APPLICATION OF THE ENVIRONMENTAL RELATIVE MOLDINESS INDEX IN FINLAND

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SUMMARY

The Environmental Relative Moldiness Index (ERMI) is a metric based on quantitative PCR (qPCR) targeting a multitude of 36 different fungal species and groups in house dust. It was initially developed to quantify mold contamination in homes in the United States, but has been applied more recently also in other countries. In this study we determined the applicability of the ERMI to quantifying mold and moisture damage in Finnish homes. Our results show that the ERMI was significantly associated with certain observations of visible mold in Finnish homes, but not with moisture damage. We then modified the ERMI towards Finnish conditions taking into consideration the prevalence and levels of mold species in Finnish homes with and without moisture damage. The Finnish ERMI (FERMI) is a simplified metric based on 10 mold species. This metric showed significant associations with observations of visible mold, mold odor and moisture damage in Finnish homes.

INTRODUCTION

Moisture problems in Finnish homes and schools are common and there is need to prioritize renovation actions based on the severity or extent of the moisture problem and/or the related health hazard. Exposure to water-damaged, moldy buildings has been linked to adverse respiratory health, including both exacerbation and development of asthma /1-3/. Identifying 'abnormal' mold exposures might be critical in efforts to reduce the disease burden of asthma due to dampness and moisture damage in buildings.

Cultivation-based determination of viable fungi and bacteria from indoor samples such as building material or air samples is the most commonly used method for quantifying microbes in support of building investigations. The cultivation-based approach has known limitations, such as extended analyses durations, limited reproducibility of short-term air samples, and detects only alive and cultivable microbes. There is need for improved and objective metrics for quantifying mold contamination in homes.

The U.S. Environmental Protection Agency (U.S. EPA) together with the U.S. Department of Housing and Urban Development established a metric to quantify mold contamination in U.S. homes called the Environmental Relative Moldiness Index (ERMI) /4/. The ERMI is a DNA-based technology that measures 36 indicator molds in floor dust samples with

quantitative PCR: 26 are species commonly found at higher levels in water-damaged homes and 10 are species commonly found in U.S. homes, independent of water damage /5/. The ERMI metric has been used in many studies in and more recently also outside the U.S. /6-12/. However, the applicability of the ERMI metric in different countries or regions with differences in climatic conditions and building stock, and characteristics of moisture problems has been challenged. The purpose of the current analysis was to determine if the ERMI metric might be applied to quantifying moisture damage and mold contamination in Finnish homes.

MATERIALS AND METHODS

Study population and home inspections

The LUKAS2 is an on-going birth cohort study in Eastern Finland /13/, consisting of a general population sample of homes in rural and suburban areas in this region. 144 dust samples collected at the child's early age were included in the current analyses; the protocol for dust collection has been previously described /13/. A building engineer performed detailed inspection of the study homes to assess moisture damage, visible mold and other dampness indicators /14,15/. The inspections followed a standardized protocol and utilized standardized checklists and questionnaires /16,17/. Visual observations in individual rooms and areas in the home were complemented by recording of surface moisture, visible mold, and mold odor. Moisture damage observations were graded based on extent and severity. The inspector recorded detailed estimates for individual damages in each location separately and also made an overall assessment of the whole house.

Quantitative PCR analysis and ERMI determination

Dust samples were stored frozen at -20 °C until shipment in dry ice to U.S. EPA. Dust samples were sieved and DNA was extracted from 5 mg of dust. The concentration of each mold was determined by mold specific quantitative PCR analysis at U.S. EPA /18/. All primer and probe sequences used in the assays have been published /19/.

The ERMI value for each home was calculated by taking the sum of the logs of the concentrations of the 26 Group 1 species (s1) and subtracting the sum of the logs of the concentrations of 10 Group 2 species (s2) /4/.

$$ERMI = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j})$$

Development of a Finnish ERMI (FERMI) and statistical analyses

In order to improve the ERMI metric towards Finnish conditions, we selected a subsample of LUKAS2 homes with severe moisture damage (n=20) and reference homes that had no signs of moisture damage (n=42). As in the definitions of the original ERMI, we calculated geometric means ratios of moisture damaged versus reference homes for the individual mold species to define Group 1 (moisture damage associated) and Group 2 (background) mold species. T-test or one-way ANOVA were used to compare mean ERMI and FERMI values to moisture damage observations in the sample of 144 LUKAS2 study homes.

