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Microbial exposures in moisture damaged schools – an occupational health risk?

Mid-term report of the MIKOKO project

DISCUSSION PAPER

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**NATIONAL INSTITUTE
FOR HEALTH AND WELFARE**

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ISBN 978-952-302-548-6 (printed)
ISBN 978-952-302-549-3 (online publication)
ISSN 2323-363X (online publication)
<http://urn.fi/URN:ISBN:978-952-302-549-3>

Juvenes Print – Finnish University Print Ltd
Tampere, Finland 2015

Foreword

Next to homes, schools are indoor spaces in which not only pupils, but also teachers spend a majority of their everyday time, and moisture and dampness problems in Finnish school are frequent. This research project (acronym MIKOKO) studies associations between moisture damage and microbial exposures in schools, and ultimately the related health effects in teachers. Moreover, this study explores the contribution of the school environment to the everyday microbial exposure of teachers. The proposed activities will not only highlight the public health relevance of moisture problems in schools, but also provide tools for objectively assessing adverse microbial exposure situations in the school environment.

The MIKOKO research project is being implemented in two phases, phase 1 (10/2014-12/2015) and phase 2 (01/2016-06/2017). Here, we present an outline of this project and report on activities implemented during phase 1, carried out with funding contributed by Työsuojelurahasto (grant no. 114139), Academy of Finland (grant no. 252718) and Terveyden ja hyvinvoinninlaitos (THL).

Abstract

Martin Täubel, Hanna Leppänen, Anne Hyvärinen. Microbial exposures in moisture damaged schools – an occupational health risk? National Institute for Health and Welfare (THL). Discussionpaper 27/2015. 21 pages. Helsinki, Finland 2015.

ISBN 978-952-302-548-6 (printed); ISBN 978-952-302-549-3 (online publication)

The quality of indoor air is a globally emerging environmental health issue, as we spend over 90% of our time in various indoor environments. One of the major factors impacting on the quality of indoor air is the building moisture and dampness, and the associated growth of microbes. While the adverse health impacts of moisture and dampness are well established, the quest for identifying the causal agents and understanding the pathophysiological mechanisms of ill health in damp buildings is still on-going.

Next to homes, schools are indoor spaces in which not only pupils, but also teachers spend a majority of their everyday time, and moisture and dampness problems in Finnish school are frequent. This project studies associations between moisture damage and microbial exposures in schools, and the related health effects in teachers. Moreover, this study explores the contribution of the school environment to the everyday microbial exposure of teachers. The proposed activities will not only highlight the public health relevance of moisture problems in schools, but also provide tools for objectively assessing adverse microbial exposure situations in the school environment. Such tools will allow for a better identification of schools with severe damages and related abnormal and potentially health hazardous microbial exposure situations, and ultimately give way to more targeted remediation actions.

The project is being implemented in two phases, phase 1 (10/2014-12/2015) which has been financially facilitated by Työsuojelurahasto and the Academy of Finland and is reported herein, and phase 2 (1/2016-6/2017). Phase 1 of this research has been implemented according to plan and the tasks that were pre-defined for this project phase have been accomplished, including i) finalization of extensive sample collection efforts in more than 100 classrooms of SISU schools and in 90 teachers' homes in the SISUhomes study; and ii) processing and in depth microbial data generation from in total more than 600 settled dust and air samples, using both quantitative and qualitative (next generation amplicon sequencing) DNA-based approaches for the characterisation of microbial exposures.

Preliminary results from quantitative PCR analyses comparing microbial exposure situations of teachers in their classrooms to their home are intriguing, indicating that indeed rather than only considering absolute quantitative levels, exploring the differences in the microbial composition of exposures in homes versus schools and specifically in moisture damaged versus non-moisture damaged environments may be highly relevant when exploring associations with moisture damage and trying to understand impacts on teachers' health. Thus, the approach proposed and followed in this project to analyse in depth the fungal and bacterial communities in moisture damaged versus non-damaged schools using next generation sequencing is promising. Such analyses will be a key component of phase 2 of this research, to be implemented during 2016/2017.

