.
7. Indoor air microbial exposure is highly variable. This should be taken into account when exploring indoor microbe related health effects.
8. Settled dust sampling appears to be a good option for assessing the microbial exposure of indoor air, since it reflects well the

quantitative microbial composition and seasonal variation of indoor air. Reservoir dusts predict better microbial levels in indoor air and are generally more reproducible than samples of indoor air.

9. Repeated sampling campaigns of settled dust and in addition of floor dust are recommend for thorough microbial exposure assessment, including qualitative and quantitative aspects, when it is not feasible to collect longer term air samples. In rural environments, complementing sampling in homes with sampling in microbe-rich environments that are frequently visited and study subjects spend time in, such as cowsheds, is recommended.

In summary, the present study has provided new information on the associations of microbial levels in a variety of different indoor environments with different housing characteristics, lifestyle and environmental factors. The findings of the present thesis contribute to the understanding of the great variation of microbial markers in different living environments and different sample types, as well as of geographical and temporal variation of microbial exposure.

Understanding the etiology of asthma and other allergic diseases, as well as clarifying the ambiguous role of microbes in these disease outcomes represent major challenges to microbiological exposure assessment. The current thesis attempts to contribute to tackling this challenge.

References

Abraham, J.H., Gold, D.R., Dockery, D.W., Ryan, L., Park, J.H. and Milton, D.K. (2005) Within-home versus between-home variability of house dust endotoxin in a birth cohort, *Environ Health Perspect*, 113, 1516-1521.

Adams, R.I., Amend, A.S., Taylor, J.W. and Bruns, T.D. (2013) A unique signal distorts the perception of species richness and composition in high-throughput sequencing surveys of microbial communities: a case study of fungi in indoor dust, *Microb Ecol*, 66, 735-741.

Adams, R.I., Miletto, M., Taylor, J.W. and Bruns, T.D. (2013) Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances, *ISME J*, 7, 1262-1273.

Adams, R.I., Tian, Y., Taylor, J.W., Bruns, T.D., Hyvarinen, A. and Taubel, M. (2015) Passive dust collectors for assessing airborne microbial material, *Microbiome*, 3, UNSP 46.

Adhikari, A., Kettleson, E.M., Vesper, S., Kumar, S., Popham, D.L., Schaffer, C., Indugula, R., Chatterjee, K., Allam, K.K., Grinshpun, S.A. and Reponen, T. (2014) Dustborne and airborne Gram-positive and Gram-negative bacteria in high versus low ERMI homes, *Sci Total Environ*, 482-483, 92-99.

Alwis, K. and Milton, D. (2006) Recombinant factor c assay for measuring endotoxin in house dust: Comparison with LAL, and (1 -> 3)-beta-D-glucans, *Am J Ind Med*, 49, 296-300.

Amend, A.S., Seifert, K.A., Samson, R. and Bruns, T.D. (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics, *Proc Natl Acad Sci U S A*, 107, 13748-13753.

An, H., Mainelis, G. and Yao, M. (2004) Evaluation of a high-volume portable bioaerosol sampler in laboratory and field environments, *Indoor Air*, 14, 385-393.

Andersen, B., Frisvad, J.C., Sondergaard, I., Rasmussen, I.S. and Larsen, L.S. (2011) Associations between fungal species and water-damaged building materials, *Appl Environ Microbiol*, 77, 4180-4188.

Axelsson, B.O., Saraf, A. and Larsson, L. (1995) Determination of ergosterol in organic dust by gas-chromatography mass-spectrometry, *J Chromatogr B Biomed Sci Appl*, 666, 77-84.

Barberan, A., Dunn, R.R., Reich, B.J., Pacifici, K., Laber, E.B., Menninger, H.L., Morton, J.M., Henley, J.B., Leff, J.W., Miller, S.L. and Fierer, N. (2015) The ecology of microscopic life in household dust, *Proc. R. Soc. Lond. Ser. B. Biol. sci*, 282, 212-220.

Barnig, C., Reboux, G., Roussel, S., Casset, A., Sohy, C., Dalphin, J. and de Blay, F. (2013) Indoor dust and air concentrations of endotoxin in urban and rural environments, *Lett Appl Microbiol*, 56, 161-167.

Bartlett, K., Kennedy, S., Brauer, M., Van Netten, C. and Dill, B. (2004a) Evaluation and a predictive model of airborne fungal concentrations in school classrooms, *Ann Occup Hyg*, 48, 547-554.

Bartlett, K., Kennedy, S., Brauer, M., van Netten, C. and Dill, B. (2004b) Evaluation and determinants of airborne bacterial concentrations in school classrooms, *J Occup Environ Hyg*, 1, 639-647.

Baxi, S.N., Muilenberg, M.L., Rogers, C.A., Sheehan, W.J., Gaffin, J., Permaul, P., Kopel, L.S., Lai, P.S., Lane, J.P., Bailey, A., Petty, C.R., Fu, C., Gold, D.R. and Phipatanakul, W. (2013) Exposures to molds in school classrooms of children with asthma, *Pediatr Allergy Immunol*, 24, 697-703.

Bischof, W., Koch, A., Gehring, U., Fahlbusch, B., Wichmann, H.E. and Heinrich, J. (2002) Predictors of high endotoxin concentrations in the settled dust of German homes, *Indoor Air*, 12, 2-9.

Blanc, P.D., Eisner, M.D., Katz, P.P., Yen, I.H., Archea, C., Earnest, G., Janson, S., Masharani, U.B., Quinlan, P.J., Hammond, S.K., Thorne, P.S., Balmes, J.R., Trupin, L. and Yelin, E.H. (2005) Impact of the home indoor environment on adult asthma and rhinitis, *J Occup Environ Med*, 47, 362-372.

Bolte, G., Bischof, W., Borte, M., Lehmann, I., Wichmann, H.E. and Heinrich, J. (2003) Early endotoxin exposure and atopy development in infants: results of a birth cohort study, *Clin Exp Allergy*, 33, 770-776.

Borras-Santos, A., Jacobs, J.H., Taubel, M., Haverinen-Shaughnessy, U., Krop, E.J., Huttunen, K., Hirvonen, M.R., Pekkanen, J., Heederik, D.J., Zock, J.P. and Hyvarinen, A. (2013) Dampness and mould in schools and respiratory symptoms in children: the HITEA study, *Occup Environ Med*, 70, 681-687.

Braun-Fahrlander, C., Gassner, M., Grize, L., Neu, U., Sennhauser, F.H., Varonier, H.S., Vuille, J.C. and Wuthrich, B. (1999) Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community, *Clin Exp Allergy*, 29, 28-34.

Brodie, E.L., DeSantis, T.Z., Parker, J.P.M., Zubietta, I.X., Piceno, Y.M. and Andersen, G.L. (2007) Urban aerosols harbor diverse and dynamic bacterial populations, *Proc Natl Acad Sci U S A*, 104, 299-304.

Bufford, J.D., Reardon, C.L., Li, Z., Roberg, K.A., DaSilva, D., Eggleston, P.A., Liu, A.H., Milton, D., Alwis, U., Gangnon, R., Lemanske, R.F., Jr. and Gern, J.E. (2008) Effects of dog ownership in early childhood on immune development and atopic diseases, *Clin Exp Allergy*, 38, 1635-1643.

Burge, H.A., Pierson, D.L., Groves, T.O., Strawn, K.E. and Mishra, S.K. (2000) Dynamics of airborne fungal populations in a large office building, *Curr Microbiol*, 40, 10-16.

Cai, G.-., Broms, K., Malarstig, B., Zhao, Z.-., Kim, J.L., Svardsudd, K., Janson, C. and Norback, D. (2009) Quantitative PCR analysis of fungal DNA in Swedish day care centers and comparison with building characteristics and allergen levels, *Indoor Air*, 19, 392-400.

