Science of the Total Environment 1047 (2026) 182020

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Water vapour as a carrier of mould metabolites in indoor air: an explanation for the sick building syndrome and severe morbidity

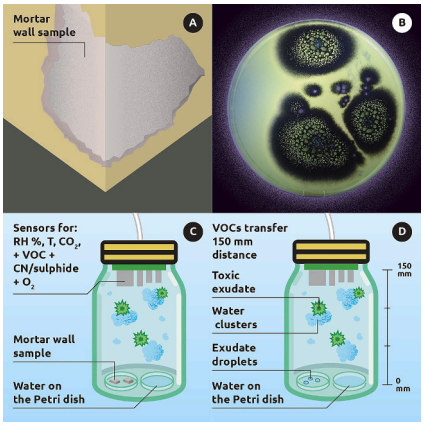
Kirsi Vaali [a,*], Michael Sulyok [b], Jari Louhelainen [d], Marika Mannerström [e], Tuula Heinonen [e], Rudolf Krska [b,c], Leena A. Räsänen [f,g], Arto Visala [h], Panu Harmo [h], Mirja Salkinoja-Salonen [h,i]

[a] SelexLab, Rauhalantie 168, 02440, Luoma, Finland
[b] BOKU University, Institute of Bioanalytics and Agro-Metabolomics, Department of Agricultural Sciences, Konrad Lorenzstr. 20, 3430, Tulln, Austria
[c] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom
[d] Liverpool John Moores University, Faculty of Science, James Parsons Building, Byrom Street, Liverpool, L3 3AF, United Kingdom
[e] The Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpönkatu 34, 33014, Tampere University, Finland
[f] Co-op Bionautit, Viikinkaari 9, 00790, Helsinki, Finland
[g] Department of Microbiology, P.O. Box 4, University of Helsinki, 00014, Finland
[h] Department of Electrical Engineering and Automation, Aalto University, Espoo, Finland
[i] Department of Chemistry, University of Helsinki, Finland

HIGHLIGHTS

- Students' IAQ symptoms disappeared in healthy, reappeared in 24 h in damaged schools
- Emission of the school wall mortars was studied in glass chambers.
- Water vapour carries VOC and non-volatile substances into indoor air.
- The wall mortars studied with KCN/NaSH revealed alternative oxidase moulds.
- Emitted cytotoxic substances from moulds explicate symptoms of the students.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Volatile organic compounds
Non-volatile compounds
Mycotoxins
Indoor air quality

ABSTRACT

Indoor air quality (IAQ) is a possible but unproven culprit for sick building syndrome (SBS). We earlier showed that emitted mould exudates are carried into ambient air by water clusters. When nine students (7–12 years) moved from the damaged school to reference schools, their symptoms attenuated but reappeared, within the same day, when transferred to schools with known IAQ problems. A self-made experimental recording of glass

* Corresponding author.
E-mail addresses: kirsi.vaali@selexlab.fi (K. Vaali), michael.sulyok@boku.ac.at (M. Sulyok), J.Louhelainen@ljmu.ac.uk (J. Louhelainen), marika.mannerstrom@gmail.com (M. Mannerström), tuula.h.heinonen@gmail.com (T. Heinonen), rudolf.krska@boku.ac.at (R. Krska), leena.rasanen@bionautit.fi (L.A. Räsänen), arto.visala@aalto.fi (A. Visala), panu.harmo@gmail.com (P. Harmo), mirja.salkinoja-salonen@helsinki.fi (M. Salkinoja-Salonen).

https://doi.org/10.1016/j.scitotenv.2026.182020
Received 5 March 2026; Received in revised form 19 June 2026; Accepted 30 June 2026
Available online 15 July 2026

Symptoms
Water vapour

chambers and sensors of volatile organic compounds (VOC) and non-volatiles, relative humidity, CO_2 and CN/sulphide were applied to mimic IAQ of the school. Mortar samples of the damaged school wall, when cultured on malt agar, grew alternative oxidase fungi (aox-fungi), which emitted exudates. Exudates were analysed by LC-MS/MS and tested on human BJ cells. LC-MS/MS analysis of the cultivated mortars showed that the mould-derived droplets hanging underneath culture lids transferred in water, being the same as the exudates' contents on top of mould growths. Boric acid added to the mortar cultivation, promoted the emission of mould metabolites, which were shown to be lethal to BJ cells. The results support presence of aox-fungi in the mortars, which was closest to the damaged area of the building. Cytotoxicity of the mould exudates in cultivation varied time-dependently, within two weeks, as indicated by BJ cells. The present results and earlier reported cytotoxic water vapour of this school air are the contact points between the mould exudates, damaged building and clinical symptoms: the missing link in the mystery of SBS. Analysing water vapour will reveal piggy-bagged toxic substances.

1. Introduction

Traditional culture-based analysis of settled dust does not necessarily reveal the origin of an indoor air quality (IAQ) problem. Moulds belonging to *Fusarium, Stachybotrys*, *Trichoderma, Chaetomium*, *Penicillium* genera are known to emit mycotoxins as liquid exudates (Gottschalk et al., 2008; Gareis and Gottschalk, 2014; Castagnoli et al., 2018; Salo et al., 2019; Andersson et al., 2020) but the exudate droplets emitted by moulds have attracted little attention in IAQ investigations (Andersson et al., 2020) although connection of microbial VOC and mouldy buildings is a well-known phenomenon (Polizzi et al., 2009; Al Hallak et al., 2023). We continue our school study from 2020, in which $n = 47$ students reported symptoms, and samples of water vapour from this school were shown to be cytotoxic to human THP-1 cells (Hyvonen et al., 2020). Respective results, cytotoxic water vapour and reported symptoms, were earlier observed in our article of adults (Vaali et al., 2022a, 2023). Students in the studied damaged school (Hyvonen et al., 2020) had suffered severe symptoms, which disappeared when they changed schools, but re-emerged when they transferred to other school (s) with reported IAQ problems.