Keywords: moisture damage, mold damage, moisture damage microbes, schools, teachers, respiratory health, indoor air exposures

Tiivistelmä

Martin Täubel, Hanna Leppänen, Anne Hyvärinen. Mikrobialtistuminen kosteusvaurioituneissa kouluissa – työterveysriski opettajille? Terveyden ja hyvinvoinnin laitos (THL). Työpaperi 27/2015. 21 sivua. Helsinki 2015.

ISBN 978-952-302-548-6 (painettu); ISBN 978-952-302-549-3 (verkkojulkaisu)

Sisäilman laatu on maailmanlaajuisesti kasvava ympäristöterveydellinen kysymys, sillä me vietämme yli 90 % ajastamme erilaisissa sisäympäristöissä. Yksi suurimmista sisäilman laatuun vaikuttavista tekijöistä ovat rakennusten kosteusvauriot sekä niihin liittyvä mikrobikasvu. Kosteus- ja homevaurioiden aiheuttamat haitalliset terveysvaikutukset ovat hyvin todennettuja, mutta epäselvää on, mitkä ovat niitä aiheuttavat tekijät sekä mitkä ovat ne patofysiologiset mekanismit, jotka ovat näiden haitallisten vaikutusten taustalla. Kotien ohella koulut ovat sisäympäristöjä, joissa oppilaiden lisäksi myös opettajat viettävät suurimman osan arkipäivistään. Kosteus- ja homevaurioiden tiedetään olevan yleisiä suomalaisissa koulurakennuksissa. Tämä tutkimus selvittää kouluissa havaittujen kosteusvaurioiden ja mikrobialtistuksen yhteyttä opettajien haitallisiin terveysvaikutuksiin. Lisäksi arvioidaan, mikä osa koulussa tapahtuvalla altistumisella on opettajien päivittäiseen mikrobiologiseen kokonaisaltistukseen. Tutkimus ei pelkästään korosta koulujen kosteusvaurioiden kansanterveydellistä merkitystä, vaan se myös tarjoaa työkaluja, joilla haitallista mikrobialtistusta voidaan määrittää objektiivisesti. Näiden työkalujen avulla pystytään tunnistamaan paremmin ne koulurakennukset, joissa on vakavia kosteusvaurioita ja niihin liittyvää poikkeavaa ja mahdollisesti terveydelle haitallista mikrobialtistusta. Näin mahdolliset korjaustoimenpiteet pystytään kohdentamaan oikein.

Projekti koostuu kahdesta osasta, tässä raportoidusta vaiheesta 1 (10/2014–12/2015), joka on Työsuojelurahaston ja Suomen Akatemian rahoittama sekä vaiheesta 2 (1/2016–6/2017). Vaihe 1 on saatu päätökseen ja projekti etenee aikataulun mukaisesti. Projektin vaiheessa 1 aikaansaadut tehtävät ovat: 1) Laaja näytteenotto 100 luokkahuoneessa (SISU koulututkimuksessa) ja 90 opettajan kodissa (SISUhome – tutkimuksessa) 2) 600 laskeutuneen pöly- ja ilmanäytteen esikäsittely ja syvälinen mikrobialtistuksen selvittäminen, käyttäen sekä kvantitatiivisia (qPCR) että kvalitatiivisia (seuraavan sukupolven amplicon -sekvensointi) DNA-pohjaisia menetelmiä.

Alustavat kvantitatiiviset PCR -tulokset opettajien mikrobialtistuksen eroista koulussa ja kotona ovat erittäin mielenkiintoisia. Tulokset osoittavat, että pelkän määrällisen mikrobipitoisuuden mittaamisen sijaan on erittäin tärkeää keskittyä mikrobikoostumuksen eroihin kotona ja kouluissa, erityisesti kosteusvaurioituneissa ja vaurioitumattomissa tiloissa. Tämä voi olla merkittävää kosteusvaurioiden ja opettajien terveysvaikutusten yhteyden arvioinnissa. Näin ollen tätä lupaavaa lähentymistapaa jatketaan tässä projektissa, jotta sekä bakteerien että sienien koostumus voidaan määrittää syvälinen kosteusvaurioituneissa verrattuna vaurioitumattomissa kouluissa. Tähän tullaan käyttämään seuraavan sukupolven sekvensointitekniikkaa ja se tulee olemaan avainasemassa tutkimuksen vaiheessa 2, joka toteutetaan ajalla 2016/2017.

