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Article

Effects of ventilation improvement on measured and perceived indoor air quality in a school building with a hybrid ventilation system

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Abstract: This paper describes a case study of ventilation as well as measured and perceived indoor air quality (IAQ) in a Finnish comprehensive school with a hybrid ventilation system and reported IAQ problems. An operational error was found when investigating the ventilation system that prevented air from coming into classrooms, except for short periods of high carbon dioxide (CO_2) concentrations. However, results indicated that hybrid ventilation system was able to provide adequate ventilation and sufficient IAQ once properly designed and maintained. After ventilation operation was improved, occupants reported less unpleasant odors and stuffy air. The amount of total volatile organic compounds (TVOC) and some single volatile organic compounds (VOCs) decreased. Indoor mycobiota was observed in settled dust in the classrooms, from which ventilation improvement eliminated the dominant, opportunistic human pathogen species *Trichoderma citrinoviride* found before improvement.

Keywords: ventilation; hybrid ventilation; indoor air quality; mycobiota; indoor air questionnaire; school building; *Trichoderma citrinoviride*

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1. Introduction

In Finland, moisture damage and ventilation disadvantages are the most common problems as they are reported in more than 50% of school buildings [1]. A recent Finnish study found that 58% of Finnish schools suffer from insufficient ventilation [2]. School environments are often complex and involve several interconnected factors that affect occupants' health [3-5]. Current evidence shows that classroom conditions are significantly associated with teachers' respiratory symptoms [6]. Kielb et al. [7] found that one or more perceived symptoms were most strongly associated with reported dust and dust reservoirs, mold and moldy odors, and paint odors. Symptoms of sick building syndrome (SBS) were associated with perceptions of stuffy air, dry air, and electricity [8]. Further, teachers´ perceptions of neuro-physiological symptoms, e.g. headache, fatigue, and difficulty concentrating, were significantly increased with every 100 ppm increase in maximum classroom CO_2 concentrations [9].

According to epidemiological studies, in general, higher ventilation rates (up to 25-40 L/s per person) reduce negative health outcomes, and with minimum rates of ventilation (above 6–7L/s), some (mainly acute) health outcomes can be avoided [10]. In their review study, Sundell et al. [11] reported that lower ventilation rates might increase the incidence of respiratory infections, asthmatic symptoms, inflammation, and short-term sick leave. Correspondingly, teachers working at schools with good perceived IAQ have decreased risk for short-term sick leave (one to three days) [12]. In addition, it is well documented that both thermal conditions and IAQ affect students' performance [13].

Cellulolytic fungi that require high water content to survive, such as the genus *Trichoderma*, are well adapted to colonize water-damaged buildings. Members of this genus are often found on wet manufactured wood and gypsum boards from schools and public buildings [14,15]. Building materials contaminated with *Trichoderma* species emit high amounts of conidia into indoor air [15]. Conidia and hyphal fragments containing toxic peptaibols have been shown to provoke histamine release and disrupt the membranes of exposed target cells [15,16]. Exposure to viable conidia emitted from potentially pathogenic *Trichoderma* species, such as *T. longibrachiatum* and *T. citrinoviride*, represents an additional health risk [14,17]. Measurement of cultivable conidia from pathogenic and toxigenic fungi in settled dust in schools is an easy method to determine the potential health risk associated with changes in ventilation and fluctuations in indoor air pressure [18].

Ventilation plays a major role in creating a healthy and pleasant indoor environment, especially in modern airtight buildings. A hybrid ventilation system aims at combining the benefits of mechanical and natural ventilation. This study was conducted as part of the Finnish "EURA" and "TOXICPM" research projects (see acknowledgements) concerning IAQ and ventilation in new and renovated school buildings and microbial toxin transport mechanisms. In Helsinki, Finland, only a few public school buildings have hybrid ventilation systems. The owner of the building investigated in this study (City of Helsinki, Urban Environment Division, Buildings and Public Areas, Built Assets Management; later in the paper called as Built Assets Management of City of Helsinki), had experienced difficulties related to maintaining sufficient ventilation and good IAQ.

The aim of our study was to investigate the functionality of a hybrid ventilation system in a newly built school building with poor perceived IAQ and to determine the effects of ventilation system improvement on measured and perceived IAQ. The occurrence of toxic and potentially pathogenic *Trichoderma* species in settled dust sampled from a school building with ventilation troubles is described before and after the building's ventilation system was improved.

2. Materials and Methods

2.1. Building Characteristics

The studied school building is located in Helsinki, Southern Finland. It was built in 2009, and since 2010, several IAQ-related investigations and repairs have been conducted in the building. For example, according to numerous investigation reports and information from Built Assets Management of City of Helsinki, flooring was replaced because the concrete slabs were moist, local moisture damage was repaired, ventilation was adjusted, and air leaks were sealed. At the time of this research, the occupants had reported severe IAQ-related symptoms and discomfort during the past few years in different sections of the building, especially the section of the building under study. Approximately 700 students and 70 staff members worked in the school.

The building featured two separate ventilation systems: mechanical supply and extract ventilation with heat recovery in one section of the building, and fan-assisted natural ventilation (hybrid ventilation) in two identical sections. One section consisted of two floors. However, it should be noted that the building sections with different ventilation systems were not completely separated. Air was mixed between the sections since the main entrance of the building was located in the mechanically ventilated section, which one had to pass through to reach the hybrid-ventilated sections.

This study focused on one hybrid-ventilated section of the building, which consisted of two equal areas in the first and second floor, and was served by one air handling unit. Approximately 30% of the school staff worked in that section. Occupants had reported the most severe symptoms and discomfort in the studied section, especially the first floor. The studied section consisted of a first floor lobby surrounded by eight classrooms and toilets. The layout is presented in **Figure 1**.