All material in the world is in three phases (gas, solid, liquid) and earlier methods have used only two phases, gas and solid phases (often tVOC and microbe assays). Our novel idea is a method, which is based on the liquid phase, and sampling of the water vapour, which results we evaluate with cytotoxicity tests by using human cells. To study relative humidity (RH%) and emissions of a building, we added small pieces of wall materials and water into a glass chamber and measured air quality with sensors attached to the top of the chamber. It is generally not realized that building-derived non-volatile compounds due to their very low vapour pressure, can be detected from air. This assumption that non-volatile compounds are absent from inhalational air has, for decades, hindered progress in IAQ research. However, we have already in 2018 demonstrated that non-volatile chemicals or fungal secondary metabolites (molecular weight 300–1500 g/mol), when placed at the bottom of a glass chamber, could be detected at the top of the chamber by condensing (Selkäinaho et al., 2018). After placing each of the various chemicals/metabolites separately to the bottom of a chamber, we collected samples from top of a Peltier cooler by condensation process (Selkäinaho et al., 2018). It appeared from petri agar-cultured samples of the wall mortar that purified water could transfer these non-volatiles up into air, and we had shown that common cleaning agents or fungal semi-ochemical, 1-octen-3-ol (MW 128 g/mol) as well as ochratoxin A, with markedly higher molecular weight, were transferred by water clusters (Selkäinaho et al., 2018). For example, the calculated boiling point of aflatoxin B1 is approximately 528 °C and that of ochratoxin A is approximately 632 °C. They are only transported to the VOC sensor if they are in droplets of evaporating water. This finding addressed us to go further to the mystery of IAQ, and later we have hypothesized that toxic substances from mould exudates can also be carried into air by water vapour (Vaali et al., 2025). Before these publications, no mechanical explanation had been given, how mould exudates were transferred via air from top of colonies to culture lids. However, van Oss et al. had presented that at room temperature, airborne water vapour moves as clusters (Van Oss et al., 2001, 2005). Water (molecular weight of 18 g/mol) can lift heavier molecules up into air when in clustered form. Water is, surprisingly, an effective hydrophobic carrier. When water molecules form clusters, the hydrogen atoms point outwards producing a layer of the most hydrophobic solvent known (Van Oss et al., 2005; Decker and van Holde, 2011). Therefore, hydrophobic microbial exudates can be carried up by water clusters. The work of van Oss suggests that water–air interfaces and water clustering phenomena may facilitate the mobilization and airborne transfer of compounds, which volatility alone would not predict substantial gas-phase occurrence.

There are two kinds of moulds: those with five or three protein complexes in the mitochondria. Those with five complexes produce energy efficiently and grow quickly, whereas those with three complexes grow slowly and are called alternative oxidase or (*aox*)-fungi. In the research area of IAQ, *aox*-fungi are hardly known. However, there are several reports of *aox*-fungi in plants, in stress signaling, fungal pathogenesis and drug resistance, which are associated in *Aspergillus* spp. (Tian et al., 2020) AOX is highly conserved structure among *Aspergillus* spp. (Li et al., 2011), it is a ubiquitous enzyme in plants and agriculture, in pathogenic fungi, and it's function is to transfer electrons from ubiquinol directly to oxygen, and to reduce O_2 to H_2O *(Tian et al., 2020)*. *Aox*-fungi use mitochondrial complexes I, II, and III, as a route for energy production. As *aox*-fungi are deficient in complex IV and V, therefore, *aox*-fungi are tolerant to compounds that inhibit complex IV (Kirimura et al., 2000; Rogov et al., 2014). To reveal the presence of *aox*-fungi in the school wall material samples, we used cyanide (KCN) and sulphide (NaHS) as tools to show presence of *aox*-fungi in mortar samples. NaHS mimicked sewer gas; appearing in buildings when ventilation is halted and restarted.

Boron compounds are used in building materials; crawl space or attics are typically insulated with cellulose with boron additive. Boron is used as antibacterial agents and fire retardants; but boron compounds are less effective against moulds (Andersson Aino et al., 2022; Mikkola et al., 2015; Vornanen-Winqvist et al., 2020; Salo et al., 2015). Lopez Hurtado writes: “It is well known that lignocellulosic materials allow mould growth” (Lopez Hurtado et al., 2016). Also, from the US, Godish and Godish show how strong mould growth can be found in cellulosic materials, they showed that 3 of 4 buildings have strong mould growth and discuss about the unacceptable risk of danger for humans (Godish and Godish, 2006). Chemicals like boron could promote growth of *aox*-fungi and exudates from *aox*-fungi could be a source of IAQ problems. Therefore, we studied mortar samples of the school's walls when cultured on malt agar with and without boric acid. The cultured moulds emitted visible exudates, which accumulated on top of colonies and were hanging from the top lids, a phenomenon that has been presented earlier in vitro in biomass extracts and/or guttation droplets from buildings, containing toxic compounds (Salo et al., 2019; Andersson et al., 2020). It must be also noted that fungi are often believed to produce mycotoxins, but only the secondary metabolites with cytotoxic capacity may be called mycotoxins. Using animal cells such cytotoxicity has been shown before: toxicological profile of *Chaetomium globosum*

was confirmed in the boar sperm motility inhibition assay (BSMI), sperm membrane integrity damage assay (SMID), and in inhibition of cell proliferation (ICP) assay (Salo et al., 2020). Therefore, in the present study, moulds cultured from wall mortar produced exudates that were subsequently analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and tested for cytotoxicity using human BJ cells.

2. Materials and methods

2.1. Retrospective study of the students

The original study report (2020) of this school (§1) examined students aged 6–15 years (Hyvonen et al., 2020). Four years later, students 9/47 were willing to participate in this retrospective follow-up study with no exclusion criteria.