Avainsanat: kosteusvauriot, homevauriot, kosteusvauriomikrobit, koulut, opettajat, hengitysterveys, sisäilma-altistuminen

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Background

The quality of indoor air is a globally emerging environmental health issue. As we spend over 90% of our time in various indoor environments, it is essential to understand and control any health hazards involved. There are pressures to improve the energy efficiency of buildings, and thus there is a need to solve the complex pursuits to both achieve energy saving aims and maintain good indoor air. Perhaps the major factor affecting the quality of indoor air is the building moisture and dampness, and the associated growth of “mould”, i.e., fungi, bacteria and other microorganisms (1,2). These are strongly linked with ill health, including increased risk of asthma exacerbation and development (2). The prevalence of dampness and moisture damage varies between 20-50% in different countries, partly depending on the metrics used, and the burden of illness and of economy are substantial (3).

The public health importance of ‘building mould’ is only partially understood since the causal agents of the various health effects as well as their pathophysiological mechanisms are still insufficiently known. The most evident health-related consequence of microbial growth in moisture-damaged indoor environments are increased concentrations of microbial agents, measured as elevated levels of fungi, bacteria and their structural components (e.g. 4), along with a alteration of the microbial profile towards potentially more harmful species (5,6). Attempts to identify causal agents have thus far failed, which may be due to limitations in detection methods for microbes that in the past have mostly been restricted to cultivable and viable microbes. It is evident today, that not one single microbe or one single microbial constituent will be able to explain the diverse, adverse health outcomes observed in resident of damp buildings, but that rather specific characteristics within complex exposures trigger ill health. Recent advances in microbial methods towards DNA based methods with highest resolution – next generation sequencing approaches - are likely to advance the field and allow for the identification of single microbial groups, microbial consortia or overall microbial diversity that link to moisture damage and adverse health outcomes in exposed residents. First studies applying such methods to indoor settings have opened our eyes for the enormous richness and diversity of bacteria and fungi in indoor spaces (e.g.7-13). The studies conducted so far were mostly descriptive in nature and attempted to elucidate factors shaping the indoor microbiome. Health implications of indoor bacterial and fungal assemblages and its diversity have been suggested in recent studies (14,15) and interactions between human and indoor microbiota seem evident (16). Further investigations are warranted with a defined focus on the assessment of moisture problems in buildings and its impact on the indoor microbiome.

Next to homes, schools are indoor spaces in which not only pupils, but also teachers spend a majority of their everyday time. The European, multi-centre HITEA study coordinated has provided a minimum estimate for moisture and mould problems in Finnish schools of 24% (17). Dampness and mould in the school environment has been associated with respiratory symptoms and asthma among school children (18-20). Recent analyses from the HITEA study in schools in three European countries – Finland, the Netherlands and Spain – indicate that teachers from moisture damaged schools are at increased risk for upper and lower respiratory tract symptoms (21). Teachers from moisture damaged schools reported more asthma symptoms and the effects were found to be stronger among teachers who had been working for more than five years in the same schools. In addition, work-related wheeze and chest tightness and nasal symptoms were more commonly reported from teachers in moisture damaged schools. Another study associated with HITEA recently found that endotoxin exposure – a surrogate for general microbial exposure – was several-fold higher in schools compared to homes in the Netherlands and concluded that exposure at school can contribute considerably to environmental endotoxin exposure of children and teachers (22). These findings imply the public health relevance of damp and mould problems and related microbial exposures in schools as a workplace. Intervention studies involving the removal of the source of moisture problems have shown a positive effect of remediation on the occupants’ health (23,24).

Aims and objectives

Thus far, causal links between specific microbial exposure situations in damp schools and adverse health effects in teachers have not been established; neither have microbial exposure levels been detailed for school versus home environments. This project studies associations between moisture damage and microbial exposures in schools, and the related health effects in teachers. Moreover, this study explores the contribution of the school environment to the everyday microbial exposure of teachers. The proposed activities will not only highlight the public health relevance of moisture problems, but also provide tools for objectively assessing adverse microbial exposure situations in the school environment. Such tools will allow for a better identification of schools with severe damages and related abnormal and potentially health hazardous microbial exposure situations, and ultimately give way to more targeted remediation actions. In addition, we will assess the effectiveness and impact of moisture damage repairs on the reduction of microbial exposures in teachers' workplaces.