Cai, G., Malarstig, B., Kumlin, A., Johansson, I., Janson, C. and Norback, D. (2011) Fungal DNA and pet allergen levels in Swedish day care centers and associations with building characteristics, *J Environ Monit*, 13, 2018-2024.

Campo, P., Kalra, H.K., Levin, L., Reponen, T., Olds, R., Lummus, Z.L., Cho, S.H., Khurana Hershey, G.K., Lockey, J., Villareal, M., Stanforth, S., Lemasters, G. and Bernstein, D.I. (2006) Influence of dog ownership and high endotoxin on wheezing and atopy during infancy, *J Allergy Clin Immunol*, 118, 1271-1278.

Casas, L., Tischer, C., Wouters, I.M., Valkonen, M., Gehring, U., Doekes, G., Torrent, M., Pekkanen, J., Garcia-Esteban, R., Hyvarinen, A., Heinrich, J. and Sunyer, J. (2013a) Endotoxin, extracellular polysaccharides, and beta(1-3)-glucan

concentrations in dust and their determinants in four European birth cohorts: results from the HITEA project, *Indoor Air*, 23, 208-218.

Casas, L., Torrent, M., Zock, J.P., Doekes, G., Forns, J., Guxens, M., Taubel, M., Heinrich, J. and Sunyer, J. (2013b) Early life exposures to home dampness, pet ownership and farm animal contact and neuropsychological development in 4 year old children: A prospective birth cohort study, *Int J Hyg Environ Health*, 216, 690-697.

Celedon, J.C., Milton, D.K., Ramsey, C.D., Litonjua, A.A., Ryan, L., Platts-Mills, T.A.E. and Gold, D.R. (2007) Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood, *J Allergy Clin Immunol*, 120, 144-149.

Chen, C.M., Thiering, E., Doekes, G., Zock, J.P., Bakolis, I., Norback, D., Sunyer, J., Villani, S., Verlato, G., Taubel, M., Jarvis, D. and Heinrich, J. (2012a) Geographical variation and the determinants of domestic endotoxin levels in mattress dust in Europe, *Indoor Air*, 22, 24-32.

Chen, N.T., Su, Y.M., Hsu, N.Y., Wu, P.C. and Su, H.J. (2012b) Airborne fungi and bacteria in child daycare centers and the effectiveness of weak acid hypochlorous water on controlling microbes, *J Environ Monit*, 14, 2692-2697.

Chew, G.L., Douwes, J., Doekes, G., Higgins, K.M., van Strien, R., Spithoven, J. and Brunekreef, B. (2001) Fungal extracellular polysaccharides, beta (1 -> 3)-glucans and culturable fungi in repeated sampling of house dust, *Indoor Air*, 11, 171-178.

Chew, G.L., Rogers, C., Burge, H.A., Muilenberg, M.L. and Gold, D.R. (2003) Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics, *Allergy*, 58, 13-20.

Cho, S.J., Cox-Ganser, J.M. and Park, J. (2016) Observational scores of dampness and mold associated with measurements of microbial agents and moisture in three public schools, *Indoor Air*, 26, 168-178.

Daisey, J., Angell, W. and Apte, M. (2003) Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information, *Indoor Air*, 13, 53-64.

Dales, R.E., Miller, D. and McMullen, E. (1997) Indoor air quality and health: Validity and determinants of reported home dampness and moulds, *Int J Epidemiol*, 26, 120-125.

Dallongeville, A., Le Cann, P., Zmirou-Navier, D., Chevrier, C., Costet, N., Annesi-Maesano, I. and Blanchard, O. (2015) Concentration and determinants of molds and allergens in indoor air and house dust of French dwellings, *Sci Total Environ*, 536, 964-972.

Dannemiller, K.C., Gent, J.F., Leaderer, B.P. and Peccia, J. (2016) Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children, *Indoor Air*, 26, 179-192.

Dannemiller, K.C., Mendell, M.J., Macher, J.M., Kumagai, K., Bradman, A., Holland, N., Harley, K., Eskenazi, B. and Peccia, J. (2014) Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development, *Indoor Air*, 24, 236-247.

Dekoster, J.A. and Thorne, P.S. (1995) Bioaerosol concentrations in noncomplaint, complaint, and intervention homes in the midwest, *Am Ind Hyg Assoc J*, 56, 573-580.

Dharmage, S., Bailey, M., Raven, J., Abeyawickrama, K., Cao, D., Guest, D., Rolland, J., Forbes, A., Thien, F., Abramson, M. and Walters, E.H. (2002) Mouldy houses influence symptoms of asthma among atopic individuals, *Clin Exp Allergy*, 32, 714-720.

Dharmage, S., Bailey, M., Raven, J., Mitakakis, T., Thien, F., Forbes, A., Guest, D., Abramson, M. and Walters, E.H. (1999) Prevalence and residential determinants of fungi within homes in Melbourne, Australia, *Clin Exp Allergy*, 29, 1481-1489.

doPico, G. (1986) Health effects of organic dusts in the farm environment. Report on diseases, *Am J Ind Med*, 10, 261-265.

Dotterud, L., Vorland, L. and Falk, S. (1995) Viable fungi in indoor air in homes and schools in the Sor-Varanger community during winter, *Pediatr Allergy Immunol*, 6, 181-186.

Douwes, J., Doekes, G., Montijn, R., Heederik, D. and Brunekreef, B. (1997) An immunoassay for the measurement of (1 -> 3)-beta-D-glucans in the indoor environment, *Mediators Inflamm*, 6, 257-262.

Douwes, J., Siebers, R., Wouters, I.M., Doekes, G., Fitzharris, P. and Crane, J. (2006) Endotoxin, (1 -> 3)-beta-D-glucans and fungal extra-cellular polysaccharides in New Zealand homes: A pilot study, *Ann Agric Environ Med*, 13, 361-365.

Douwes, J., van der Sluis, B., Doekes, G., van Leusden, F., Wijnands, L., van Strien, R., Verhoeff, A. and Brunekreef, B. (1999) Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: Relations with culturable fungi, reported home dampness, and respiratory symptoms, *J Allergy Clin Immunol*, 103, 494-500.

Douwes, J., Versloot, P., Hollander, A., Heederik, D. and Doekes, G. (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin, *Appl Environ Microbiol*, 61, 1763-1769.

Douwes, J., Zuidhof, A., Doekes, G., van der Zee, S.C., Wouters, I., Boezen, M.H. and Brunekreef, B. (2000) (1-->3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children, *Am J Respir Crit Care Med*, 162, 1348-1354.

Ege, M.J., Mayer, M., Normand, A.C., Genuneit, J., Cookson, W., Braun-Fahrlander, C., Heederik, D., Piarroux, R. and von Mutius, E. (2011) Exposure to environmental microorganisms and childhood asthma, *N Engl J Med*, 364, 701-709.

Fierer, N., Liu, Z., Rodriguez-Hernandez, M., Knight, R., Henn, M. and Hernandez, M.T. (2008) Short-term temporal variability in airborne bacterial and fungal populations, *Appl Environ Microbiol*, 74, 200-207.

Findlay, R., Moriarty, D. and White, D. (1983) Improved method of determining muramic acid from environmental-samples, *Geomicrobiol J*, 3, 135-150.

Flannigan, B., Samson, R. and Miller, D. (2011) Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control, Second edition. SRC Press.

Foarde, K. and Berry, M. (2004a) Comparison of biocontaminant levels associated with hard vs. carpet floors in nonproblem schools: Results of a year long study, *J Expo Anal Environ Epidemiol*, 14, S41-S48.

Fox, A., Harley, W., Feigley, C., Salzberg, D., Sebastian, A. and Larsson, L. (2003) Increased levels of bacterial markers and CO2 in occupied school rooms, *J Environ Monit*, 5, 246-252.

References

Fox, A., Harley, W., Feigley, C., Salzberg, D., Toole, C., Sebastian, A. and Larsson, L. (2005) Large particles are responsible for elevated bacterial marker levels in school air upon occupation, *J Environ Monit*, 7, 450-456.