2.2. The mortar samples from the school walls and cultures of the samples

There was a period of sewer and hot water leakage into the basement, and no mechanical air inlet, the exhaust air was erroneously recycled back indoors. The concrete walls were levelled with mortar (sand, cement, paint). Samples H1, H2, were taken along corridor wall, H3 from a staircase wall, close by piano from which toxic swab samples were taken earlier (Hyvonen et al., 2020), (Fig. 2). Malt agar (MA) plates (2% malt in ddH_2O) or MA with boric acid 2000 mg/L (MA + B), were prepared and autoclaved (20 min, 121 °C). The mortars (1 g) were blended with 1 L of MA or MA + B, cultured for 14d.

2.3. The exudate droplets produced by cultured moulds from walls

Moulds emitted fluorescing droplets (Graphical abstract Fig. 1B), on the surface of the colonies, also hanging under lids of the dishes (Andersson et al., 2020). These were harvested and stored in glass vials (−18 °C), diluted in methanol until subjected to LC-MS/MS analysis.

2.4. Toxic secondary metabolites in the exudates

The analysis was carried out using a 1290 Series HPLC System (Agilent, Waldbronn, Germany) coupled to a QTrap 5500 LC-MS/MS System (Applied Biosystems SCIEX, Foster City, CA) equipped with Turbo Ion Spray electrospray ionization source (Sulyok et al., 2020). Chromatographic separation was performed at 25 °C on a Gemini® C_{18}-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C_{18} 4x3mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US). Confirmation of positive metabolite identification was carried out by the acquisition of two MS/MS signals per analyte in the time scheduled

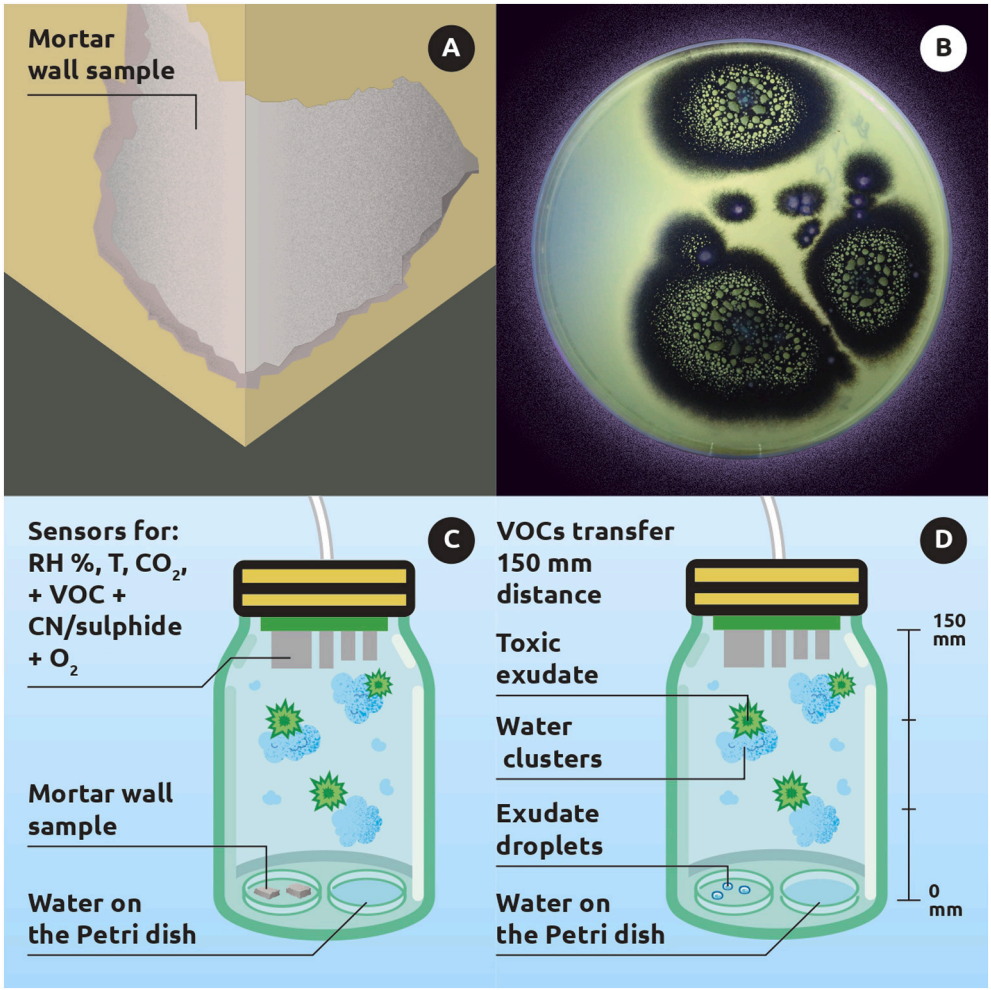


Fig. 1. The overall description of the study methods. A) Samples of mortars (H1, H2, H3) from the school's walls were cultured on malt agar, with and without boric acid. B) Cultures emitted liquid exudates, droplets were found on the colonies, and hanging undersides of the cover lid, both were analysed by LC-MS/MS. The exudates (yellow) emitted by the colonies (dark) were most clearly visible under fluorescent light. C) The measuring chamber was equipped with sensors, attached to the inside surface of the lid. Experiments in glass chambers were performed with wall mortars (H1, H2, H3), or from D) exudate droplets (H2, H3). VOC and non-volatiles from the emitted exudates (illustrated with green stars), carried by water vapour (light blue clouds), migrated 150 mm up to the sensors. These experiments demonstrate how water vapour acted as a carrier for the mould-produced liquid exudates into inhalation air.

multiple reaction monitoring mode, yielding 4.0 identification points (European Commission decision 2002/657). Retention times and ion ratios had to agree with the values of authentic standards within 0.03 min and 30% rel., respectively. Quantitation was based on external calibration using serial dilutions of a multi-analyte stock solution. The accuracy was verified on a continuous basis by participation in the proficiency testing scheme organized by BIPEA (Gennevilliers, France) with a current rate of z-scores between −2 and 2 of >96% (>2100 results submitted). Exudate droplets were diluted 1 + 3 using acetonitrile/water/acetic acid 49.5/49.5/1, injection volume 5 μL.