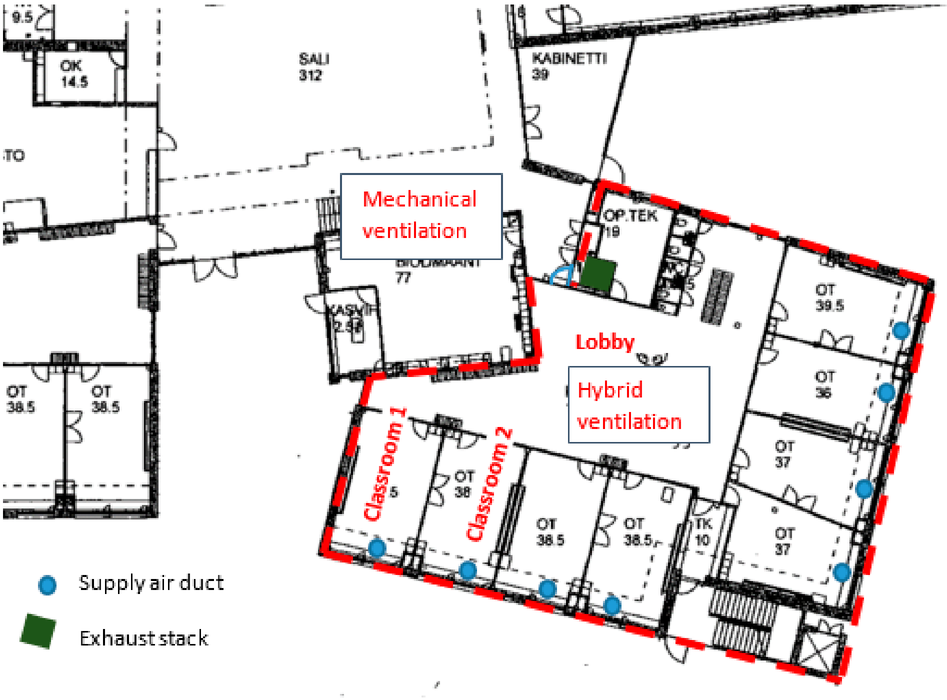

Figure 1. Studied building section (first floor). Measurements were conducted mainly in Classrooms 1 and 2.

Supply air was taken into the building section and filtered by an air handling unit located in the cellar underneath the building section. Fans assisting air income were designed for on-demand use, but due to the prolonged IAQ problems, they were running constantly at full speed. Supply air entered a chamber with two corridors. Each classroom of the building section (altogether, 16 classrooms on two floors) had its own supply air duct beginning from these underground corridors. The layout of the supply air corridors and their location in relation to the section of the building under study are shown in **Figure 2**.

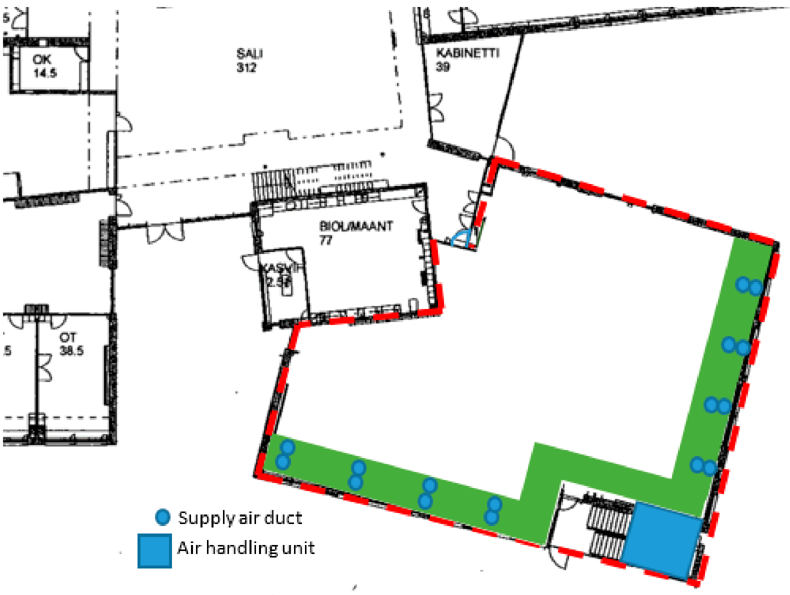

Figure 2. Location of the underground supply air chamber: air handling unit, corridors, and terminal units of supply air ducts leading to the first- and second-floor classrooms.

Classroom supply air rates were adjusted by motorized dampers located at the beginning of the supply air ducts. The dampers were designed to be a minimum of 20% open in basic situations to provide base ventilation for the classrooms. Classroom-specific carbon dioxide (CO_2) sensors controlled the dampers after the CO_2 level exceeded approximately 500 ppm in order to increase the supply air rate on demand.

Air was brought to the classrooms through grilles under the windows, transferred from the classrooms to the lobby via grilles in the partitions, and then extracted outdoors from the lobby via a large exhaust stack, as presented in **Figure 3**.

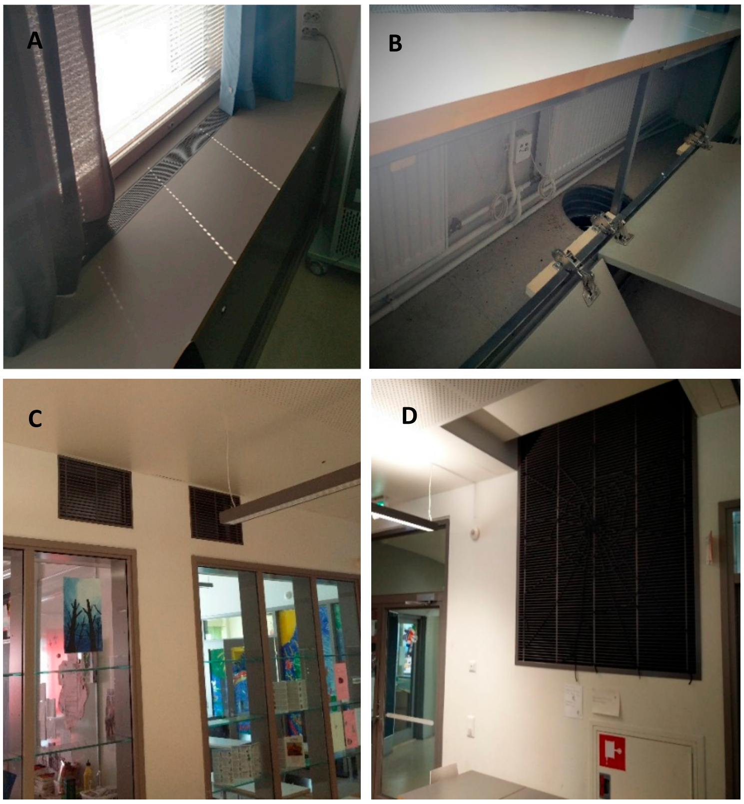

Figure 3. (A and B) Supply air grill and duct in the classrooms, (C) transfer air grilles in the partitions between classrooms and the lobby, and (D) exhaust stack in the lobby.