The hypotheses in our study are:

- *Exposure to environmental microbes in schools exceeds in quantitative terms and/or is different in qualitative terms to exposure situations in residential homes. Schools affected by moisture damage and dampness with a related increase and/or change in microbial content represent an occupational health hazard for teachers working in such schools.*
- *Novel methods in sampling and in characterizing microbial communities allow for an identification of components within these microbial communities that differentiate indoor situations characterized by moisture damage from non-damage situations.*
- *Based on this information, quantitative DNA-based methods can be developed as diagnostic tools to identify abnormal and health hazardous indoor microbial exposure situations.*
- *Thorough remediation of moisture and mould damage leads to a reduction in microbial exposure levels or change in microbial community characteristics and to an alleviation of adverse health outcomes in teachers.*

The objectives of the study are:

- 1) to provide an in-depth characterisation of fungal and bacterial communities in moisture damaged versus non-moisture damaged schools, using next generation sequencing approaches and standardized samples of settled dust and indoor air;
- 2) to identify fungal and bacterial profiles/factors that are specific to moisture damage situations by using next generation sequencing data;
- 3) to apply/develop quantitative, DNA based methods (qPCR) for the fungal and/or bacterial targets identified under point 2, in order to provide tools for an objective assessment of abnormal microbial exposure situations relating to moisture and mould damage;
- 4) to compare - in a subset of study subjects (teachers) – microbial exposure situations in their working and home environment, in order to evaluate the contribution of school exposures to the total exposure to environmental microbes;
- 5) to compare microbial exposure situations in schools before and after remediation of the moisture damages and its source.

Materials and methods

Study material

This research emphasizes on materials from two studies that have been or are being carried out in Finnish schools within their own funding frameworks. These studies have in common that they include index and reference school buildings, i.e. schools with and without moisture damage and dampness problems. The damage status in these schools has been established via standardized walk through school building inspections. In both studies, extensive collection of exposure samples as well as rich datasets on teachers' health will allow for in-depth analyses of the link between moisture damage, microbial exposures and teachers' health.

The *SISU study (School intervention study)* has collected severely moisture damaged schools across Finland, with a part of these schools having been repaired and visited before and after moisture damage renovations. The general field work of this study and basic analyses focusing on microbial toxins have been financially supported by the Academy of Finland (Microbial toxins indoors – gripping an emerging issue; Grant No. 252718; project ended 31.08.2015). In total, 11 moisture damaged and 5 non-damaged schools have been recruited to the study. Seven of the moisture damage schools have undergone renovations. Detailed exposure and health assessments have been conducted before (all 16 schools) and after remediation (7 schools).

In total, more than 200 teachers are taking part in the SISU study. Exposure samples have been collected from over 100 classrooms and teachers' lounges in the study schools; airborne settled dust as well as active air samples have been collected. In total, approx. 180 settled dust and close to 100 active air samples are available for in-depth exposure studies. Questionnaires and symptom diaries have been applied to both, pupils and teachers. Additional clinical sampling (exhaled NO, blood serum, nasal lavage) is being performed from teachers in the course of school visits by nurses and researchers with medical background, to allow determination of immunological parameters. The herein described research is joined with research efforts carried out at Työterveyslaitos (TTL, group of Professor Harri Alenius), focusing on immunotoxicological and system-biological aspects of exposures in moisture damaged schools. This study at TTL is currently on-going with the support of TSR.

The *SISUhome study* is an addition to the SISU study in schools and aims to determine the contributions of the home and the school environment to the total microbial exposure indoors for teachers. SISUhome has recruited more than 100 teachers participating in the SISU school study. Electrostatic dust fall collectors have been distributed to these teachers' homes and settled dust has been collected in an identical fashion as done in SISU schools/classrooms. The aim is to assess exposure to microbes in the teachers' homes in addition to their working environment, in order to be able to evaluate the contribution of the school environment to overall and specific microbial exposures. Questionnaires on home characteristics and health status of these teachers have been distributed to the study participants.