2.5. Cytotoxicity of microbial exudates

The exudates (10 μL) were disinfected with 140 μL of 96% ethanol and evaporated for 72 h and reconstituted in 250 μL MEM with 10% FBS, 2 mM L-Glutamine, 1% NEAA (Carlsbad, CA). Human BJ fibroblasts, ATCC (LGC Promochem AB, Sweden), were cultured for 24 h on 96-well plates, 4000 cells/well, these were exposed to the exudates for 48 h, in 2–3 parallels. Culture medium served as a negative control. Neutral red (NR) dye was added, incubated for 3 h, washed and absorbance measured at 540 nm (Borenfreund and Puerner, 1985; Mannerstrom et al., 2017). Viable BJ cells incorporate NR. The results were calculated as viability percentage compared to the negative control, (100% alive).

2.6. Measurement of VOC and non-volatile emissions from the building wall in chambers

The mortar samples, 5 g dry weight, were blended in 1.0 mL/ddH_2O, pH 12 (pH-test strips, Dosatest, BDH, Germany). The chambers (840 mL) were equipped with sensors for humidity (RH%), temperature (°C), and CO_2 (ppm) by Sensirion SCD30 (GitHub HQ, San Francisco, USA). VOC and non-volatiles were assayed by (SGP30), oxygen (Oxygen MIX8410-O_2, M1[21S00]28, Shenzhen Headquarters, Shenzhen, China), and HCN/H_2S (Sensor 4HCN-50, max 50 ppm, PN 064-0000-000 SN: 064002210097, Euro-Gas Management Services, Devon, UK). Oxygen (20%vol) was stable throughout experiments. Sensors were inserted through the cap, 150 mm above the chamber's bottom and placed in a ventilation hood. A self-made data recording program collected data 1×/min. Toluene 1 μL was used as an internal standard for the tVOC sensor.

3. Results

3.1. Symptoms of the students

Each of the nine students from the severely damaged school (§1), changed school several times (everyone was in 2–5 different school buildings), altogether in 10 different buildings, and their IAQ reports were congruent. Their symptoms were persistent and severe: hepatitis, nausea, headaches, epilepsy, IBD, chronic pains, hypermobile joints. For diagnosis, even liver biopsies were needed to be performed. They also reported symptoms from the annex building (§6) where desks, books, and piano were relocated from the damaged school (§1). When 6 students moved to a reference school (§2), 5 reported no symptoms, while student #2 was suspected to react to some exposure while travelling on the school-bus. Student #5 developed inflammatory bowel disease, anaemia, scoliosis, and grew 12 cm in 6 months (§1). In his new school (§8), he was symptom-free, like his sibling, student #6. One year later, student #5 moved to a secondary school (§9), in which there were known IAQ problems, and his symptoms re-emerged on the same day. The symptoms disappeared when he moved to a separate building (§10). Student #7 developed epilepsy, aggression and fatigue. After she moved to the reference school, her aggression subsided, epilepsy symptoms alleviated, and anti-epileptic drug therapy was discontinued (§2). Student #8 developed hypermobile joints with symptoms of fatigue as earlier reported in children exposed to moulds (Tuuminen et al., 2019). Liver biopsy confirmed the diagnosis of cirrhosis in student #9, his serum ALAT was 2400 IU/L. His symptoms gradually lessened while at home but reappeared in the annex building (§6). He changed to the reference school (§2), and while he is still on medication, he is asymptomatic, and when re-biopsied in January 2024, showed no signs of inflammation. All the students are presented in Table 1.

3.2. Content of the exudates

Mortar samples were collected at different sites (Fig. 2) of the damaged school (§1) and cultured to produce exudates. The exudates were harvested from top of the microbial colonies or from the top lids and analysed by LC-MS/MS. The exudates from top of colonies and lids contained the same secondary metabolite concentrations (Table 2, blue font). Mortar sample H1, taken most distant from the damaged area, did not emit exudates (Fig. 2). When cultured on malt agar containing boric acid 2000 mg/L (MA + B), mortars H2 and H3 emitted more than double the amounts of secondary metabolites (4/14 and 8/14, respectively), compared to MA culture without boric acid (Table 2, orange marks). Production of one Trichoderma metabolite from H3 sample decreased, when cultured on MA + B compared to MA (Table 2). Of the analysed metabolites 28/42 were detected at insignificant concentrations in the exudates (Supplement 1).

3.3. Cytotoxicity of the exudates

Cultured on MA + B, two different exudates from the same H3 mortar, sampled one week apart each other, were the most cytotoxic to BJ cells. The cytotoxicity of H3 exudates varied markedly (10% and 91%) as a function of time. The H2 droplets cultured on MA were not cytotoxic, but when cultured on MA + B, neutral red (NR) uptake increased to 29 ± 8%, indicating cellular stress (Mannerstrom et al., 2020). Droplet-producing strains, *Aspergillus calidoustus* and *Aspergillus versicolor* were used as reference in the cytotoxicity tests (Table 3).

3.4. The wall mortar samples studied in chambers

These chamber experiments aimed to determine whether VOC and non-volatile compounds were emitted from the school wall mortar into indoor air. The emission of VOC and non-volatiles from petri dish-cultured samples of the wall mortar, in response to RH %, was measured by sensors in a closed glass chamber. To simplify the plots, oxygen is not presented as it did not appear to play any role in these recordings. However, temperature results are shown, although these played a minor role.