2.2. Study Design

According to the building's management, occupants had reported significant discomfort, stuffy air, and unpleasant odors, especially in the section of the building under study. Bad odors and displeasing IAQ were easily observed during the researchers' first visits to the building. The causes of occupants´ symptoms and discomfort were not identified, even after several investigations, but were suspected to be impurities that infiltrated the building through air leakages.

Ventilation function was investigated and reported previously [19] using pressure difference measurements, tests, and observations of the dampers in the supply air corridor with varied CO_2 concentration; by air flow measurements in the classrooms; and by pressure difference measurements in the classrooms with varied door positions and damper settings.

The ventilation system was found to be severely malfunctioning since the supply air dampers were completely closed due to a guidance system error. Supply air was enabled to enter the ducts and classrooms only during short periods when CO_2 levels exceeded the limit value. Thus, the CO_2 concentrations were sustained at an acceptable level, but the classrooms still had no base ventilation. There was a complete lack of supply air in the classrooms when they were unoccupied. The supply air duct corridor and a duct damper that is opened as required for base ventilation are shown in **Figure 4**.

Figure 4. Supply air duct corridor and classroom-specific ducts' terminal units. Dampers are opened 20%, as designed.

Negative pressures up to -30 Pa were measured before ventilation improvement, which might have enabled infiltration through the building envelope (e.g., from the crawl space). After inspections and adjustments, the ventilation system was shown to provide sufficient air flow rates and pressure differences were close to 0 Pa. A positive pressure difference across the envelope also occurred in some classrooms.

In addition, the ventilation system was found to be complex and barely controllable. The form of the supply air corridors (one straight and one angular) caused pressure loss and thus divided supply air unequally among the classrooms. Furthermore, the classroom door positions affected the pressure relations throughout the whole building section. Thus the hybrid ventilation system was shown to have some prerequisites for appropriate operation. Constant provision of the demanded supply air could be supported by some technical changes to the adjustments and control system.

2.3. Measurements in the School

Measurements were performed in May 2016 with the initial ventilation system and in March 2017 after improvement. They were conducted mainly in the two classrooms (Classrooms 1 and 2) in which occupants had reported the most severe symptoms and discomfort, according to the Built Assets Management of City of Helsinki. Additional measurements were conducted in the lobby and some other classrooms in the section of the building under study. The measurement methods are presented in Table 1.

Table 1. Measurement methods, devices and their accuracy, measurement place and duration.

Measured factor	Device	Accuracy	Place	Time
Pressure difference across the envelope	KIMO CP101, logger Grant 1000	1.5% of reading ±3 Pa	Classrooms 1 and 2	Continuous, 1 week (May 2016)
	Envic dp-101s-pd2, logger Grant 1000	3% of reading ± 0.2 m/s	Classrooms 1 and 2	Continuous, 2 weeks (March 2017)
Temperature (T)	Rotronic CL11	±0.3 °C	Classrooms 1 and 2	Continuous (1-2 weeks)
Relative humidity (RH)	Rotronic CL11	±3% (10 ... 95%)	Classrooms 1 and 2	Continuous (1-2 weeks)
Carbon dioxide (CO_2)	Rotronic CL11	±(30 ppm + 5% of reading)	Classrooms 1 and 2	Continuous (1-2 weeks)

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Formaldehyde	FM-801	±10 ppb at 40, 80, 160 ppb	Classroom 2	Continuous
Particulate matter 2.5 µm ($PM_{2.5}$)	MIE pDR-1500	±5%	Classroom 2	Continuous
Volatile organic compounds (VOCs)	Tenax TA, TD-GC-MS	±20% (average)	Classrooms 1 and 2	40 min
Mycobiota of settled dust			Classrooms 1 and 2, lobby	Cultivated for 4 weeks
Perceived indoor air quality	Örebro (MM40)—questionnaire (Finnish Institute of Occupational Health (FIOH))		Occupants of the whole building	2-week response time

2.4.2. Pressure Differences across the Building Envelope

Pressure differences across the building envelope were measured in Classrooms 1 and 2 continuously for one week before and two weeks after the ventilation improvement. A plastic tube with a copper core was placed outside by a window that was not normally open. A measurement device and logger were placed inside near the window.

2.4.3. Indoor Air Quality (IAQ) Measurements

Temperature (T), relative humidity (RH), and CO_2 concentrations were measured in Classrooms 1 and 2 for a one-week period before and a two-week period after ventilation improvement. Measurement devices were placed on the teacher's desk in the front of the room, away from the teacher's breathing zone when seated and as close to the horizontal central area of the room as possible.

Volatile organic compounds (VOCs) were measured in Classroom 2 before and after the ventilation improvement. VOC sampling and analysis were carried out according to the ISO 16000-6 standard [20]. Air samples were taken from the central area of an empty, closed room in the main working zone at a height of 1.5 m. Samples were collected in Markes International Ltd. (Llantrisant, UK) stainless steel tubes packed with Tenax TA (60/80 mesh) and Tenax TA-Carbograph 5TD using GilAir Plus air sampling pumps (Sensidyne, St. Petersburg, FL, USA) at a flow rate of 200 mL/min for 40 min.