Frankel, M., Timm, M., Hansen, E.W. and Madsen, A.M. (2012) Comparison of sampling methods for the assessment of indoor microbial exposure, *Indoor Air*, 22, 405-414.

Gehring, U., Douwes, J., Doekes, G., Koch, A., Bischof, W., Fahlbusch, B., Richter, K., Wichmann, H.E. and Heinrich, J. (2001a) Beta(1-->3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds, *Environ Health Perspect*, 109, 139-144.

Gehring, U., Heinrich, J., Jacob, B., Richter, K., Fahlbusch, B., Schlenvoigt, G., Bischof, W. and Wichmann, H.E. (2001b) Respiratory symptoms in relation to indoor exposure to mite and cat allergens and endotoxins. Indoor factors and genetics in asthma (INGA) Study Group, *Eur Respir J*, 18, 555-563.

Genuneit, J. (2012) Exposure to farming environments in childhood and asthma and wheeze in rural populations: a systematic review with meta-analysis, *Pediatr Allergy Immunol*, 23, 509-518.

Gereda, J.E., Klinnert, M.D., Price, M.R., Leung, D.Y.M. and Liu, A.H. (2001) Metropolitan home living conditions associated with indoor endotoxin levels, *J Allergy Clin Immunol*, 107, 790-796.

Gessner, M.O. and Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes, *Appl Environ Microbiol*, 59, 502-507.

Giovannangelo, M., Gehring, U., Nordling, E., Oldenwening, M., Terpstra, G., Bellander, T., Hoek, G., Heinrich, J. and Brunekreef, B. (2007a) Determinants of house dust endotoxin in three European countries - the AIRALLERG study, *Indoor Air*, 17, 70-79.

Giovannangelo, M., Nordling, E., Gehring, U., Oldenwening, M., Bellander, T., Heinrich, J., Hoek, G. and Brunekreef, B. (2007b) Variation of biocontaminant levels within and between homes - the AIRALLERG study, *J Expo Sci Environ Epidemiol*, 17, 134-140.

Giovannangelo, M., Gehring, U., Nordling, E., Oldenwening, M., van Rijswijk, K., de Wind, S., Hoek, G., Heinrich, J., Bellander, T. and Brunekreef, B. (2007c) Levels and determinants of beta(1 -> 3)-glucans and fungal extracellular polysaccharides in house dust of (pre-)schoolchildren in three European countries, *Environment International*, 33, 9-16.

Golofit-Szymczak, M. and Gorny, R.L. (2010) Bacterial and fungal aerosols in air-conditioned office buildings in Warsaw, Poland-the winter season, *Int J Occup Saf Ergon*, 16, 465-476.

Gorny, R.L., Dutkiewicz, J. and Krysinska-Traczyk, E. (1999) Size distribution of bacterial and fungal bioaerosols in indoor air, *Ann Agr Env Med*, 6, 105-113.

Gorny, R. (2004) Filamentous microorganisms and their fragments in indoor air - A review, *Ann Agr Env Med*, 11, 185-197.

Grant, W.D. and West, A.W. (1986) Measurement of ergosterol, diaminopimelic acid and glucosamine in soil - evaluation as indicators of microbial biomass, *J Microbiol Methods*, 6, 47-53.

Hanson, B., Zhou, Y., Bautista, E.J., Urch, B., Speck, M., Silverman, F., Muilenberg, M., Phipatanakul, W., Weinstock, G., Sodergren, E., Gold, D.R. and Sordillo, J.E. (2016) Characterization of the bacterial and fungal microbiome in indoor dust and outdoor air samples: a pilot study, *Environ Sci Process Impacts*, 18, 713-724.

Haugland, R., Vesper, S., inventors and U.S. Environmental Protection Agency, assignee. (2003) Method of identifying and quantifying specific fungi and bacteria. US patent 6387652. 2003.

Haugland, R., Varma, M., Wymer, L. and Vesper, S. (2004) Quantitative PCR analysis of selected Aspergillus, Penicillium and Paecilomyces species, *Syst Appl Microbiol*, 27, 198-210.

Haverinen, U., Husman, T., Toivola, M., Suonketo, J., Pentti, M., Lindberg, R., Leinonen, J., Hyvarinen, A., Meklin, T. and Nevalainen, A. (1999) An approach to management of critical indoor air problems in school buildings, *Environ Health Perspect*, 107, 509-514.

Haverinen-Shaughnessy, U., Borras-Santos, A., Turunen, M., Zock, J.P., Jacobs, J., Krop, E.J., Casas, L., Shaughnessy, R., Taubel, M., Heederik, D., Hyvarinen, A., Pekkanen, J., Nevalainen, A. and HITEA study group. (2012) Occurrence of moisture problems in schools in three countries from different climatic regions of Europe based on questionnaires and building inspections - the HITEA study, *Indoor Air*, 22, 457-466.

Haverinen-Shaughnessy, U., Hyvarinen, A., Pekkanen, J., Nevalainen, A., Husman, T., Korppi, M., Halla-Aho, J., Koivisto, J. and Moschandreas, D. (2005) Occurrence and characteristics of moisture damage in residential buildings as a function of occupant and engineer observations, *Indoor Built Environ*, 14, 133-140.

Heinrich, J., Gehring, U., Douwes, J., Koch, A., Fahlbusch, B., Bischof, W., Wichmann, H.E. and Grp, I.N. (2001) Pets and vermin are associated with high endotoxin levels in house dust, *Clin Exp Allergy*, 31, 1839-1845.

Heinrich, J., Holscher, B., Douwes, J., Richter, K., Koch, A., Bischof, W., Fahlbusch, B., Kinne, R.W. and Wichmann, H.E. (2003) Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment, *J Expo Anal Environ Epidemiol*, 13, 152-160.

Hirvonen, M., Ruotsalainen, M., Roponen, M., Hyvarinen, A., Husman, T., Kosma, V., Komulainen, H., Savolainen, K. and Nevalainen, A. (1999) Nitric oxide and proinflammatory cytokines in nasal lavage fluid associated with symptoms and exposure to moldy building microbes, *Am J Respir Crit Care Med*, 160, 1943-1946.

Hirvonen, M., Ruotsalainen, M., Savolainen, K. and Nevalainen, A. (1997) Effect of viability of actinomycete spores on their ability to stimulate production of nitric oxide and reactive oxygen species in RAW264.7 macrophages, *Toxicology*, 124, 105-114.

Hoang, C.P., Kinney, K.A., Corsi, R.L. and Szaniszlo, P.J. (2010) Resistance of green building materials to fungal growth, *Int Biodeterior Biodegrad*, 64, 104-113.

Holst, G., Host, A., Doekes, G., Meyer, H.W., Madsen, A.M. and Sigsgaard, T. (2015) Determinants of house dust, endotoxin, and beta-(1 -> 3)-D-glucan in homes of Danish children, *Indoor Air*, 25, 245-259.

Hong, H., Pruden, A. and Reardon, K.F. (2007) Comparison of CE-SSCP and DGGE for monitoring a complex microbial community remediating mine drainage, *J Microbiol Methods*, 69, 52-64.

Horner, W.E., Worthan, A.G. and Morey, P.R. (2004) Air- and dustborne mycoflora in houses free of water damage and fungal growth, *Appl Environ Microbiol*, 70, 6394-6400.

Hospodsky, D., Qian, J., Nazaroff, W.W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H. and Peccia, J. (2012) Human occupancy as a source of indoor airborne bacteria, *PLoS One*, 7, e34867.

Hyvärinen, A., Meklin, T., Vepsalainen, A. and Nevalainen, A. (2002) Fungi and actinobacteria in moisture-damaged building materials - concentrations and diversity, *Int Biodeterior Biodegrad*, 49, 27-37.