By disrupting the healthy microbiota in buildings with substances that inhibit mitochondrial complex IV, slowly growing *aox*-fungi may gain a competitive advantage and proliferate. Therefore, mortars of the school's walls were tested for the presence of alternative oxidase (*aox*)-fungi. Mortar samples from the damaged school wall were added into glass chambers (840 ml) to mimic IAQ of building air, and to reveal how water vapour carriers mould-emitted exudates into air.

The goal for these studies was to show if use of experimental tools (NaHS, KCN) in the glass chamber can reveal the presence of *aox*-fungi in the mortar samples of the studied school. As shown earlier, VOCs migrate independently to the detection source, whereas non-volatile organic compounds require a water carrier for detection (Vaali et al., 2025). Since indoor RH % in Finland typically is round 40%, to see generation of VOC and non-volatiles from mortar samples, we added water to maintain RH at 70–80% inside the experimental chamber. Chamber was often opened for 1 h to release the produced gases, but closing the chamber revealed continued emission of VOC, non-volatiles and CO_2 as indication of alive moulds on the mortars. Neither sulphide (NaHS, 250–950 μg) nor cyanide (KCN, 450 μg) inhibited VOC and non-volatiles emission from samples H1 and H2 (Figs. 3A, B and 4),

proposing the presence of *aox*-fungi in the samples. Emissions of VOC and non-volatiles from H3 sample were not affected by sulphide, but when studied in the presence of high dose cyanide (1000 μg), VOC and non-volatile production was inhibited. However, we later discovered that H3 should had been studied for longer than 50 h, so the result was unclear (Fig. 5).

3.5. Droplet emission from the mortars studied in the chamber

Cultured mortar sample H2 produced one 9 μL droplet which was transferred to an empty petri dish on the bottom of the chamber. The droplet emitted VOC and non-volatiles reaching a plateau until the chamber was ventilated for 1 h, during which VOC and non-volatiles escaped into the laboratory air (Fig. 6). Addition of water elevated RH %, which was paralleled by production of VOC and non-volatiles. This supported our earlier result from H3 droplet (Vaali et al., 2025). The experiment mimics a situation when a classroom's ventilation is interrupted, leading to VOC and non-volatiles accumulation on the classroom's surfaces.

4. Discussion

4.1. Water vapour results and symptoms of the students

The present study indicates how mortar samples H1, H2 and H3 from the school wall; and the H2 exudate, emitted VOC and non-volatiles into air of the school (§1). These emissions were studied in glass chambers which performed an isolated environment and mimicked indoor air, this

Table 1
Symptoms reported by nine students from the damaged school (§1), and their subsequent schools. The schools from which there were symptom reports were indicated with yellow, when there were no reports (grey), and when the symptom reports were unavailable (red). Supplies, piano and furniture were transferred from the school (§1) to the school (§6), this action transformed the school (§6) into a potential source of mould.

Case	Years at School §1	Sex	Year born	Age (2020)	School year	Schools, numbered 1 to 6	Symptoms at the schools	Diagnosis
#1	6.5y.	F	2008	12 y.	Years 1-5 => May 2020	At the damaged school (§1)	Headaches, stomach and generalised pains, chronic recurrent cold, epistaxis	
					Year 6 Sept. 2020 =>	Reference school (§2)	No symptoms	
					Year 7 Sept. 2021 =>	Air purifier in use in the school (§3)	Suffered from headaches, stomach aches, nausea, chronic recurrent cold, epistaxis, concentration problems	
					Year 7 Dec. 2021 =>	Reference school (§4)	The symptoms ceased immediately, if certain lessons had been delivered in damaged classrooms, then symptoms reemerged.	
#2	4.5 y.	M	2010	9 y.	Years 1-4 => May 2020	At the damaged school (§1)	Migraine, stomach aches, vomiting, ended during summer vacation 2020*	Migraine, investigated at the Uni. Hospital, gastroscopies, antibiotics for parasites, medication resistance, many medications Referral for psychiatric evaluation No psychiatric disorder was detected
				10 y.	Year 5 Aug. 2020=>	Reference school (§2)	Symptoms connected to bus travel to school and remained unabated	
					Year 5	At school (§5), IAQ not reported	Profuse vomiting	
				11 y.	Year 6 Aug. 2021 =>	Air purifier in use in the school (§3)	Symptoms continued	
				11 y.	Year 6	Relocation to an annex (§6)	Symptoms continued	
				12 y.	Year 6 Dec. 2021=>	Reference school (§4)	The symptoms ceased on the first school day	
#3	3.5 y.	F	2011	9 y.	Years 1-2	At the damaged school (§1)	Chronic recurrent cold, epistaxis	
				10 y.	Year 3 Aug. 2021 =>	Relocation to the school's annex (§6)	Chronic recurrent cold, epistaxis	
				10 y.	Year 3 Dec. 2021	Reference school (§4)	No symptoms	
#4	1.5 y.	M	2013	7 y.	Year 1	At school's annex (§6), but lunch and sports were at the school (§1)	Severe epistaxis when attending school (§6) During exercise, upset stomach at school (§1)	
				8 y.	Year 2 Dec. 2021	School (§7)	No symptoms	
#5	2 y.	M	2009	9 y.	Years 1-2	At the damaged school (§1)	Stomach aches, headache, epistaxis, atopy, chronic recurrent cold	IBD, height growth 12 cm/6 month scoliosis, iron-deficient anaemia
				11 y.	Year 3 Sept. 2020 =>	Reference school (§8)	No symptoms	
				12 y.	Year 4 Aug. 2021 =>	At a damaged school (§9)	From day 1, headaches, otitis, epistaxis	
				12 y.	Year 4 Oct. 2021 =>	Separated from others to study in another space, not a school building (§10)	Disappearance of the symptoms from the 1st week	
#6	1 y.	F	2011	8 y.	Year 1	At the damaged school (§1)	Chronic recurrent cold, epistaxis, headaches	
				9 y.	Sept. 2020 =>	Reference school (§8)	No symptoms	