RESULTS AND DISCUSSION

The ERMI has been developed to quantify mold contamination in homes in the U.S. /4/. It is a metric that is based on quantitatively measured mold species or groups that are measured from floor dust and are either linked to conditions of moisture and mold damage

(Group 1 species) or are considered normal 'background' molds (Group 2). Table 1 lists the 36 mold species/groups that compose the ERMI metric and are allocated either into Group 1 (26) or Group 2. Given that these definitions were based on a sample of U.S. homes /5/ we aimed to modify the ERMI towards applicability to Finnish conditions, utilized a subset from LUKAS2 composed of homes with severe moisture damages in main living areas (n=20) and of non-moisture damaged reference homes (n=42). We observed clear differences in prevalence and levels of the individual mold species and also their allocation into Group 1 and Group 2 molds. We used a similar logic in creating the Finnish ERMI as was used previously in defining the original ERMI and reallocated the mold species into 'moisture damage' and 'background' molds based on Finnish conditions. Molds with clear association with moisture and mold damage were allocated into group 1 (mold species with a GM ratio between damaged and non-damaged homes above 1.5 were considered good moisture damage indicators), and we also considered that this association should be independent of season of floor dust sampling. Mold species that showed no association with moisture damage (GM ratio of 1 or below) independent of season of dust sampling and that were also well prevalent (>50%) in Finnish house dust samples were included into Group 2. By doing so we created the FERMI metric that consists of 10 mold species (7 Group 1 molds, and 3 Group 2 molds) (Table 1). The calculation of the FERMI followed the original ERMI approach (see methods section) with some minor modification:

$$FERMI = \sum_{i=1}^{7} \log_{10}(s_{1i}) - \sum_{j=1}^{3} \log_{10}(s_{1j}) + 14.42.$$

When applied to the full sample of 144 homes from the LUKAS2 cohort the FERMI metric was found to be significantly and consistently associated with observations of visible mold in various location and – this being a clear improvement compared to the original ERMI metric – also of moisture damage recorded in the living room, the child's main living areas and the whole house (Table 2), as well as with mold odor observed in the whole house (data not shown).

The results of our study are encouraging in that the FERMI appears to be a promising tool to confirm inspection based observations of mold and moisture damage in homes in Finland in an objective way. However, our findings will have to be confirmed in other studies in Finland, before an application of the FERMI in research or practical settings can be recommended. Our work highlights that differences in climatic conditions, building stock and moisture damage characteristics between different countries does not permit an uncritical use of microbial metrics that have been developed elsewhere. Prior testing of such metrics to confirm or reject their applicability to local conditions is crucial.

In conclusion, we show here that remodeling the ERMI scale to account for local microbial flora and moisture damage characteristics in Finland resulted in a metric with greater potential to objectively rate homes with moisture and mold damage in this specific setting.

Table 1. Mold species/groups targeted with qPCR as associated with moisture damage or as background molds in the ERMI and the Finnish ERMI (FERMI).

Environmental Relative Moldiness Index (ERMI)	**Finnish Environmental Relative Moldiness Index (FERMI)**
Group 1 – Moisture damage associated mold species	
Aspergillus flavus	*Aspergillus ochraceus*
Aspergillus fumigatus	*Aspergillus versicolor*
Aspergillus niger	*Chaetomium globosum*
Aspergillus ochraceus	*Cladosporium sphaerospermum*
Aspergillus penicillioides	*Penicillium corylophilum*
Aspergillus restrictus	*Penicillium crustosum* group
Aspergillus sclerotiorum	*Penicillium chrysogenum*
Aspergillus sydowii	
Aspergillus unquis	
Aspergillus versicolor	
Aureobasidium pullulans	
Chaetomium globosum	
Cladosporium sphaerospermum	
Eurotium amstelodami	
Paecilomyces variotii	
Penicillium brevicompactum	
Penicillium corylophilum	
Penicillium crustosum group	
Penicillium purpurogenum	
Penicillium spinulosum	
Penicillium variabile	
Scopulariopsis brevicaulis	
Scopulariopsis chartarum	
Stachybotrys chartarum	
Trichoderma viride	
Wallemia sebi	
Group 2 – Background mold species	
Acremonium strictum	*Alternaria alternata*
Alternaria alternata	*Cladosporium cladosporioides* 1
Aspergillus ustus	*Epicoccum nigrum*
Cladosporium cladosporioides 1	
Cladosporium cladosporioides 2	
Cladosporium herbarum	
Epicoccum nigrum	
Mucor group	
Penicillium chrysogenum	
Rhizopus stolonifer	

Table 2. Comparison of mean ERMI and FERMI values in LUKAS2 homes in which observations of visible mold or more generally moisture damage were made in the living room, the child's main living areas, or the whole house.