Hyvärinen, A., Reponen, T., Husman, T., Ruuskanen, J. and Nevalainen, A. (1993) Characterizing mold problem buildings - concentrations and flora of viable fungi, *Indoor Air*, 3, 337-343.

Hyvärinen, A., Roponen, M., Tiittanen, P., Laitinen, S., Nevalainen, A. and Pekkanen, J. (2006a) Dust sampling methods for endotoxin - an essential, but underestimated issue, *Indoor Air*, 16, 20-27.

Hyvärinen, A., Sebastian, A., Pekkanen, J., Larsson, L., Korppi, M., Putus, T. and Nevalainen, A. (2006b) Characterizing microbial exposure with ergosterol, 3-hydroxy fatty acids, and viable microbes in house dust: Determinants and association with childhood asthma, *Arch Environ Occup Health*, 61, 149-157.

Hyvärinen, A., Vahteristo, M., Meklin, T., Jantunen, M., Nevalainen, A. and Moschandreas, D. (2001) Temporal and spatial variation of fungal concentrations in indoor air, *Aerosol Sci Technol*, 35, 688-695.

Institute of Medicine (US) Committee on Damp Indoor Spaces and Health. (2004). Washington, DC: The National Academies Press.

Jacob, B., Ritz, B., Gehring, U., Koch, A., Bischof, W., Wichmann, H.E., Heinrich, J. and INGA-Study Grp. (2002) Indoor exposure to molds and allergic sensitization, *Environ Health Perspect*, 110, 647-653.

Jacobs, J.H., Krop, E.J., Borras-Santos, A., Zock, J.P., Täubel, M., Hyvärinen, A., Pekkanen, J., Doekes, G., Heederik, D.J. and HITEA schools study consortium. (2014a) Endotoxin levels in settled airborne dust in European schools: the HITEA school study, *Indoor Air*, 24, 148-157.

Jacobs, J.H., Krop, E.J.M., de Wind, S., Spithoven, J. and Heederik, D.J.J. (2013) Endotoxin levels in homes and classrooms of Dutch school children and respiratory health, *Eur Respir J*, 42, 314-322.

Jacobs, J., Borras-Santos, A., Krop, E., Taubel, M., Leppanen, H., Haverinen-Shaughnessy, U., Pekkanen, J., Hyvarinen, A., Doekes, G., Zock, J. and Heederik, D. (2014b) Dampness, bacterial and fungal components in dust in primary schools and respiratory health in schoolchildren across Europe, *Occup Environ Med*, 71, 704-712.

Johansson, E., Vesper, S., Levin, L., LeMasters, G., Grinshpun, S. and Reponen, T. (2011) Streptomycetes in house dust: associations with housing characteristics and endotoxin, *Indoor Air*, 21, 300-310.

Jovanovic, S., Felder-Kennel, A., Gabrio, T., Kouros, B., Link, B., Maisner, V., Piechotowski, I., Schick, K.H., Schrimpf, M., Weidner, U., Zollner, I. and Schwenk, M. (2004) Indoor fungi levels in homes of children with and without allergy history, *Int J Hyg Environ Health*, 207, 369-378.

Kaarakainen, P., Rintala, H., Meklin, T., Karkkainen, P., Hyvarinen, A. and Nevalainen, A. (2011) Concentrations and diversity of microbes from four local bioaerosol emission sources in Finland, *J Air Waste Manag Assoc*, 61, 1382-1392.

Kaarakainen, P., Rintala, H., Vepsalainen, A., Hyvarinen, A., Nevalainen, A. and Meklin, T. (2009) Microbial content of house dust samples determined with qPCR, *Sci Total Environ*, 407, 4673-4680.

Kalatoor, S., Grinshpun, S.A., Willeke, K. and Baron, P. (1995) New aerosol sampler with low wind sensitivity and good filter collection uniformity, *Atmos Environ*, 29, 1105-1112.

Kanchongkittiphon, W., Mendell, M.J., Gaffin, J.M., Wang, G. and Phipatanakul, W. (2015) Indoor environmental exposures and exacerbation of asthma: an update to the 2000 review by the Institute of Medicine, *Environ Health Perspect*, 123, 6-20.

Kärkkäinen, P.M., Valkonen, M., Hyvärinen, A., Nevalainen, A. and Rintala, H. (2010) Determination of bacterial load in house dust using qPCR, chemical markers and culture, *J Environ Monit*, 12, 759-768.

Karvonen, A.M., Hyvarinen, A., Gehring, U., Korppi, M., Doekes, G., Riedler, J., Braun-Fahrlaender, C., Bitter, S., Schmid, S., Keski-Nisula, L., Roponen, M., Kaulek, V., Dalphin, J.-., Pfefferle, P.I., Renz, H., Buechele, G., von Mutius, E., Pekkanen, J. and PASTURE Study Grp. (2012) Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas, *Clin Exp Allergy*, 42, 1246-1256.

Karvonen, A.M., Hyvarinen, A., Roponen, M., Hoffmann, M., Korppi, M., Remes, S., von Mutius, E., Nevalainen, A. and Pekkanen, J. (2009) Confirmed moisture damage at home, respiratory symptoms and atopy in early life: A birth-cohort study, *Pediatrics*, 124, E329-E338.

Kembel, S.W., Meadow, J.F., O'Connor, T.K., Mhuireach, G., Northcutt, D., Kline, J., Moriyama, M., Brown, G.Z., Bohannan, B.J.M. and Green, J.L. (2014) Architectural design drives the biogeography of indoor bacterial communities, *Plos One*, 9, e87093.

Kenny, L., Bowry, A., Crook, B. and Stancliffe, J. (1999) Field testing of a personal size-selective bioaerosol sampler, *Ann Occup Hyg*, 43, 393-404.

Kettleson, E., Kumar, S., Reponen, T., Vesper, S., Meheust, D., Grinshpun, S.A. and Adhikari, A. (2013) Stenotrophomonas, Mycobacterium, and Streptomyces in home dust and air: associations with moldiness and other home/family characteristics, *Indoor Air*, 23, 387-396.

Kilburg-Basnyat, B., Peters, T.M., Perry, S.S. and Thorne, P.S. (2015) Electrostatic dust collectors compared to inhalable samplers for measuring endotoxin concentrations in farm homes, *Indoor Air*, 26, 724-733.

Klanova, K. (2000) The concentrations of mixed populations of fungi in indoor air: rooms with and without mould problems; rooms with and without health complaints, *Cent Eur J Public Health*, 8, 59-61.

Koch, A., Heilemann, K.J., Bischof, W., Heinrich, J. and Wichmann, H.E. (2000) Indoor viable mold spores - a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany), *Allergy*, 55, 176-180.

Korthals, M., Ege, M., Lick, S., von Mutius, E. and Bauer, J. (2008) Occurrence of Listeria spp. in mattress dust of farm children in Bavaria, *Environmental Research*, 107, 299-304.

Kotimaa, M., Husman, K., Terho, E. and Mustonen, M. (1984) Airborne molds and actinomycetes in the work-environment of farmers lung patients in Finland, *Scand J Work Environ Health*, 10, 115-119.

Krop, E.J.M., Jacobs, J.H., Sander, I., Raulf-Heimsoth, M. and Heederik, D.J.J. (2014) Allergens and beta-glucans in Dutch homes and schools: characterizing airborne levels, *Plos One*, 9, e88871.

Lacey, J. and Crook, B. (1988) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens, *Ann Occup Hyg*, 32, 515-533.

Lai, P.S., Sheehan, W.J., Gaffin, J.M., Petty, C.R., Coull, B.A., Gold, D.R. and Phipatanakul, W. (2015) School Endotoxin Exposure and Asthma Morbidity in Inner-city Children, *Chest*, 148, 1251-1258.