Before ventilation improvement, analyses were conducted at Aalto University, and after improvement, they were conducted at the Finnish Institute of Occupational Health (FIOH) due to reorganization of the project resources. In the Aalto University analysis, total volatile organic compounds (TVOCs) and single compounds with concentrations over 1 µg/m^3 were analyzed, while in the FIOH analysis, concentrations less than 1 µg/m^3 were also covered. At Aalto University, the samples were desorbed using a thermal desorption unit (TD-100, Markes International Ltd.) and analyzed using a gas chromatograph (Clarus 580, Perkin-Elmer Ltd., Beaconsfield, UK) equipped with a Clarus 600T (Perkin-Elmer Ltd.) mass selective detector. VOCs were quantified by the scan (50–400 m/z) mode. TVOC concentrations were determined from TVOC area (*n*-hexane to *n*-hexadecane) and calculated as toluene equivalents, and individual compounds' concentrations were calculated either using pure reference compounds or as toluene equivalents. The concentrations of single compounds were also determined from the chromatogram before and after the TVOC area. In the case of such compounds, the quantitative results were as indicative.

Reference compounds and the NIST 2011 Mass Spectral Library automated mass spectral deconvolution and identification system (AMDIS) was used for identification during analysis at Aalto University, and at FIOH, where samples collected using Tenax TA-Carbograph 5TD steel tubes were analyzed, the Wiley database was also used. The detection limit was 0.2 µg/m^3 (not included in sum concentration).

The formaldehyde concentration of indoor air was measured using an FM-801 formaldehyde meter (GrayWolf Sensing Solution, Sheldon, IA, USA). Fine particulate matter ($PM_{2.5}$) was measured using a MIE pDR-1500 (Thermo Fisher Scientific, Franklin, MA, USA) nephelometer equipped with a $PM_{2.5}$ size-selective inlet cyclone. Formaldehyde and $PM_{2.5}$ were measured continuously for a one-week period before the ventilation in Classroom 2 was improved to determine the indoor conditions while the ventilation system was dysfunctional. Measurement devices were placed in the back of the room at a height of 1.5 m and as close to the central area of the room as possible. The occupancy rate was very low during the measurements because the semester was ending.

2.4.4. Characterization of Mycobiota in Indoor Dust

Mycobiota in indoor dust was obtained from the settled dust collected from Classrooms 1 and 2 and the lobby. These mycobiota were characterized in three stages, as described in [18]: sampling of dust, rapid toxicity screening of single colonies, and characterization and identification of the fungal isolates.

Dust samples were wiped into a clean plastic bag (Minigrip: Amerplast, Tampere, Finland) from ca. 30 × 30 cm^2 surfaces 1–2 m above floor level. The dust (ca. 10 mg) was spread with a sterile cotton swab onto malt extract agar (MEA) plates (malt extract 15 g: Sharlab, Barcelona, Spain; agar 12 g: Amresco, Solon, Ohio, USA, in 500 mL of H_2O). Culture plates were inoculated, sealed, and cultivated at 22 °C for four weeks.

For initial toxicity screening, 10–20 mg of biomass (wet weight) from each colony of the original culture plates was looped into 0.2 mL of ethanol and heated in a water bath for 10 min at 80 °C. The obtained ethanolic lysates were exposed to porcine spermatozoa and kidney tubular epithelial cells (PK-15, Finnish Food Safety Authority, EVIRA, Helsinki, Finland). The lysate was considered toxic when 2.5 vol% decreased boar sperm motility or 5 vol% decreased proliferation of PK-15 cells by >50% compared to the sham exposed control. Boar sperm motility inhibition assay (BSMI) measuring motility inhibition (i.e., inability of resting sperm cells exposed for one day at room temperature to respond to induction of motility) is described in [21]. The inhibition of cell proliferation (ICP) assay with PK-15 cells and determination of EC_{50} concentrations followed the methods described by Bencsik et al. [22]. Colonies that displayed toxicity were streaked pure and identified to the genus or species level.

Fungal colonies were grouped into eight morphotypes based on their morphology on MEA, ability to grow at 37 °C, light microscopy results for conidia and conidiophores, and responses in the two toxicity assays, BSMI and ICP. The isolates were compared to the reference strains from the HAMBI culture collection or identified according to the process described by Samson et al. [23]. A representative of the morphotype of toxigenic *Trichoderma* able to grow at 37 °C was identified by a sequence analysis of the ribosomal RNA gene cluster's internal transcribed spacer (ITS) region [24].

2.4.5. Indoor Air Questionnaire

Occupants' indoor air-related symptoms and discomfort were recorded with the standardized Indoor Air Questionnaire of FIOH twice during the research: in May 2016, before the ventilation improvement, and in March 2017, after 8 months of working in the building after the improvement.

The questionnaire was based on the Örebro Indoor Climate Questionnaire (MM40) [25] and asks respondents to recall environmental problems that had occurred during the past three months. It consists of four different sections: (1) work environment; (2) work arrangements; (3) employees' allergy history; and (4) work-related symptoms.

Staff members working throughout the school were requested to participate during the two weeks allotted for responses. The principal of the school was responsible for delivering the questionnaires to staff members, and FIOH collected and reported the answers.

Potentially significant differences between the two questionnaires were analyzed at Aalto University by SPSS statistical software (SPSS Finland Oy, Espoo, Finland) with a chi-squared test.

3. Results and Discussion

3.1 Pressure Differences across the Building Envelope

Pressure differences across the building envelope in Classrooms 1 and 2 before and after the ventilation improvement are shown in **Figure 5**.