#7	4 y.	F	2009	10 y.	Years 1-4	At the damaged school (§1)	A series of epileptic seizures flashes of anger in school §1, eyeglasses 2019, weight increase 38 kg => 46 kg (in 6 months) In 2019 increase in BMI	Epilepsy, the seizures were drug resistant Serum sample was opalescent and white
				11 y.	Aug. 2021-	Reference school (§2)	No more need for eyeglasses	No symptoms nor epilepsy medication, 2023
#8	2 y.	M	2009	10 y.	Years 1-2	At the damaged school (1)	Epistaxis, abrasion of the fingertips	Hypermobile joints
				11 y.	Aug. 2020-2021	Reference school (§2)	No symptoms	
#9	4 y.	M	2009	10 y.	Years 1-4 Sept. 2019	At the damaged school (§1) At home	Epistaxis, headaches Liver biopsy was performed on 2/2020.	Increased serum liver enzyme ALAT 2400 IU/mL The liver was believed to be permanently damaged.
				11 y.	Sept. 2020 =>	From the school (§1) moved to the annex (§6)	Immediate symptoms as before.	
				11-12 y.	Jan. 2021 =>	Reference school (§2)	Symptoms declined gradually	
				13-14 y.	2022-2023	Reference school (§2)	No symptoms	Liver medication continued in Oct 2023. Prednisolone, ursochol, pantoprazole, vitamin D, calcium Confirmation by biopsy, recovery from the liver cirrhosis 1/2024

Please use the above description of the schools as in the legend.

(§1) A damaged elementary school
(§2) The reference elementary school
(§3) Upper grade school in the same county as (1)
(§4) An upper grade school
(§5) An upper grade school
(§6) An annex into which desks, books and piano were transferred from the school (§1).
(§7) An upper grade school
(§8) An elementary school
(§9) An upper grade school
(§10) A separate building, where the boy (#5) studied.

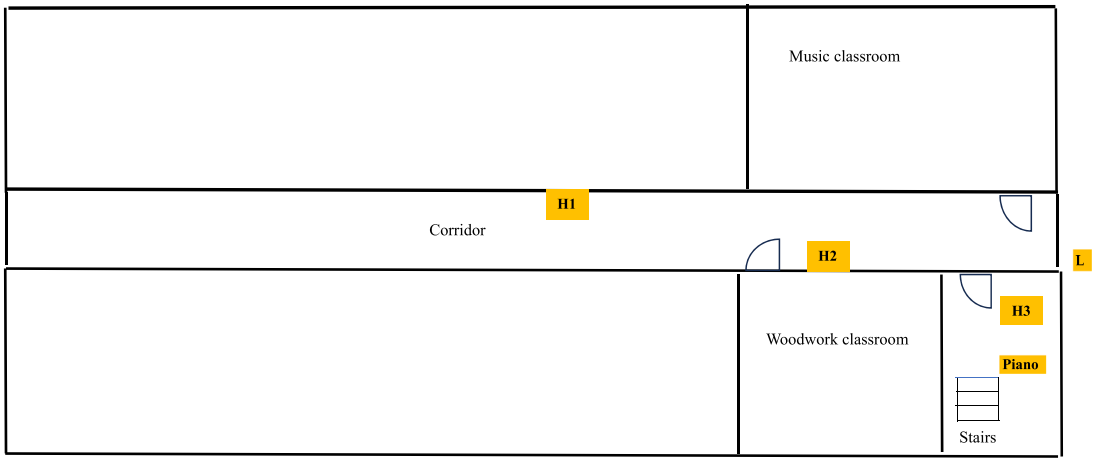


Fig. 2. Floorplan of the school (§1) at the basement level ("1st floor"). A leakage from a hot water pipe (L) had occurred at the end of the building. The place where the piano was located is marked ("Piano"). The swab specimen from the top surface of the piano caused 32% BJ cell death ($n = 6$), compared to the reference swab specimens 1% BJ cell death (n = 6), these results are from our earlier publication (Hyvonen et al., 2020). In the present study, the sample H1 was from the wall facing the kitchen corridor, H2 was from the woodwork classroom wall close to the skirting board. H3 was from the damaged site at the floor level of the staircase wall.

was a self-made method for studies of exposure in the building. Furthermore, LC-MS/MS analysis and human BJ cell test results, when put together confirmed that droplets emitted mycotoxins. The present study strongly support our earlier hypothesis that it is water vapour which carries mould-derived droplet into the inhaled air (Vaali et al., 2025), and we were able to follow up 9/47 of the severely diseased students who studied in 10 different school buildings. Their symptoms disappeared, when they moved to schools from which there was no reported IAQ problems, but their symptoms re-emerged, within a day, after they moved to school buildings from which there were reports of IAQ problems. The symptom reports showed how acutely people can identify toxicity of indoor air.

Our 2020 published study of students ($n = 47$) attending this damaged school (§1), reported severe symptoms, when compared to students ($n = 56$) from a school of a neighbouring small town (Hyvonen et al., 2020). Although no clear mould growth in the school building (§1)

Table 2
The building mortars, samples H2 and H3, produced secondary metabolites in exudate droplets when cultivated on malt agar (MA) and malt agar with boric acid (MA + B).