The *HITEA study (Health Effects of Indoor Pollutants; www.hitea.eu)* is a multi-center, international study that has been implemented during 2008-2013, financed under the EC Framework Programme 7, and was coordinated by the applicant of this research, Doc. Anne Hyvärinen. The study part on indoor exposures in schools has been conducted in identical set-up in Finland, The Netherlands and Spain, representing three climatic regions in Europe. In total, more than 60 schools with and without moisture damage and over 600 teachers participated in the early phases of the study, revealing associations between moisture damage and dampness in schools and adverse respiratory health in teachers (see background). For this current research, we utilize exposure samples from 6 schools in Eastern Finland (4 index, 2 reference schools) that were followed up in a longitudinal fashion over more than one school year. Exposure samples were taken at three points in time (winter 2009, spring 2009, winter 2010) from approx. 60 classrooms and

teachers' lounges in the study schools. A total of 180 settled dust samples collected with EDCs (see detailed description of EDC samples below) are available for in depth characterization of microbial exposures in moisture damaged versus non-damaged Finnish schools. Health data that have been collected from the teachers in these schools include respiratory health questionnaire data, information on lung function (spirometric measurement) and airway inflammation (exhaled NO), inflammatory cytokines in serum and nasal lavage, and total IgG in serum.

Building investigations and microbial sampling

Information on building structures, history, materials and observations of moisture damage and dampness from HITEA and SISU schools are received from standardized technical investigations that were performed by trained civil engineers. The inspections included walkthroughs utilizing pre-designed checklists for the collection of observations of moisture damage, dampness and visible mould, including their extent and severity. Non-destructive measurements included hand-held moisture detectors, relative humidity, temperature and CO₂ monitoring devices. Standardized questionnaires were utilized to collect detailed information on characteristics (building structure, materials, HVAC system, etc.), condition and maintenance of the school buildings. Moisture damage status of homes in the SISUhome study is assessed via questionnaire.

Inhalation exposure to microbes occurs mainly via airborne route; hence air samples are thought to characterize best these exposures. Due to the great variations in air concentrations and poor reproducibility, however, settled dust samples with better reproducibility are collected in addition (25-27). Exposure samples that have been or are being collected in these two school studies and in SISUhome study and that will be utilized in the herein proposed research are:

- Airborne settled dust, passively collected with EDCs (electrostatic dust fall collector; 28): electrostatic dust wipes will be placed on elevated surfaces like shelves or cupboards for a period of 8 weeks; the sample collected represents an integrated sample of exposure over a standardized period of time;
- Settled dust from elevated surfaces, collected with a vacuum cleaner into a dust sampling nylon sock (27): the advantage of this sample material compared to EDC dust is that larger sample amounts can be collected, while as a drawback the age/history of the dust is unknown.
- Active air samples, collected with Button inhalable aerosol sampler (29): active air samples are collected in SISU schools over a period of 2 school days (12 hours), at a flow rate of 4L per minute that allows for collection of the actual inhalable dust fraction in indoor air. In each school samples will be collected in three classrooms and one outdoor location.

Microbial analyses methods

- Next generation sequencing on Illumina MiSeq platform (350bp, paired end) is performed for an in-depth characterization of fungal and bacterial communities in indoor air and airborne settled dust collected with EDCs. Bioinformatics processing of sequence information largely rely on QIIME software pipeline (www.qiime.org) with built in modules from an in-house pipelines and are conducted at THL, Department of Health Protection. Reference based OTU picking is done using greengenes database for 16S data and UNITE database for ITS fungal data. The resulting OTU tables are further utilized in downstream processing steps.
- Quantitative real-time PCR (qPCR) is performed at THL, Living Environment and Health Unit that has many years' experience and a strong track record in developing and applying qPCR as a measure for environmental microbial exposure from several sample types (30-34). QPCR is used to quantify microbial exposures in moisture damaged and non-damaged schools (HITEA, SISU),

before and after renovation (SISU), and in homes of teachers (SISUhome). qPCR analyses target fungal and bacterial species/groups that represent the relevant indoor microflora, eg. the general assays universal fungal, gram positive and gram negative bacteria, as well as more specific assays for *Aspergillus versicolor*, *Cladosporium* spp., *Streptomyces* ssp., *Penicillium/Aspergillus* group, etc.. In addition, 2-3 qPCR assays are developed or optimized based on earlier published assays for the most promising microbial targets that have been identified via MiSeq sequencing to be relevant with respect to moisture damage and dampness indoors.