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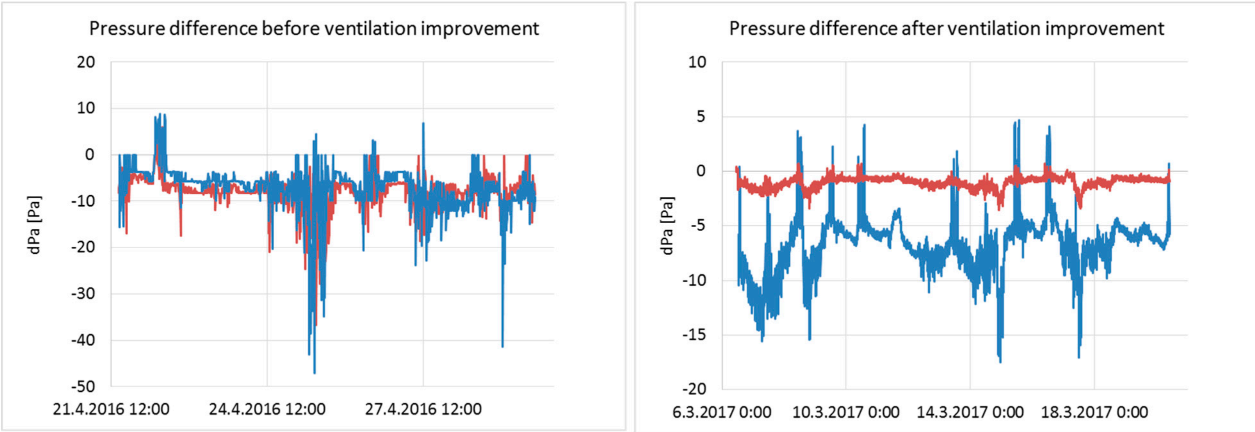


Figure 5. Pressure differences across the envelope in Classrooms 1 (red) and 2 (blue) before and after ventilation improvement.

In the initial ventilation system operation, pressure differences varied between -47.1 and 8.9 Pa and between -36.7 and 6 Pa (averages: -7.0 and -7.9 Pa) in Classrooms 1 and 2, respectively. The pressure differences were more stable after ventilation improvement, varying between -17.5 and 4.7 Pa and between -3.6 and 0.7 Pa (averages: -6.8 and -1.0 Pa), respectively.

3.2 IAQ Measurements

The T, RH and CO_2 of indoor air before and after ventilation improvement during the entire measurement period are presented in **Figure 6**, and during school occupancy hours from 8am to 5pm in **Table 2**.

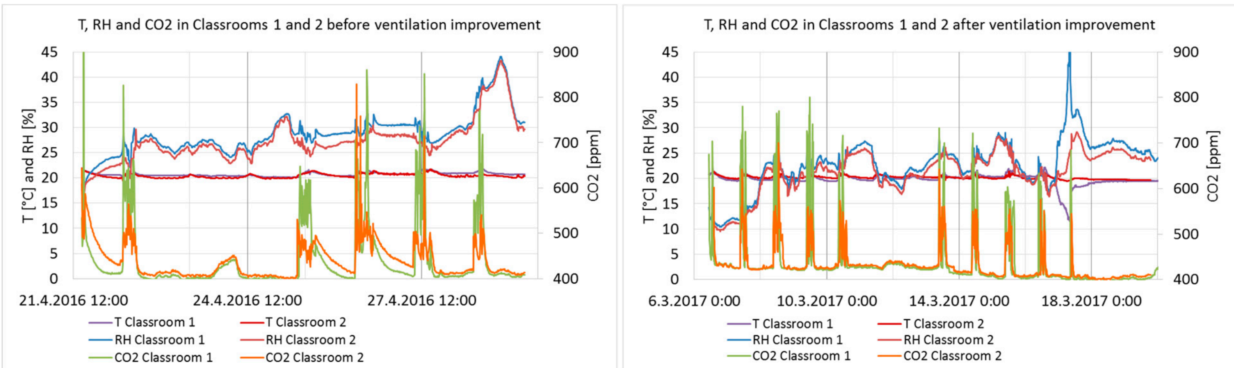


Figure 6. T, RH and CO_2 of indoor air in Classrooms 1 and 2 before and after ventilation improvement.

Table 2. Minimum, maximum and average values of RH, T and CO_2 in Classrooms 1 and 2 before and after ventilation improvement during school occupancy hours from 8am to 5pm.

		Classroom 1			Classroom 2		
		RH (%)	T (°C)	CO_2 (ppm)	RH (%)	T (°C)	CO_2 (ppm)
Before	Min	18	20	394	16	20	402
	Max	40	22	1431	38	22	829
	Average	29	21	488	27	21	458
After	Min	11	12	394	10	20	400
	Max	46	22	801	29	21	700
	Average	23	20	464	22	20	450

The maximum CO_2 concentrations according to the Finnish Classification are 750 ppm for Category I, 900 ppm for Category II, and 1200 ppm for Category III [26]. The stability of the conditions must be 95% for Category I and 90% for Category II. Category I is defined as the best possible IAQ, Category II as good IAQ, and Category III as the minimum requirements of Finnish regulations. In Category I, it is recommended that RH not drop below 20% for long periods [26]. No recommendations exist for the other categories. The CO_2 concentration and RH levels before and after ventilation improvement fell within Category I. Lack of base ventilation in the classrooms before ventilation improvement is not reflected in the CO_2 concentrations because of the temporary air dilution caused by the CO_2 sensors. Temperatures were stable and at a target level, approximately 21 °C.

TVOC and VOC concentrations in the lobby, Classroom 2 and Classroom 1 are shown in **Table 3**.

Table 3. TVOC and VOC concentrations before and after ventilation improvement.

	Lobby	Classroom 2		Classroom 1	Regulatory thresholds	FIOH thresholds
μg/m³	2016	2016	2017	2017		
TVOC	42	71,5	10	20	400	100
Acetone	21	2	7	4	50	
Decanal	5	2	1	0,8	50	3
Nonanal	11	2	1	1	50	5
Benzaldehyde	3	2	0,9	1	50	2
Toluene		25	6	7	50	4
Acetic acid		2			50	
Decamethylcyclopentasiloxane		15			50	10
Octanal	3				50	2
alpha-Pinene	3				50	8
Ethyl acetate	5			1	50	
Benzene			0,8	0,6	50	1
Xylene (p,m)			0,4	0,4	50	6
1-methoxy-2-propanol			0,9		50	3
1-butanol				0,6	50	4
2-ethyl-1-hexanol				0,4	10	4
2-propanol				3	50	

TVOC and VOC concentrations were well below the national action values [27]. However, FIOH's recommendation [28] show limit values above which the concentrations might indicate the existence of an exceptional indoor source for impurities, and the need for additional environmental investigations. Several single VOC concentrations in the lobby and in Classroom 2 before improvement exceeded these thresholds. It should be noted that these threshold values are not regulatory limit values, but are based on typical concentrations in office environments with mechanical ventilation.