Row	Sample	Culture Medium	Source	*Pen andr. A*	*Pen andr. B*	*Pen andr. C*	*Pen andr. D*	*Pen / Fus chrysogin*	*Pen citr.hybr.*	*Pen citreoind.*
1	H2	MA	Lid	8800	34300	38000	4300	1500	1500	< LOD
2	H2	MA+B	Lid	**6300**	**23000**	**29700**	**4200**	**1800**	**800**	< LOD
3	H2	MA+B	Gr	**5800**	**23700**	**26400**	**4100**	**1400**	**500**	< LOD
4	H3	MA	Gr	15300	13300	15000	1300	400	6500	57800
5	H3	MA+B	Gr	23700	40000	45800	6600	2500	29500	36000
6	H3	MA+B	Lid rinse	1200	2600	2100	260	50	1600	2400

Row	Sample	Culture Medium	Source	*Pen meleagrin*	*Pen NP1243*	*Pen NP8689*	*Pen quinol. A*	*Pen roquef. C*	*Pen roquef. D*	*Trichod. trichodim.*
7	H2	MA	Lid	130800	11700	< LOD	< LOD	1500	100	300
8	H2	MA+B	Lid	**277900**	46000	< LOD	< LOD	**5300**	**200**	200
9	H2	MA+B	Gr	**263900**	29200	< LOD	< LOD	**7800**	**200**	300
10	H3	MA	Gr	218200	68100	79800	< LOD	1900	300	750
11	H3	MA+B	Gr	323900	47100	42800	9200	6700	1100	90
12	H3	MA+B	Lid rinse	63500	8200	32200	420	1900	400	400

The droplets harvested from the microbial colonies or from the lids were assayed for metabolites by LC-MS/MS (ng/mL). Results from colonies and the lids showed similar metabolite results (bold blue font, compare row 2 to 3, and 8 to 9). This demonstrates that the transfer of toxins occurs in liquid form and is mediated by water vapour. The presence of boric acid at least doubled the emission of Penicillium metabolites, which can be interpreted as evidence for the development of resistance (orange), but not in Trichoderma (green). With respect to the results of the H2 sample, row 1 should be compared to row 2, and 7 to 8, and for H3 sample row 4 to 5, and 10 to 11. Lid = liquid droplets accumulated on the inside surface of the petri dishes' coverlids, collected by micropipette and diluted in methanol. Gr = liquid exudate collected from the top of colony. Pen. = Penicillium, Fus. = Fusarium, andr. = andrastin, citr. hybr. = citreohybridinol, citreoind. = citreoindole, quinol. A = quinolactacin A, roquef. = roquefortine, Tricodim. = Trichodimerol, LOD = limit of detection.

Table 3
Samples of cultured wall mortars emitted exudates which were cytotoxic to human BJ cells.

Mould plate	Description of the sample	BJ cell death % (Decreased NR uptake)	BJ cell stress % (Increased NR uptake)
MA	*Aspergillus. calidoustus*, MH4	18.9 ± 1.8	No increase
MA + B	*Aspergillus versicolor,* GAS226	29.4 ± 0.9	No increase
MA + B	H3, collected 30th September 2020	91.3 ± 2.5	No increase
MA + B	H3, collected 7th October 2020	10.2 ± 0.7	No increase
MA + B	H2, collected 17th September 2020	No decrease	29.0 ± 8.0
MA	H2, collected 7th October 2020	No decrease	1.9 ± 2.7
Control	Glass tube without mould droplets, controls processed with ethanol evaporation	No decrease	0.0 ± 2.2

Exudates were collected at different time points and measured for cytotoxicity with human BJ cells by using neutral red (NR) uptake assay. The results are given as a percentage of BJ cell lethality (measured as decreased NR uptake due to BJ cell death). The increased incorporation of NR dye into lysosomes as compared to control is considered as a stress response caused e.g. by swelling of lysosomes. Controls were treated equally, with highly purified water. Exudates emitted by the cultured H3 mortar samples were the most cytotoxic, increasing cell death to 91%. The effect in H2 samples was mainly stress-inducing as shown by 29 ± 8% increase in NR uptake. *A calidoustus* MH4 and *A versicolor* GAS226 served as reference strains. Malt agar cultivation (MA), MA + 2000 mg/L of boric acid (MA + B).

was detected, seven samples of water vapour from the school (§1) showed marked death of human THP-1 cells (monocyte cells converted to macrophages) in culture (Hyvonen et al., 2020). The THP-1 test had been validated over four years with >3000 samples (University of Tampere, at an OECD-GLP accredited laboratory FICAM). The death of the THP-1 cells was based on a mitochondrial activity test of living, metabolically active human cells (Mannerstrom et al., 2020). Although mould-exposed individuals typically complain of repeated infections in the beginning of the sick building syndrome (SBS) (Valtonen, 2017; Maaranen et al., 2025), such acute reappearance of the symptoms in so many students cannot be of only infectious origin. Furthermore, their long-term and severe symptoms were individual (Table 1). These severe symptom reports resembles the Cleveland study, in which there was exposure to *Stachybotrys* (Etzel et al., 1998), which is known to produce toxic exudates (Gareis and Gottschalk, 2014).

4.2. Simulated building-derived toxicity in glass chambers from building materials

All these mortar-samples (H1,H2,H3) from the school walls, without culture, emitted VOC and non-volatiles, when studied in glass chambers (Figs. 3, 4, 5). The VOC and non-volatile emissions were not inhibited by the addition of NaHS supporting a vision that mortars did contain *aox*-fungi. Moreover, addition of cyanide (KCN, 450 μg) to the glass chamber of H1 or H2, only transiently inhibited VOC and non-volatiles production, suggesting that *aox*-fungi of the school's walls contributed to the toxicity of the indoor air. It is conceivable that the non-toxic moulds from mortar samples died due to the KCN, and the later appeared VOC