Health assessment in teachers

The health status of teachers in SISU is assessed using symptom questionnaire information collected prior and post remediation in the schools. In addition, a marker for lung inflammation (exNO), basic blood parameters and levels of inflammatory cytokines in serum will be used to assess teachers' health. In SISU home study, the health status of the teachers will be assessed via questionnaire. In HITEA, the collected health data include respiratory health questionnaire, information on lung function (spirometric measurement) and airway inflammation (exhaled NO), inflammatory cytokines in serum and nasal lavage, and total IgG in serum. The health questionnaires both in HITEA and SISU were developed including items on demographic characteristics, relevant exposures (at home) and especially respiratory health. Questions on respiratory health are based on the validated International Study of Asthma and Allergy in Children (ISAAC) Questionnaire (35). Ethical issues in this research were considered; both HITEA and SISU studies have received ethical approval by the local ethical committee (Pohjois-Savon Sairanhoitopiirin Tutkimuseettinen Toimikunta). All teachers participating provided informed consent prior to health measurements.

Time line of the project

This research project is being implemented in two core phases: phase I (01.10.2014-31.12.2015; the period reported herein and financially supported by grants of TSR (No. 114139) and the Academy of Finland (No. 252718)), and phase II (01.01.2016-30.06.2017; financial support is currently being applied for this period). The time schedule and specific tasks referring to this research project are detailed in Table 1 below.

Table 1. Time schedule, phases, and specific tasks of the MIKOKO study.

	2014				phase I				phase II				2017	2018
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4		
Field work, Exposure and Health assessment teachers														
Exposure&Health assessment SISU schools (post renovation)	→				→	→								
Exposure assessment SISUhome study					→									
Laboratory analyses														
MiSeq sequencing from SISU; sample processing					→	→	→							
MiSeq sequencing from HITEA; confirmation study									→	→				
qPCR assay development									→	→	→			
qPCR analyses SISU, HITEA, SISUhome, HITEA					→	→	→	→	→	→	→			→ in-depth health analyses
Data analyses, reporting, dissemination														
Microbial communities analyses; identification of factors relating to moisture damage									→	→				
Compare exposure levels school vs. home						→	→							
Before/after remediation, effect on exposure						→	→	→	→					
Dissemination of research results									→	→	→	→		

In the following, we specify the tasks relating to phases 1 and 2 of the MIKOKO study. Note that this current project report refers exclusively to phase 1 of the MIKOKO study; phase 2 will be implemented – if financial support can be secured – from the beginning of 2016 onwards.

Key tasks defined for phase 1 are as follows:

- 1) Finalization of field work efforts in SISU schools – exposure and health assessments, before and after renovation.
- 2) Recruitment and field work in SISUhomes study – we target 100 teachers from the SISU schools, who will be asked to sample their classroom and home environment to allow microbial exposure assessment in these two environments.
- 3) Sample processing, DNA extraction, and next generation sequencing (Illumina MiSeq) of settled dust and air samples in SISU study.
- 4) Compare microbial exposure levels for SISU teachers in schools versus their homes in the SISUhomes study.
- 5) Evaluate the effect of moisture damage renovations on microbial exposure levels in SISU schools.

Phase 2 of the study links to following key tasks:

- 1) Sequencing data analyses and reporting of the microbial community analyses from SISU schools; identification of factors relating to moisture damage.
- 2) Confirmation sequencing study using sample materials from HITEA schools.
- 3) Optimisation/development of qPCR assays based on SISU microbial community findings in relation to moisture damage.
- 4) Completion of the quantitative microbial exposure analyses in SISU, SISUhomes and HITEA schools.

5) Dissemination of project results: publication, presentation of results in national and international conferences, dissemination in social media, press releases.

Following the implementation of phases 1 and 2 and based on the results of the MIKOKO research project we anticipate follow-up studies on this topic that will focus on linking the extensive exposure dataset generated under the current project to rich health datasets that were also collected in SISU and HITEA projects.