Lappalainen, M.H.J., Roponen, M., Hyvarinen, A., Nevalainen, A., Laine, O., Pekkanen, J. and Hirvonen, M.R. (2008) Exposure to environmental bacteria may have differing effects on tumour necrosis factor-alpha and interleukin-6-producing capacity in infancy, *Clin Exp Allergy*, 38, 1483-1492.

Larsson, L., Szponar, B. and Pehrson, C. (2004) Tobacco smoking increases dramatically air concentrations of endotoxin, *Indoor Air*, 14, 421-424.

Larsson, L., Szponar, B., Ridha, B., Pehrson, C., Dutkiewicz, J., Krysinska-Traczyk, E. and Sitkowska, J. (2008) Identification of bacterial and fungal components in tobacco and tobacco smoke, *Tob Induc Dis*, 4, 4-9625-4-4.

Lehtonen, M., Reponen, T. and Nevalainen, A. (1993) Everyday activities and variation of fungal spore concentrations in indoor air, *Int Biodeterior Biodegrad*, 31, 25-39.

Leung, M.H.Y. and Lee, P.K.H. (2016) The roles of the outdoors and occupants in contributing to a potential pan-microbiome of the built environment: a review, *Microbiome*, 4, 21.

Li, C.S. and Kuo, Y.M. (1994) Characteristics of airborne microfungi in subtropical homes, *Sci Total Environ*, 155, 267-271.

Lignell, U., Meklin, T., Putus, T., Vepsalainen, A., Roponen, M., Torvinen, E., Reeslev, M., Pennanen, S., Hirvonen, M.R., Kalliokoski, P. and Nevalainen, A. (2005) Microbial exposure, symptoms and inflammatory mediators in nasal lavage fluid of kitchen and clerical personnel in schools, *Int J Occup Med Environ Health*, 18, 139-150.

Lignell, U., Meklin, T., Rintala, H., Hyvarinen, A., Vepsalainen, A., Pekkanen, J. and Nevalainen, A. (2008) Evaluation of quantitative PCR and culture methods for detection of house dust fungi and streptomycetes in relation to moisture damage of the house, *Lett Appl Microbiol*, 47, 303-308.

Lignell, U., Meklin, T., Putus, T., Rintala, H., Vepsalainen, A., Kalliokoski, P. and Nevalainen, A. (2007) Effects of moisture damage and renovation on microbial conditions and pupils' health in two schools - a longitudinal analysis of five years, *J Environ Monit*, 9, 225-233.

Lindsley, W.G., Schmechel, D. and Chen, B.T. (2006) A two-stage cyclone using microcentrifuge tubes for personal bioaerosol sampling, *J Environ Monit*, 8, 1136-1142.

Liu, L.J., Krahmer, M., Fox, A., Feigley, C.E., Featherstone, A., Saraf, A. and Larsson, L. (2000) Investigation of the concentration of bacteria and their cell envelope components in indoor air in two elementary schools, *J Air Waste Manag Assoc*, 50, 1957-1967.

Loo, C.K.J., Foty, R.G., Wheeler, A.J., Miller, J.D., Evans, G., Stieb, D.M. and Dell, S.D. (2010) Do questions reflecting indoor air pollutant exposure from a questionnaire predict direct measure of exposure in owner-occupied houses? *Int J Environ Res Publ Health*, 7, 3270-3297.

Madureira, J., Paciencia, I., Ramos, E., Barros, H., Pereira, C., Teixeira, J.P. and Fernandes, E.d.O. (2015) Children's health and indoor air quality in primary schools and homes in Portugal-study design, *J Toxicol Environ Health A*, 78, 915-930.

Madureira, J., Pereira, C., Paciencia, I., Teixeira, J.P. and Fernandes, E.d.O. (2014) Identification and levels of airborne fungi in Portuguese primary schools, *J Toxicol Environ Health A*, 77, 816-826.

Malmberg, P., Raskandersen, A. and Rosenhall, L. (1993) Exposure to microorganisms associated with allergic alveolitis and febrile reactions to mold dust in farmers, *Chest*, 103, 1202-1209.

Matheson, M.C., Dharmage, S.C., Forbes, A.B., Raven, J.M., Woods, R.K., Thien, F.C., Guest, D.I., Rolland, J.M., Haydn Walters, E. and Abramson, M.J. (2003) Residential characteristics predict changes in Der p 1, Fel d 1 and ergosterol but not fungi over time, *Clin Exp Allergy*, 33, 1281-8.

McKenzie, J.H., Alwis, K.U., Sordillo, J.E., Kalluri, K.S. and Milton, D.K. (2011) Evaluation of lot-to-lot repeatability and effect of assay media choice in the recombinant Factor C assay, *J Environ Monit*, 13, 1739-1745.

Meadow, J.F., Altrichter, A.E., Kembel, S.W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'Connor, T.K., Womack, A.M., Brown, G.Z., Green, J.L. and Bohannan, B.J.M. (2014) Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source, *Indoor Air*, 24, 41-48.

Meklin, T., Haugland, R.A., Reponen, T., Varma, M., Lummus, Z., Bernstein, D., Wymer, L.J. and Vesper, S.J. (2004) Quantitative PCR analysis of house dust can reveal abnormal mold conditions, *J Environ Monit*, 6, 615-620.

Meklin, T., Husman, T., Vepsalainen, A., Vahteristo, M., Koivisto, J., Halla-Aho, J., Hyvarinen, A., Moschandreas, D. and Nevalainen, A. (2002) Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools, *Indoor Air*, 12, 175-183.

Meklin, T., Hyvarinen, A., Toivola, M., Reponen, T., Koponen, V., Husman, T., Taskinen, T., Korppi, M. and Nevalainen, A. (2003) Effect of building frame and moisture damage on microbiological indoor air quality in school buildings, *AIHA J*, 64, 108-116.

Meklin, T., Potus, T., Pekkanen, J., Hyvarinen, A., Hirvonen, M. and Nevalainen, A. (2005) Effects of moisture-damage repairs on microbial exposure and symptoms in schoolchildren, *Indoor Air*, 15, 40-47.

Mendell, M.J., Mirer, A.G., Cheung, K., Tong, M. and Douwes, J. (2011) Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence, *Environ Health Perspect*, 119, 748-756.

Mentese, S., Rad, A.Y., Arisoy, M. and Gullu, G. (2012) Multiple comparisons of organic, microbial, and fine particulate pollutants in typical indoor environments: diurnal and seasonal variations, *J Air Waste Manage Assoc*, 62, 1380-1393.

Miletto, M. and Lindow, S.E. (2015) Relative and contextual contribution of different sources to the composition and abundance of indoor air bacteria in residences, *Microbiome*, 3, UNSP 61.

Mille-Lindblom, C., von Wachenfeldt, E. and Tranvik, L. (2004) Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death, *J Microbiol Methods*, 59, 253-262.

Miller, J.D., Laflamme, A.M., Sobol, Y., Lafontaine, P. and Greenhalgh, R. (1988) Fungi and fungal products in some Canadian houses, *International Biodeterioration*, 24, 103-120.

Milton, D., Alwis, K., Fisette, L. and Muilenberg, M. (2001) Enzyme-linked immunosorbent assay specific for (1 -> 6) branched, (1 -> 3)-beta-D-glucan detection in environmental samples, *Appl Environ Microbiol*, 67, 5420-5424.

Mimura, T. and Romano, J.C. (1985) Muramic acid measurements for bacterial investigations in marine environments by high-pressure liquid chromatography, *Appl Environ Microbiol*, 50, 229-237.

Moniruzzaman, S., Engman, L.H., James, P., Sigsgaard, T., Thorne, P.S., Sundell, J. and Bornehag, C.G. (2012) Levels of endotoxin in 390 Swedish homes: determinants and the risk for respiratory symptoms in children, *Int J Environ Health Res*, 22, 22-36.

Muyzer, G., de Waal, E. and Uitterlinden, A. (1993) Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s ribosomal-RNA, *Appl Environ Microbiol*, 59, 695-700.