Observations of visible mold in Finnish homes						
		N	**Mean ERMI value**	***p-value***	**Mean FERMI value**	***p-value***
Living room	no	141	5.43		5.33	
	yes	3	10.24	*0.52*	15.20	***0.007***
Child's main living area	no	134	5.22		4.92	
	yes	10	9.72	***0.007***	13.69	***<0.0001***
Whole house	no	100	5.14		4.51	
	yes	44	6.42	*0.17*	7.85	***0.003***
Observations of moisture damage in Finnish homes						
		N	**Mean ERMI value**	***p-value***	**Mean FERMI value**	***p-value***
Living room	no	128	5.27		4.98	
	minor	12	7.45		11.10	
	major	4	8.22	*0.21*	6.53	***0.01***
Child's main living area	no	102	5.35		4.85	
	minor	31	5.22		6.30	
	major	11	8.12	*0.22*	9.70	***0.04***
Whole house	Class 0/1	60	5.16		3.81	
	Class 2	50	5.72		5.91	
	Class ≥3	34	5.90	*0.76*	8.02	***0.007***

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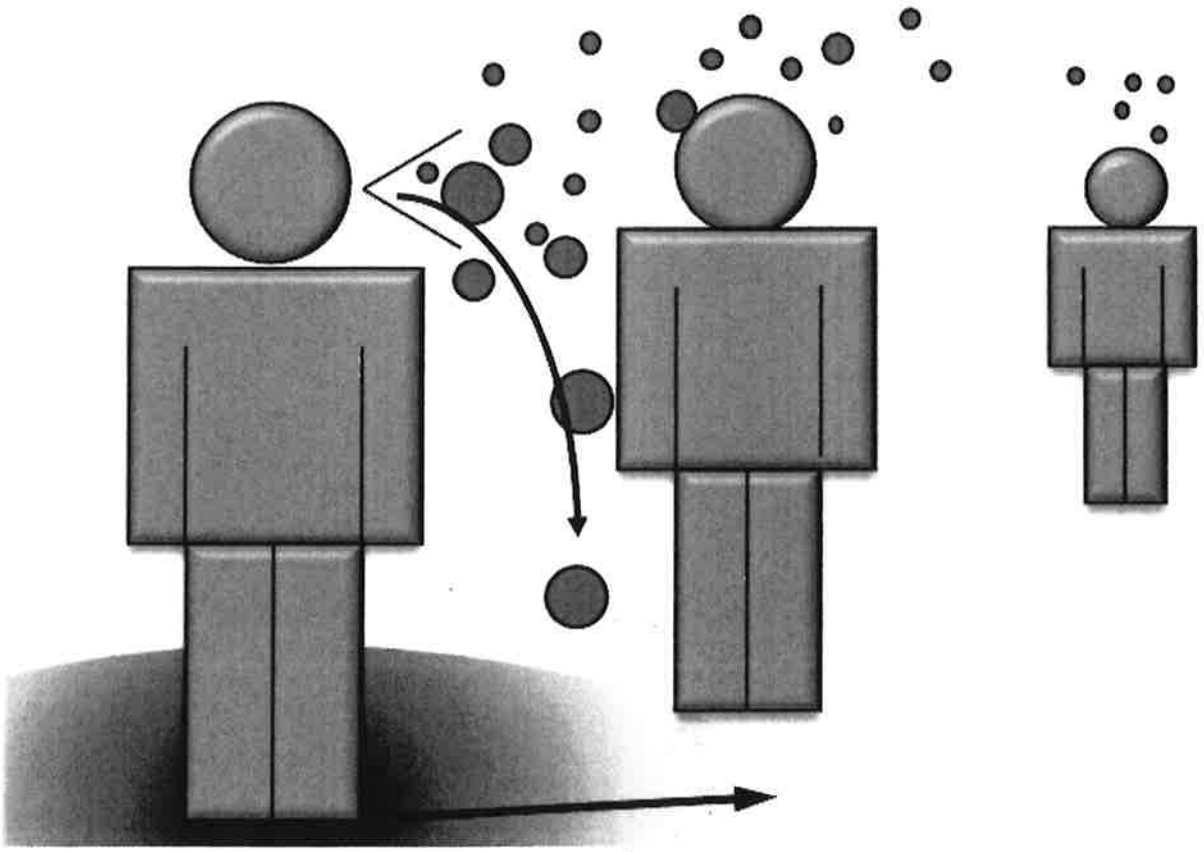

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