Project status and current results

The herein reported project status and preliminary results refer to phase 1 of the project. It needs to be stressed here that the project is currently approaching only midterm, implying that while a lot of sample collection, processing and laboratory analyses have been accomplished, the statistical analyses of the study data and the scientific reporting can only be started now, when the project enters phase 2. In the following we describe the project status and results following the list of tasks as have been defined for phase 1 of this research effort (see above).

1) Finalization of field work efforts in SISU schools – exposure and health assessments, before and after renovation.

The field work efforts in the SISU schools have been finalized, with the last of these schools having been visited for detailed exposure and health assessments during March 2015. In total, 11 moisture damaged and 5 non-damaged schools have been enrolled to the study. Seven of the moisture damage schools have undergone renovations. Detailed exposure and health assessments have been conducted before (all 16 schools) and after remediation (7 schools). Overall, more than 100 classrooms and teachers lounges have been sampled for microbes using different approaches (settled dust, active air sampling) with in total approx. 180 settled dust and close to 100 active air samples available for in-depth exposure studies. More than 200 teachers have been enrolled to the study, with health questionnaires and symptom diaries distributed. Additionally, clinical sampling (exhaled NO, blood serum, nasal lavage) has been performed from teachers in the course of school visits by nurses and researchers with medical background, to allow determination of immunological parameters. These activities were largely covered with funds from an Academy of Finland project (grant no. 252718) and complemented with TSR funding.

2) Recruitment and field work in SISUhomes study – targeting 100 teachers from the SISU schools who will be asked to sample their classroom and home environment to allow microbial exposure assessment in these two environments.

In total, 104 teachers have been recruited to the study, and electrostatic dustfall collectors (EDCs) have been sent to the study participants to be applied for collection of airborne settled dust for a duration of 4 weeks in both the teachers' homes and primary classrooms. The field work for this study module has been finalized in spring 2015. In addition to the settled dust samplers, also health questionnaires and sampling questionnaires have been distributed to the teachers. We received in total 89 sample pairs from classrooms and homes that are available for in depth microbial analyses. These efforts were implemented fully with the financial support of TSR.

3) Sample processing, DNA extraction, and next generation sequencing of settled dust and air samples in SISU study.

A total of more than 500 settled dust and air samples collected in SISU schools have been processed, DNA has been extracted and all samples have been subjected to quantitative PCR analyses (laboratory work finalized during August 2015). In addition, approx. 350 settled dust and air samples have been subjected to next generation sequencing (NGS) for the characterisation of bacterial and fungal communities in classrooms and teachers lounges. The task of NGS on Illumina Miseq has been outsourced to the service provider LGC Genomics in Germany. The sequence data have been received from the service provider in August 2015. This means that all laboratory processing and analyses efforts relating to this task have been completed; currently, data and bioinformatics processing, as well as statistical analyses of these extensive datasets are ongoing.

4) Compare microbial exposure levels for SISU teachers in schools versus their homes in the SISUhomes study.

From 104 teachers that participated in this study, we received samples from both home and classroom of 89 teachers, in addition to extensive health and home exposure questionnaires. 178 EDC samples were processed to extract dust and subsequently DNA, and quantitative PCR was applied to measure several bacterial and fungal species or groups, in order to compare microbial exposure levels and composition in homes versus schools. We measured total fungal DNA, *Penicillium* spp./*Aspergillus* spp. group (Pen/Asp group, including several of the most common indoor fungal species), *Cladosporium herbarum* (a mold with strong outdoor context), as well as Gram positive and Gram negative bacteria. All sample processing and laboratory analyses to this task have been completed. First statistical analyses of these data have led to following preliminary observations:

- Generally, fungal and bacterial levels in settled dust were statistically significantly higher in the home environment compared to classrooms. This is true for total fungal content, more outdoor related fungi (*C. herbarum*) as well as for the indoor prominent fungi (*Pen/Asp* group). For bacteria, the effect was more pronounced for Gram positive compared to Gram negative bacteria. We believe that the finding of lower microbial levels observed in classrooms compared to home environments likely relates to more efficient and fully mechanical ventilation strategies with filtered incoming air in all study schools, but further analyses need to be done to confirm this. Also other factors, such as smooth floors rather than carpeted floors being typical in schools, potentially more frequent cleaning activities, no shoes and outdoor clothes on in classrooms and no pets – all of these vehicle for tracking in outdoor particles and microbial material - may contribute to the lower microbial levels generally observed in the school environments.
- Interestingly, when comparing the microbial content with qPCR in classrooms versus homes, there are not only differences in general levels, but also clear differences in the composition of the exposure. For example the total fungal content in homes is more dominated by *Penicillium* spp. and *Aspergillus* spp. in relation to the total fungal content, and the bacterial content of homes is more dominated by Gram positive bacteria versus Gram negative bacteria, compared to the situation in classrooms. These findings were statistically significant, and indicate that compositional, qualitative aspects will be relevant in addition to quantitative aspects, when comparing school and home environments with respect to their microbial exposure situations and potential impact on teachers' health.
- When comparing the general microbial exposure levels in settled dust in moisture damaged versus non-damaged or renovated schools, we observe mostly somewhat higher microbial levels in moisture damaged schools, however, these findings do not reach statistical significance. Further work will be done utilizing also active air samples and targeting more specific fungal and bacterial groups in the qPCR analyses. This finding is in agreement with earlier studies that show that microbial exposure levels in moisture damaged school environments tend to be elevated, but differences to 'normal' situations are not always significant and/or consistent across studies and countries (eg. 37-38). It is obvious that next to amount also the microbial composition of the exposure may be crucial in both relating to moisture damage and to health effects observed in teachers in moisture damaged schools. Thus, the approach followed in this project (phase 2) to analyse in depth the fungal and bacterial communities in moisture damaged versus non-damaged schools using next generation sequencing is highly promising.

- We also observe large variation between individual schools with respect to their mean bacterial (up to 10-fold differences) and fungal (up to >30-fold difference) levels, and these data will be analysed also against symptom questionnaire data collected from teachers and pupils in these schools, to investigate effects of microbial exposure on teachers' health, even independent of moisture damage.

5) Evaluate the effect of moisture damage renovations on microbial exposure levels in SISU schools.

More than 500 settled dust and active air samples collected in 16 SISU schools before and 7 schools after renovation have been subjected to quantitative PCR analyses using both bacterial and fungal quantitative PCR assays. We measured total fungal DNA, *Penicillium* spp./*Aspergillus* spp. group (*Pen/Asp* group, including several of the most common indoor fungal species), *Cladosporium herbarum* (a mold with strong outdoor context), as well as Gram positive and Gram negative bacteria. All sample processing and laboratory analyses to this task have been completed. At current, the datasets are being prepared for subsequent statistical analyses, specifically investigating the impact of moisture damage aspects and moisture damage interventions on quantitative microbial levels in schools. This analysis work has so far been largely financed through the Academy of Finland grant, but will have to be completed in phase 2 of the project.

Conclusions

Phase 1 of this research has been implemented according to plan and the project is well on schedule. The tasks that were pre-defined for this project phase have been accomplished, including i) finalization of extensive sample collection efforts in SISU schools and SISUhomes studies; and ii) sample processing and microbial data generation from in total more than 600 settled dust and air samples from classrooms and teachers lounges, including quantitative PCR analyses as well as microbial community analyses using next generation sequencing (NGS).

The data analyses and reporting in this project is currently on-going and will be a focus in phase 2 of this research. Preliminary results from quantitative PCR analyses of the SISUhomes study that compares microbial exposure situations of teachers in their classrooms to their homes are intriguing: we observed generally higher microbial levels in airborne settled dust in homes compared to classrooms, but also find statistically significant differences in the composition of bacterial and fungal exposures in homes versus classrooms. Microbial exposure situations may vary heavily in different schools with several-fold difference in fungal and bacterial levels. Based on first analyses from settled dust and targeting more general groups of microbes with qPCR, moisture damage in the schools seems to have a rather subtle and small effect on the microbial levels. Further work will be done utilizing also active air samples and targeting more specific fungal and bacterial groups. Interpreting these indicative findings together, this underlines the importance of considering not only amount but also compositional, qualitative aspects of the microbial exposures in the school environment, when exploring associations with moisture damage and teachers' health. Thus, the approach proposed and followed in this project to analyse in depth the fungal and bacterial communities in moisture damaged versus non-damaged schools using next generation sequencing and also from active air samples is highly promising. These analyses will be a key component of phase 2 of this research, which currently is applied funding for and is planned to be implemented during 2016/2017.

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