TVOC concentrations were higher in the lobby and in Classroom 2 before the ventilation improvement compared to concentrations in Classrooms 1 and 2 after improvement. In Classroom 2 TVOC decreases 86 %. Except for acetone, single VOC concentrations decreased as well, in particular the concentrations of toluene and decamethylcyclopentasiloxane. These compounds might be released from, for example, cleaning or cosmetic products used by the previous occupants. Typical VOC sources include building materials, coverings, and cleaning as well as cosmetic products [29,30].

The formaldehyde concentration was below 10 ppb (equivalent to approx. 12 μg/m³), which is the detection limit of the meter. The PM2.5 concentration varied between 0 and 17.6 μg/m³ (average 3.8 μg/m³), which is below the limit value of 25 μg/m³ [27].

3.2 Characterization of Mycobiota in Indoor Dust

Diverse mycobiota cultivated in indoor settled dust were sampled before and after ventilation improvement and are visualized in **Figure 7**. Fungal colonies representing the dominant morphotypes were tested for toxic and pathogenic potential and identified to the genus or species level as shown in **Table 4**. The toxic and pathogenic morphotype of the green colonies dominating the plate shown in Panel A of **Figure 7** was identified as *Trichoderma citrinoviride*. The rhizoid

 doi:10.20944/preprints201803.0007.v1

morphotype in Panels B and C was nontoxic *Rhizopus* sp. unable to grow at 37 °C. The toxic *Trichoderma* sp. colonies in Panel D differed from *T. citrinoviride* due to a lack of ability to grow at 37 ºC and association with globous conidia. The dominant morphotypes in Panels E–H were represented by a terverticilliate nontoxic *Penicillium* sp. and a nontoxic monoverticilliate *Penicillium* sp. Sporadic toxigenic, black, yellow, and light green colonies in Panels G and H were identified as fungi belonging to *Aspergillus nigri*, *Aspergillus westerdijkiae*, and *Eurotium* sp., respectively.

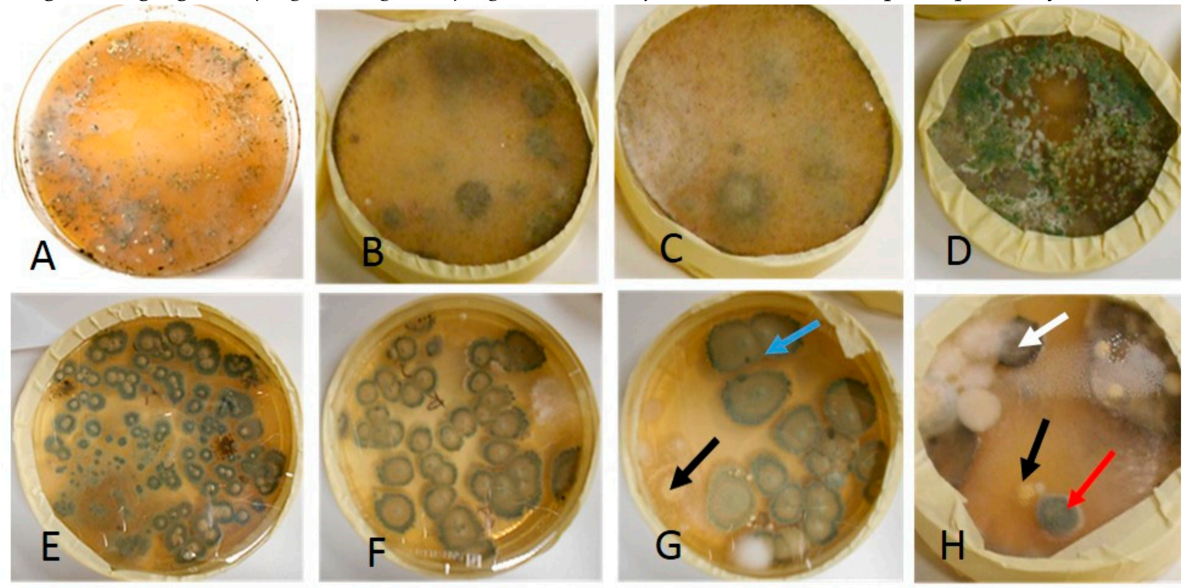
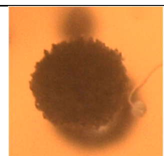
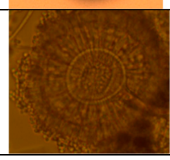
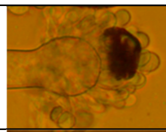
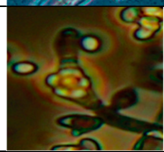


Figure 7. Fungal colonies cultivated from settled dust sampled before (upper row) and after (lower row) ventilation improvement. Panels A–C and E–G are cultures from dust samples collected from Classroom 2. Panel D is a culture from dust collected in the lobby, while Panel H is a dust culture from Classroom 1. The dust samples were cultivated on MEA and incubated for four weeks at room temperature. The plates in Panels A and D contained over 100 green *Trichoderma*-like colonies. The pates in Panels B and C were overgrown with *Rhizopus*-like colonies. The plates in the lower row (Panels A–G) contained mainly green *Penicillium* colonies (blue arrow). The plates in Panels G and H contained yellow *Aspergillus* colonies (black arrow), a black *Aspergillus* colony (white arrow), and a green *Eurotium/Aspergillus* colony (red arrow).

Table 4. The eight fungal morphotypes isolated from settled dust collected before and after ventilation improvement, characterized by toxigenicity, pathogenic potential, and conidiophore morphology.