Nevalainen, A., Partanen, P., Jaaskelainen, E., Hyvarinen, A., Koskinen, O., Meklin, T., Vahteristo, M., Koivisto, J. and Husman, T. (1998) Prevalence of moisture problems in Finnish houses, *Indoor Air*, 8, 45-49.

Nevalainen, A. and Seuri, M. (2005) Of microbes and men, *Indoor Air*, 15, 58-64.

Nevalainen, A., Taubel, M. and Hyvarinen, A. (2015) Indoor fungi: companions and contaminants, *Indoor Air*, 25, 125-156.

Nielsen, K.F. and Madsen, J.O. (2000) Determination of ergosterol on mouldy building materials using isotope dilution and gas chromatography-tandem mass spectrometry, *J Chromatogr A*, 898, 227-234.

Niemeier, R.T., Sivasubramani, S.K., Reponen, T. and Grinshpun, S.A. (2006) Assessment of fungal contamination in moldy homes: comparison of different methods, *J Occup Environ Hyg*, 3, 262-273.

Normand, A., Ranque, S., Cassagne, C., Gaudart, J., Sallah, K., Charpin, D. and Piarroux, R. (2016) Comparison of air impaction and electrostatic dust collector sampling methods to assess airborne fungal contamination in public buildings, *Ann Occup Hyg*, 60, 161-175.

Normand, A., Sudre, B., Vacheyrou, M., Depner, M., Wouters, I.M., Noss, I., Heederik, D., Hyvarinen, A., Genuneit, J., Braun-Fahrlaender, C., von Mutius, E., Piarroux, R. and GABRIEL-A Study Grp. (2011) Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings, *Occup Environ Med*, 68, 849-855.

Noss, I., Wouters, I.M., Visser, M., Heederik, D.J., Thorne, P.S., Brunekreef, B. and Doekes, G. (2008) Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment, *Appl Environ Microbiol*, 74, 5621-5627.

Noss, I., Wouters, I.M., Bezemer, G., Metwali, N., Sander, I., Raulf-Heimsoth, M., Heederik, D.J.J., Thorne, P.S. and Doekes, G. (2010) beta-(1,3)-glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays, *Appl Environ Microbiol*, 76, 1158-1167.

Nunan, N., Daniell, T., Singh, B., Papert, A., McNicol, J. and Prosser, J. (2005) Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques, *Appl Environ Microbiol*, 71, 6784-6792.

Orman, A., Korcan, E., Konuk, M., Kurt, E., Toprak, D. and Ay, A. (2006) Determination of fungal frequency and comparison of allergic symptoms related with buildings and fungi in Afyon, Turkey, *Saudi Med J*, 27, 1146-1151.

Ownby, D.R., Peterson, E.L., Williams, L.K., Zoratti, E.M., Wegienka, G.R., Woodcroft, K.J., Joseph, C.L.M. and Johnson, C.C. (2010) Variation of dust endotoxin concentrations by location and time within homes of young children, *Pediatr Allergy Immunol*, 21, 533-540.

Park, J.H., Spiegelman, D.L., Burge, H.A., Gold, D.R., Chew, G.L. and Milton, D.K. (2000) Longitudinal study of dust and airborne endotoxin in the home, *Environ Health Perspect*, 108, 1023-1028.

Park, J.H., Szponar, B., Larsson, L., Gold, D.R. and Milton, D.K. (2004) Characterization of lipopolysaccharides present in settled house dust, *Appl Environ Microbiol*, 70, 262-267.

Pasanen, A.L., Kalliokoski, P., Pasanen, P., Salmi, T. and Tossavainen, A. (1989) Fungi carried from farmers work into farm homes, *Am Ind Hyg Assoc J*, 50, 631-633.

Pasanen, A.L., Yli-Pietila, K., Pasanen, P., Kalliokoski, P. and Tarhanen, J. (1999) Ergosterol content in various fungal species and biocontaminated building materials, *Appl Environ Microbiol*, 65, 138-142.

Pasanen, A., Kasanen, J., Rautiala, S., Ikaheimo, M., Rantamaki, J., Kaariainen, H. and Kalliokoski, P. (2000) Fungal growth and survival in building materials under fluctuating moisture and temperature conditions, *Int Biodeterior Biodegrad*, 46, 117-127.

Pekkanen, J., Hyvarinen, A., Haverinen-Shaughnessy, U., Korppi, M., Putus, T. and Nevalainen, A. (2007) Moisture damage and childhood asthma: a population-based incident case-control study, *Eur Respir J*, 29, 509-515.

Pekkanen, J., Xu, B. and Jarvelin, M.R. (2001) Gestational age and occurrence of atopy at age 31 - a prospective birth cohort study in Finland, *Clin Exp Allergy*, 31, 95-102.

Perzanowski, M.S., Miller, R.L., Thorne, P.S., Barr, R.G., Divjan, A., Sheares, B.J., Garfinkel, R.S., Perera, F.P., Goldstein, I.F. and Chew, G.L. (2006) Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood, *J Allergy Clin Immunol*, 117, 1082-1089.

Pessi, A.M., Suonketo, J., Pentti, M., Kurkilahti, M., Peltola, K. and Rantio-Lehtimaki, A. (2002) Microbial growth inside insulated external walls as an indoor air biocontamination source, *Appl Environ Microbiol*, 68, 963-967.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Paulin, L., Auvinen, P., Nevalainen, A. and Rintala, H. (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture, *Appl Environ Microbiol*, 74, 233-244.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Nevalainen, A., Paulin, L., Auvinen, P., Lignell, U. and Rintala, H. (2011) Molecular profiling of fungal communities in moisture damaged buildings before and after remediation - a comparison of culture-dependent and culture-independent methods, *BMC Microbiol*, 11, 235.

Platts-Mills, T., Vaughan, J., Squillace, S., Woodfolk, J. and Sporik, R. (2001) Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study, *Lancet*, 357, 752-756.

Prussin, A. and Marr, L. (2015) Sources of airborne microorganisms in the built environment, *Microbiome*, 3, UNSP 78.

Purokivi, M., Hirvonen, M., Randell, J., Roponen, M., Meklin, T., Nevalainen, A., Human, T. and Tukiainen, H. (2001) Changes in pro-inflammatory cytokines in association with exposure to moisture-damaged building microbes, *Eur Respir J*, 18, 951-958.

Qian, J., Hospodsky, D., Yamamoto, N., Nazaroff, W.W. and Peccia, J. (2012) Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom, *Indoor Air*, 22, 339-351.

Reijula, K., Ahonen, G., Alenius, H., Holopainen, R., Lappalainen, S., Palomäki, E. and Reiman, M. (2012) Rakennusten kosteus- ja homeongelmat. Eduskunnan tarkastusvaliokunnan julkaisu, Eduskunta, 1/2012, 205 s.

Ren, P., Jankun, T.M., Belanger, K., Bracken, M.B. and Leaderer, B.P. (2001) The relation between fungal propagules in indoor air and home characteristics, *Allergy*, 56, 419-424.

Ren, P., Jankun, T.M. and Leaderer, B.P. (1999) Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county, *J Expo Anal Environ Epidemiol*, 9, 560-568.

Rennie, D.C., Lawson, J.A., Kirychuk, S.P., Paterson, C., Willson, P.J., Senthilselvan, A. and Cockcroft, D.W. (2008) Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children, *Indoor Air*, 18,447-453.

Reponen, T., Nevalainen, A., Jantunen, M. and Kalliokoski, P. (1992) Normal range criteria for indoor air bacteria and fungal spores in subarctic climate, *Indoor Air*, 2, 26-31.

Reponen, T., Singh, U., Schaffer, C., Vesper, S., Johansson, E., Adhikari, A., Grinshpun, S.A., Indugula, R., Ryan, P., Levin, L. and LeMasters, G. (2010) Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes, *Sci Total Environ*, 408, 5565-5574.