		Toxicity		Colony color	Size of conidia/spores	Morphology under light microscope
	Growth at 37ºC	BSMI	ICP	MEA	(µm)	
Aspergillus section *Nigri* 1 strain	+	-	+	Black	3.5-5	
Asp. westerdijkiae 2 strains	-	+	+	Yellow	2.5-3	

Eurotium sp. 1 strain	+	+	+	Green	5-7	
Penicillium sp. 10 strains (Terverticilliate)				Green	3.4	
Penicillium sp. 3 strains (Monoverticilliate)				Green	2.3	
Rhizopus sp. 10 strains	-	-	-	Grey	5-10	
*Trichoderma citrinoviride** 10 strains	+	+	+	Green	1.6 x 3	
Trichoderma sp. 5 strains	-	+	+	Green	4	

* Identified to species level by ITS sequence analysis

Table 5 shows that the dominant morphotypes cultivated from dust sampled from two locations before ventilation improvement in May 2016 were the potentially opportunistic human pathogen *T. citrinoviride* [31], toxic *Trichoderma* sp., and non-toxic, non-pathogenic *Rhizopus* sp. colonies. The mycobiota cultivated from settled dust collected from three locations after ventilation improvement in March 2017 was more diverse and characterized by frequent non-toxic *Penicillium* species as well as sporadic toxic and potentially pathogenic *Aspergillus* and *Eurotium* species.

Table 5. Cultivable mycobiota in settled indoor dust sampled from surfaces above floor level in four different locations of the school. Dust was sampled before and after ventilation improvement and cultivated on three plates per location.

School samples	Settled dust		
Before ventilation improvement	Sampled 31.5.2016	Number of colonies /plate	Number of plates containing a colony morphotype/all plates
Locations: Classroom 2 and lobby	*Trichoderma citrinoviride*[ab]	> 100	1/6
	Rhizopus sp.	Plate overgrown	2/6
	Trichoderma sp.[a]	>100	2/6
After ventilation improvement	Sampled 6.3.2017		
Locations: Classrooms 1 and 2 and one other classroom	*Penicillium* sp.[c]	>100-120	3/9
	Penicillium sp.[d]	10	2/9
	Aspergillus westerdijkiae[a]	2-3	2/9
	Asp. niger[ab]	1-2	3/9
	Eurotium sp.[a]	1	1/9

[a] Colonies of morphotypes that are toxic to sperm or kidney cells. [b] Colonies of potentially pathogenic morphotypes able to grow at 37°C. [c] Terverticilliate *Penicillium* species. [d] Monoverticilliate *Penicillium* species.

T. citrinoviride is a potentially opportunistic human pathogen that produces toxic peptaibols and is known to colonize water-damaged buildings [16,17,31]. To our knowledge, this is the first report of the dominant occurrence of potentially pathogenic and allergenic *T. citrinoviride* in indoor settled dust from a Finnish school building. Since settled dust is very likely derived from airborne dust, airborne exposure to viable conidia of *T. citrinoviride* is possible, which is of concern in a school building. Previous studies revealed a significant correlation between the risk of both childhood and adulthood asthma and IgG antibodies to *T. citrinoviride*, suggesting that this species may play a role in the etiology of asthma [32,33]. The cultivated settled dust sampled one year later from the same location after ventilation improvement did not exhibit *T. citrinoviride* colonies. This indicates that the ventilation improvement eradicated the airborne source of viable *T. citrinoviride* conidia. Viable conidia of toxigenic *Trichoderma* sp. colonies unable to grow at 37°C were found before ventilation improvement in dust sampled from the lobby but were absent in dust sampled from three locations after ventilation improvement. It is possible that the ventilation improvement stopped the spreading of the moisture demanding *Trichoderma* species to the indoor environment.

3.3 Indoor Air Questionnaire

Indoor air questionnaire results before and after ventilation improvement from the studied and corresponding building sections are shown in **Table 6**.

Table 6. Indoor air questionnaire (Finnish Institute of Occupational Health© 2006–2008, version 2.0) results from May 2016 and January 2017.

	Comparison Values	Studied Section		*p*-Value	Corresponding Section		*p*-Value
	[34-36]	2016	2017		2016	2017	
Background information							
Number of answers		15	16		16	17	
Answer (%)	71	79	80		84	85	
Females (%)	21	87	88		94	94	
Daily smokers (%)		13	6		0	0	
Average age (years)		41	42		41	38	
Average employment in this work place (years)		5	5		4	4	
Work environment (%)		%	%				
Draught	22	7	44	**0.037***	13	47	**0.057***
Room temperature too high	17	0	0	---	0	6	1.000*
Varying temperature	16	20	31	0.685*	19	27	0.685*
Room temperature too low	13	27	56	**0.095**	19	53	**0.041**
Stuffy air	34	53	38	0.376	80	71	0.691*
Dry air	35	13	44	0.113*	14	41	0.132*
Insufficient ventilation	32	47	31	0.379	75	59	0.325
Smell of mold	9	7	0	0.484*	7	6	1.000*
Unpleasant odour	17	40	19	0.252*	38	24	0.465*
Environmental tobacco smoke	4	0	0	---	13	0	0.227*
Noise	17	47	56	0.594	19	50	**0.063**
Dim light or reflections	14	7	13	1.000*	6	0	0.485*
Dust or dirt	25	27	25	1.000*	25	35	0.708*
Work regarded as interesting and stimulating (%)							
Often	75	73	88	0.394*	88	82	1.000*
Sometimes	20	27	13		13	18	
Seldom or never	4	0	0		0	0	
Too much work to do (%)							
Often	20	0	13	**0.081***	13	18	0.700*
Sometimes	59	40	63		56	65	
Seldom or never	21	60	25		31	18	
Opportunity to influence work conditions (%)							
Often	35	27	25	0.513*	25	24	0.577*
Sometimes	44	60	75		63	47	
Seldom or never	21	13	0		13	29	
Fellow workers help with problems in the work (%)							
Often	72	87	88	1.000*	88	76	**0.050***
Sometimes	22	13	13		0	24	
Seldom or never	6	0	0		13	0	
Allergic diseases (%)							
Asthma	8	0	0	---	19	18	1.000*