Reponen, T., Willeke, K., Grinshpun, S. and Nevalainen, A. (2001) Biological particle sampling, In: Aerosol measurement, principles, techniques and applications. Second edition. Baron BA, Willeke K eds. New York; John Wiley and Sons, 751-778.

Rintala, H. and Nevalainen, A. (2006) Quantitative measurement of streptomycetes using real-time PCR, *J Environ Monit*, 8, 745-749.

Rintala, H., Pitkaeranta, M., Toivola, M., Paulin, L. and Nevalainen, A. (2008) Diversity and seasonal dynamics of bacterial community in indoor environment, *BMC Microbiol*, 8.

Rintala, H., Pitkäranta, M. and Täubel, M. (2012) Microbial communities associated with house dust, *Adv Appl Microbiol*, 78, 75-120.

Roponen, M., Hyvarinen, A., Hirvonen, M., Keski-Nisula, L. and Pekkanen, J. (2005) Change in IFN-gamma-producing capacity in early life and exposure to environmental microbes, *J Allergy Clin Immunol*, 116, 1048-1052.

Roponen, M., Meklin, T., Rintala, H., Hyvaerinen, A. and Hirvonen, M.-. (2013) Effect of moisture-damage intervention on the immunotoxic potential and microbial content of airborne particles and on occupants' upper airway inflammatory responses, *Indoor Air*, 23, 295-302.

Ross, A.A. and Neufeld, J.D. (2015) Microbial biogeography of a university campus, *Microbiome*, 3, 66.

Rylander, R., Persson, K., Goto, H., Yuasa, H. and Tanaka, S. (1992) Airborne beta-1,3-glucan may be related to symptoms in sick buildings, *Indoor Built Environ*, 1, 263-267.

Salonen, H., Duchaine, C., Letourneau, V., Mazaheri, M., Laitinen, S., Clifford, S., Mikkola, R., Lappalainen, S., Reijula, K. and Morawska, L. (2016) Endotoxin levels and contribution factors of endotoxins in resident, school, and office environments - a review, *Atmos Environ*, 142, 360-369.

Salonen, H., Duchaine, C., Mazaheri, M., Clifford, S., Lappalainen, S., Reijula, K. and Morawska, L. (2015) Airborne viable fungi in school environments in different climatic regions a review, *Atmos Environ*, 104, 186-194.

Saraf, A., Larsson, L., Burge, H. and Milton, D. (1997) Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography mass spectrometry: comparison with fungal culture and determination of endotoxin by a Limulus amebocyte lysate assay, *Appl Environ Microbiol*, 63, 2554-2559.

Sauni, R., Uitti, J., Jauhiainen, M., Kreiss, K., Sigsgaard, T. and Verbeek, J.H. (2013) Remediating buildings damaged by dampness and mould for preventing or reducing respiratory tract symptoms, infections and asthma (Review), *Evid Based Child Health*, 8, 944-1000.

Schleifer, K.H. and Kandler, O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications, *Bacteriol Rev*, 36, 407-477.

Schram, D., Doekes, G., Boeve, M., Douwes, J., Riedler, J., Ublagger, E., von Mutius, E., Budde, J., Pershagen, G., Nyberg, F., Alm, J., Braun-Fahrlander, C., Waser, M., Brunekreef, B. and Parsifal Study Grp. (2005) Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children - the PARSIFAL Study, *Allergy*, 60, 611-618.

Schram-Bijkerk, D., Doekes, G., Boeve, M., Douwes, J., Riedler, J., Ublagger, E., von Mutius, E., Benz, M., Pershagen, G., Wickman, M., Alfven, T., Braun-Fahrlander, C., Waser, M., Brunekreef, B. and Parsifal Study Grp. (2006) Exposure to microbial components and allergens in population studies: a comparison of two house dust

collection methods applied by participants and fieldworkers, *Indoor Air*, 16, 414-425.

Sebastian, A., Harley, W., Fox, A. and Larsson, L. (2004) Evaluation of the methyl ester O-methyl acetate derivative of muramic acid for the determination of peptidoglycan in environmental samples by ion-trap GC-MS-MS, *J Environ Monit*, 6, 300-304.

Sebastian, A. and Larsson, L. (2003) Characterization of the microbial community in indoor environments: a chemical-analytical approach, *Appl Environ Microbiol*, 69, 3103-3109.

Sebastian, A., Pehrson, C. and Larsson, L. (2006) Elevated concentrations of endotoxin in indoor air due to cigarette smoking, *J Environ Monit*, 8, 519-522.

Sebastian, A., Szponar, B. and Larsson, L. (2005) Characterization of the microbial community in indoor environments by chemical marker analysis: an update and critical evaluation, *Indoor Air*, 15, 20-26.

Shelton, B., Kirkland, K., Flanders, W. and Morris, G. (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States, *Appl Environ Microbiol*, 68, 1743-1753.

Simoni, M., Cai, G., Norback, D., Annesi-Maesano, I., Lavaud, F., Sigsgaard, T., Wieslander, G., Nystad, W., Canciani, M., Viegi, G. and Sestini, P. (2011) Total viable molds and fungal DNA in classrooms and association with respiratory health and pulmonary function of European schoolchildren, *Pediatr Allergy Immunol*, 22, 843-852.

Singh, U., Levin, L., Grinshpun, S.A., Schaffer, C., Adhikari, A. and Reponen, T. (2011) Influence of home characteristics on airborne and dustborne endotoxin and beta-D-glucan, *J Environ Monit*, 13, 3246-3253.

Smedje, G. and Norback, D. (2001) Irritants and allergens at school in relation to furnishings and cleaning, *Indoor Air*, 11, 127-133.

Sordillo, J.E., Hoffman, E.B., Celedon, J.C., Litonjua, A.A., Milton, D.K. and Gold, D.R. (2010) Multiple microbial exposures in the home may protect against asthma or allergy in childhood, *Clin Exp Allergy*, 40, 902-910.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R. and Milton, D.K. (2011) Home characteristics as predictors of bacterial and fungal microbial biomarkers in house dust, *Environ Health Perspect*, 119, 189-195.

SPSS Inc. (1988) SPSS-X™ User's Guide, Chicago, IL, SPSS Inc.

Stetzenbach, L., Buttner, M. and Cruz, P. (2004) Detection and enumeration of airborne biocontaminants, *Curr Opin Biotechnol*, 15, 170-174.

Strachan, D. (1989) Hay-Fever, Hygiene, and Household Size, *Br Med J*, 299, 1259-1260.

Su, H., Wu, P., Chen, H., Lee, F. and Lin, L. (2001) Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in Southern Taiwan, *Environmental Research*, 85, 135-144.

Szponar, B., Pehrson, C. and Larsson, L. (2012) Bacterial and fungal markers in tobacco smoke, *Sci Total Environ*, 438, 447-451.

Tager, I.B., Lurmann, F.W., Haight, T., Alcorn, S., Penfold, B. and Hammond, S.K. (2010) Temporal and spatial patterns of ambient endotoxin concentrations in Fresno, California, *Environ Health Perspect*, 118, 1490-1496.

Thatcher, T. and Layton, D. (1995) Deposition, resuspension, and penetration of particles within a residence, *Atmos Environ*, 29, 1487-1497.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J. and Zeldin, D.C. (2009) Predictors of endotoxin levels in US housing, *Environ Health Perspect*, 117, 763-771.

Thorne, P.S., Kulhankova, K., Yin, M., Cohn, R., Arbes, S.J. and Zeldin, D.C. (2005) Endotoxin exposure is a risk factor for asthma - the national survey of endotoxin in United States housing, *Am J Respir Crit Care Med*, 172, 1371-1377.

Thorne, P.S., Perry, S.S., Saito, R., O'Shaughnessy, P.T., Mehaffy, J., Metwali, N., Keefe, T., Donham, K.J. and Reynolds, S.J. (2010) Evaluation of the Limulus amebocyte lysate and Recombinant Factor C assays for assessment of airborne endotoxin, *Appl Environ Microbiol*, 76, 4988-4995.