Hay fever	38	67	56	0.552*	50	41	0.611
Atopic eczema	28	40	19	0.252*	13	12	1.000*
Stress (%)							
Very much	10	7	0	0.450*	13	24	0.735*
Some	28	27	47		50	35	
None/only a little	63	67	53		38	41	
Symptoms (%)							
Fatigue	16	7	19	0.600*	19	29	0.688*
Heavy-headedness	9	20	13	0.654*	6	35	**0.085***
Headache	7	13	6	0.600*	19	29	0.688*
Difficulty concentrating	3	0	6	1.000*	6	18	0.601*
Eye irritation	17	27	31	1.000*	20	41	0.265*
Irritated, stuffy, or running nose	20	13	25	0.654*	19	35	0.438*
Hoarse/dry throat	14	13	38	0.220*	31	35	0.805
Cough	5	0	13	0.484*	6	18	0.601*
Cough disturbing sleep	1	0	6	1.000*	0	0	---
Dry or flushed facial skin	11	7	25	0.333*	13	25	0.654*
Hands: dry, itching, red skin	15	0	19	0.226*	7	24	0.338*
Shortness of breath	3	7	0	0.484*	0	6	1.000*
Wheezing	1	7	0	0.484*	0	6	1.000*
Fever or chills	2	0	0	---	7	6	1.000*
Joint pain	3	0	0	---	0	0	---
Muscular pain	4	0	0	---	0	0	---
Other		0	0	---	9	12	0.832*

Statistically significant changes at a 10% confidence interval ($p < 0.1$) are bolded. The p-values marked with * were determined by the Fisher's exact test (SPSS). Comparison values are based on analysis of the comprehensive questionnaire data collected by FIOH.

The first questionnaire was conducted in May 2016 during the warm spring/summer season, two weeks before the summer holiday began. The other questionnaire was conducted in March 2017 during cold winter season, halfway through the school semester. It is known that the occupants′ perceptions are affected by seasonal [37] and psychosocial factors [38-40], and thus the conditions for the questionnaires were not optimal. In addition, the number of responses was low for both questionnaires, preventing reliable statistical interpretation of the results. In the corresponding building section, the incidence of asthma among the occupants is relatively high, which affected the answers.

Because it was implemented in the cold season, the climate conditions of the second questionnaire differed remarkably from the first questionnaire and likely affected the responses. The only statistically significant change (at a 50% confidence interval) between the two questionnaires was that the perception of draught increased from 7% to 44% in the section of the building under study and in the corresponding section (p=0.057). However, increases in the perception of dry air and belief that the room temperature was too low were reported as well.

In the first questionnaire, before the ventilation improvement, stuffy air (53%), insufficient ventilation (47%), and unpleasant odor (40%) were more commonly reported compared to the comparison values. After the ventilation improvement, respondents' perceptions of these factors decreased to the levels of the comparison values. However, the changes in perceptions before and after the improvement were not statistically significant. An equivalent trend in perceptions can be observed in the identical section of the building, in which corresponding ventilation improvement took place according to the information received from Built Assets Management of the City of Helsinki.

5. Conclusions

The disadvantages of the previous ventilation system function were detected by occupants through sensory observations and perceived symptoms. Missing of base ventilation was not reflected in the CO_2 concentrations in classrooms' indoor air before ventilation improvement due to the short-time dilution of classroom air caused by the CO_2 sensor control. Based on the CO_2 measurement results, the ventilation system seemed to work sufficiently. However, this was proven to be a false conclusion based on observations. Thus, it can be concluded that determining ventilation function based on CO_2 concentration might lead to severe misinterpretation. A lack of base ventilation in the baseline of this study was not reflected in other IAQ measurements, but the levels of TVOC and single VOCs were significantly decreased after the ventilation improvement.

Although perceptions of unpleasant odours and stuffy air decreased after the ventilation improvement, statistically significant improvement in perceived IAQ were not observed. The low number of responses to the questionnaire and seasonal effects on participants' perceptions weakened the ability to interpret the results of the questionnaire in relation to ventilation operation.

To our knowledge, this study is the first to report the dominant occurrence of the opportunistic, potentially pathogenic species *T. citrinoviride* in indoor dust in a Finnish school building, which was found before the ventilation improvement. Airborne exposure to this species might be of concern since settled dust is very likely derived from airborne dust. After ventilation improvement *T. citrinoviride* and toxic *Trichoderma* sp. were not sampled showing the potential effect of ventilation improvement on the cultivable mycobiota in the settled dust. This indicates that the ventilation improvement stopped the spreading of the moisture demanding *Trichoderma* species to the indoor environment.

Acknowledgments: The authors warmly acknowledge the Academy of Finland (grant for TOXICPM 289161), the Finnish Work Environment Fund (grant for EURA 115376) and Business Finland (grant for Indoor air police – detection and prevention of indoor air quality problems 4098/31/2015) for funding this research. The participation of City of Helsinki and studied school is also greatly appreciated. László Kredics was supported by the GINOP-2.3.2-15-2016-00012 grant (Széchenyi 2020 Programme, Hungary) and the János Bolyai Research Scholarship (Hungarian Academy of Sciences).

Author Contributions: Camilla Vornanen-Winqvist wrote the paper and conceived, designed, and performed the experiments. Kati Järvi analyzed the questionnaire data and participated in writing the paper. Maria A. Andersson conceived, designed, and performed the mycobiota experiments, analyzed the data, and participated in writing the paper. Sander Toomla conceived, designed, and performed the ventilation experiments and contributed the measurement devices. Kaiser Ahmed designed and performed the ventilation experiments. Raimo Mikkola designed the IAQ experiments, contributed measurement devices, analyzed the VOC samples, and participated in writing the paper. Tamás Marik and László Kredics designed and performed identification of the fungal morphotypes. Heidi Salonen conceived and designed the IAQ experiments and questionnaires as well as participated in writing the paper. Jarek Kurnitski was the principal investigator responsible for the study design and participated in writing the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

IAQ	Indoor air quality
TVOC	Total volatile organic compounds
$PM_{2.5}$	Fine particulate matter, particle size 2.5 μm
RH	Relative humidity
VOC	Volatile organic compounds
T	Temperature
CO_2	Carbon dioxide
FIOH	Finnish Institute of Occupational Health
MEA	Malt extract agar
ITS	Internal transcribed spacer

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