Tischer, C., Zock, J., Valkonen, M., Doekes, G., Guerra, S., Heederik, D., Jarvis, D., Norback, D., Olivieri, M., Sunyer, J., Svanes, C., Taubel, M., Thiering, E., Verlato, G., Hyvarinen, A. and Heinrich, J. (2015) Predictors of microbial agents in dust and respiratory health in the ECRHS, *BMC Pulm Med*, 15, 48.

Toivola, M., Alm, S., Reponen, T., Kolari, S. and Nevalainen, A. (2002) Personal exposures and microenvironmental concentrations of particles and bioaerosols, *J Environ Monit*, 4, 166-174.

Toivola, M., Nevalainen, A. and Alm, S. (2004) Personal exposures to particles and microbes in relation to microenvironmental concentrations, *Indoor Air*, 14, 351-359.

Topp, R., Wimmer, K., Fahlbusch, B., Bischof, W., Richter, K., Wichmann, H.E. and Heinrich, J. (2003) Repeated measurements of allergens and endotoxin in settled house dust over a time period of 6 years, *Clin Exp Allergy*, 33, 1659-1666.

Torvinen, E., Torkko, P., Nevalainen, A. and Rintala, H. (2010) Real-time PCR detection of environmental mycobacteria in house dust, *J Microbiol Methods*, 82, 78-84.

Täubel, M., Rintala, H., Pitkäranta, M., Paulin, L., Laitinen, S., Pekkanen, J., Hyvärinen, A. and Nevalainen, A. (2009) The occupant as a source of house dust bacteria, *J Allergy Clin Immunol*, 124, 834-840.

U.S. EPA. Child-Specific Exposure Factors Handbook (Final Report). (2008) U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/096F, 2008.

van Strien, R.T., Engel, R., Holst, O., Bufe, A., Eder, W., Waser, M., Braun-Fahrlander, C., Riedler, J., Nowak, D., von Mutius, E. and Alex Study Team. (2004) Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health, *J Allergy Clin Immunol*, 113, 860-867.

Verhoeff, A., van Strien, R., van Wijnen, J. and Brunekreef, B. (1995) Damp housing and childhood respiratory symptoms - the role of sensitization to dust mites and molds, *Am J Epidemiol*, 141, 103-110.

Verhoeff, A., Vanreenenhoekstra, E., Samson, R., Brunekreef, B. and Vanwijnen, J. (1994a) Fungal propagules in house dust 1. Comparison of analytic methods and their value as estimators of potential exposure, *Allergy*, 49, 533-539.

Verhoeff, A., Vanwijnen, J., Vanreenenhoekstra, E., Samson, R., van Strien, R. and Brunekreef, B. (1994b) Fungal propagules in house dust 2. Relation with residential characteristics and respiratory symptoms, *Allergy*, 49, 540-547.

Vogel, K., Blumer, N., Korthals, M., Mittelstadt, J., Garn, H., Ege, M., von Mutius, E., Gatermann, S., Bufe, A., Goldmann, T., Schwaiger, K., Renz, H., Brandau, S.,

Bauer, J., Heine, H. and Holst, O. (2008) Animal shed Bacillus licheniformis spores possess allergy-protective as well as inflammatory properties, *J Allergy Clin Immunol, 122, 307-312*.

von Mutius, E., Braun-Fahrlander, C., Schierl, R., Riedler, J., Ehlermann, S., Maisch, S., Waser, M. and Nowak, D. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy, *Clin Exp Allergy*, 30, 1230-1234.

von Mutius, E., Schmid, S. and Grp, P.S. (2006) The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe, *Allergy*, 61, 407-413.

von Mutius, E. and Vercelli, D. (2010) Farm living: effects on childhood asthma and allergy, *Nat Rev Immunol*, 10, 861-868.

Wady, L., Shehabi, A., Szponar, B., Pehrson, C., Sheng, Y. and Larsson, L. (2004) Heterogeneity in microbial exposure in schools in Sweden, Poland and Jordan revealed by analysis of chemical markers, *J Expo Anal Environ Epidemiol*, 14, 293-299.

Waser, M., Schierl, R., Von mutius, E., Maisch, S., Carr, D., Riedler, J., Eder, W., Schreuer, M., Nowak, D. and Braun-Fahrlander, C. (2004) Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas, *Clin Exp Allergy*, 34, 389-397.

WHO. (2009) *WHO guidelines for indoor air quality: dampness and mould,* WHO Regional Office for Europe.

Wickens, K., Douwes, J., Siebers, R., Fitzharris, P., Wouters, I., Doekes, G., Mason, K., Hearfield, M., Cunningham, M. and Crane, J. (2003) Determinants of endotoxin levels in carpets in New Zealand homes, *Indoor Air*, 13, 128-135.

Wickman, M., Gravesen, S., Nordwall, S., Pershagen, G. and Sundell, J. (1992) Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children, *J Allergy Clin Immunol*, 89, 752-759.

Willeke, K. and Macher, J.M. (1999) Air sampling In J. Macher (Ed.), Bioaerosols: assessment and control, 11-1 - 11-27. Cincinnati, OH, USA: ACGIH.

Wouters, I.M., Douwes, J., Doekes, C., Thorne, P.S., Brunekreef, B. and Heederik, D.J.J. (2000) Increased levels of markers of microbial exposure in homes with indoor storage of organic household waste, *Appl Environ Microbiol*, 66, 627-631.

Wu, F.F., Wu, M., Pierse, N., Crane, J. and Siebers, R. (2012) Daily Vacuuming of mattressess significantly reduces house dust mite allergens, bacterial endotoxin, and fungal beta-glucan, *J Asthma*, 49, 139-143.

Wu, Y., Chan, C., Rao, C.Y., Lee,Chung-Te, Hsu,Hsiao-Hsien, Chiu, Y. and Chao, H.J. (2007) Characteristics, determinants, and spatial variations of ambient fungal levels in the subtropical Taipei metropolis, *Atmos Environ*, 41, 2500-2509.

Wurtz, H., Sigsgaard, T., Valbjorn, O., Doekes, G. and Meyer, H.W. (2005) The dustfall collector--a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB), *Indoor Air*, 15 Suppl 9, 33-40.

Yamamoto, N., Shendell, D.G. and Peccia, J. (2011) Assessing allergenic fungi in house dust by floor wipe sampling and quantitative PCR, *Indoor Air*, 21, 521-530.

Yamamoto, N., Hospodsky, D., Dannemiller, K.C., Nazaroff, W.W. and Peccia, J. (2015) Indoor emissions as a primary source of airborne allergenic fungal particles in classrooms, *Environ Sci Technol*, 49, 5098-5106.

Yli-Pirilä, T., Kusnetsov, J., Haatainen, S., Hänninen, M., Jalava, P., Reiman, M., Seuri, M., Hirvonen, M. and Nevalainen, A. (2004) Amoebae and other protozoa in material samples from moisture-damaged buildings, *Environ Res*, 96, 250-256.

Zumstein, E., Moletta, R. and Godon, J. (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis, *Environ Microbiol*, 2, 69-78.

HANNA LEPPÄNEN

Understanding the role of microbes in the protective as well as harmful health effects represents major challenges to microbiological exposure assessment. The current thesis attempts to contribute to tackling this challenge by evaluating the major sources and determinants of human exposure to environmental microbes indoors, by clarifying the reproducibility of the different microbial determinations, and by comparing different sample types in order to define feasible and informative way to assess microbial exposure in indoor environments.

UNIVERSITY OF
EASTERN FINLAND

uef.fi

**PUBLICATIONS OF
THE UNIVERSITY OF EASTERN FINLAND**
Dissertations in Forestry and Natural Sciences

ISBN 978-952-61-2564-0
ISSN 1798-5668