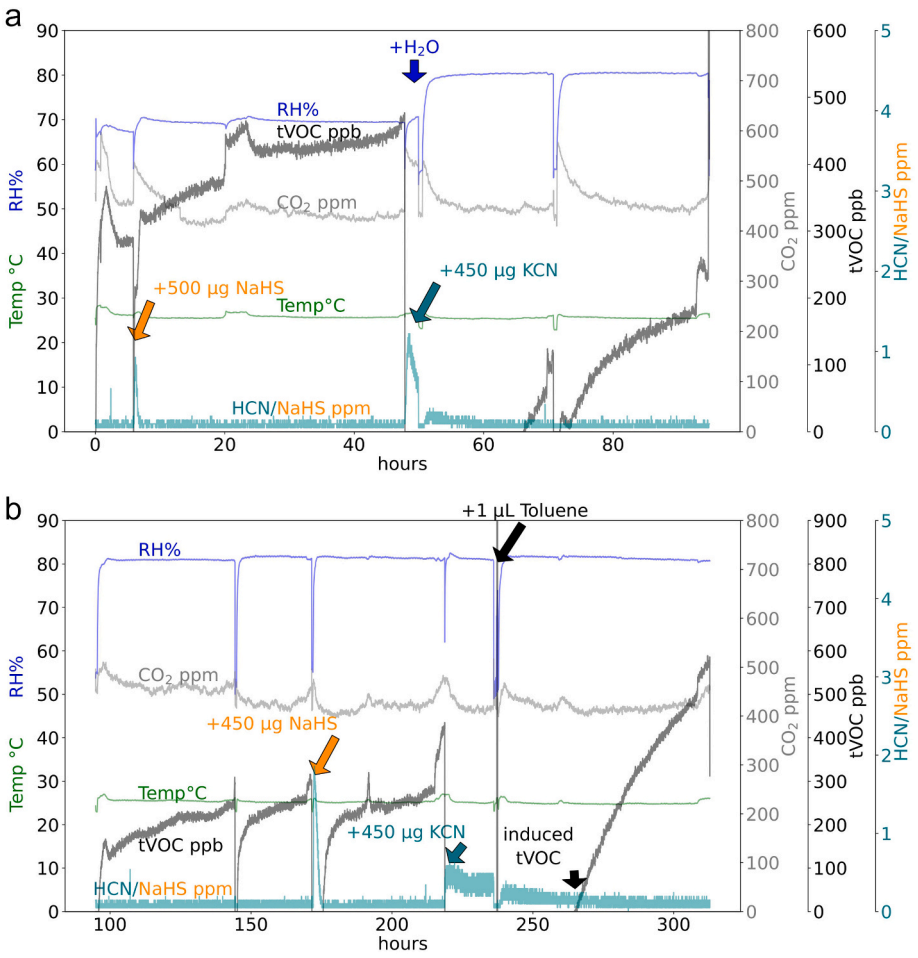


Fig. 3. The experimental data of H1 mortar sample from kitchen corridor, divided to two different figures for clarification. A) Measurement during the first 100 h. The microbiota of H1 mortar sample emitted VOC and non-volatiles in the chamber, and it was insensitive toward the first addition of 500 µg NaHS (at 6 h). However, 450 µg KCN (at 49 h) transiently inhibited VOC and non-volatiles emissions (between 49 and 67 h). The alternative oxidase (*aox*)-fungi are not sensitive to inhibition by sulphide or cyanide. B) Measurement during the last 100–300 h of the H1 sample. H1 continued to emit VOC and non-volatiles, and the second addition of 450 µg NaHS did not prevent VOC and non-volatile emission, rather at 170 h, emissions increased to 400 ppb. After the second addition of 450 µg KCN (at 220 h), emission was inhibited. At 235 h the functionality of the VOC sensor was tested and confirmed by 1 µL of toluene, which evaporated immediately. A strong reappearance of VOC and non-volatiles occurred after 270 h, as the *aox*-fungi became active.

and non-volatiles originated from the building-derived *aox*-fungi. The chamber analysis of mortar sample H3 should have been conducted over a longer period (more than two days), to fully assess the high impact of cyanide (1000 µg) on the metabolism given the slow growth of *aox*-fungi (Fig. 5).

Interestingly, based on our practical experience, there appears to be no upper limit to the amount of KCN that *aox*-fungi can tolerate; in the absence of functional complex IV, these fungi continue to grow. In Finland, school floors are washed daily with cleaning agents, which are left on surfaces without rinsing. Many modern cleaning and building products contain highly toxic chemicals, including biocides, which are documented in publicly available databases such as those maintained by the European Chemicals Agency (ECHA).

4.3. Secondary metabolites from exudates of the cultured mortars studied with LC-MS/MS

Mortars were sampled from the school walls (§1) and cultured on agar. The cultured moulds emitted exudates which accumulated on the inside surface of the culture lids and our LC-MS/MS analysis confirmed migration of the secondary metabolites from colonies to culture lids. Secondary metabolites (14/42) of the exudates were products of *Penicillium*, and one of *Trichoderma*. Earlier reports support these results: mould exudates, cultured from an office building, produced highly toxic *Penicillium expansum* (biomass 10–20 mg, ethanol extracted), was shown to be cytotoxic using porcine spermatozoa and kidney tubular epithelial cells (PK-15) (Salo et al., 2019). However, how exudates were transferred from colonies to culture lids had not been shown by the time. This is what we present in this study: it is the water vapour that carries toxic or non-toxic substances into air.

Cultured mortars (H2,H3) emitted exudates in the presence and absence of boric acid. Boron compounds are intended to prevent microbial growth in building materials. Boric acid promoted, rather than prevented, the emission of *Penicillium* metabolites in the exudate droplets isolated from mortar culture. Several mould strains have shown to be boron-tolerant (Andersson Aino et al., 2022; Mikkola et al., 2015; Vornanen-Winqvist et al., 2020). Therefore, boron compounds should not be used in building materials.

4.4. The cultured mould emitted toxic droplets

The mortar-originated moulds emitted droplets, the toxicity of which was assayed using human BJ cells. The H2 droplet, in the presence of boric acid, increased NR uptake and cell number by 29 ± 8% showing a sign of stress, an indication of future cell death (Table 3). On boron-containing agar, H3 droplets exhibited the highest cytotoxicity to BJ

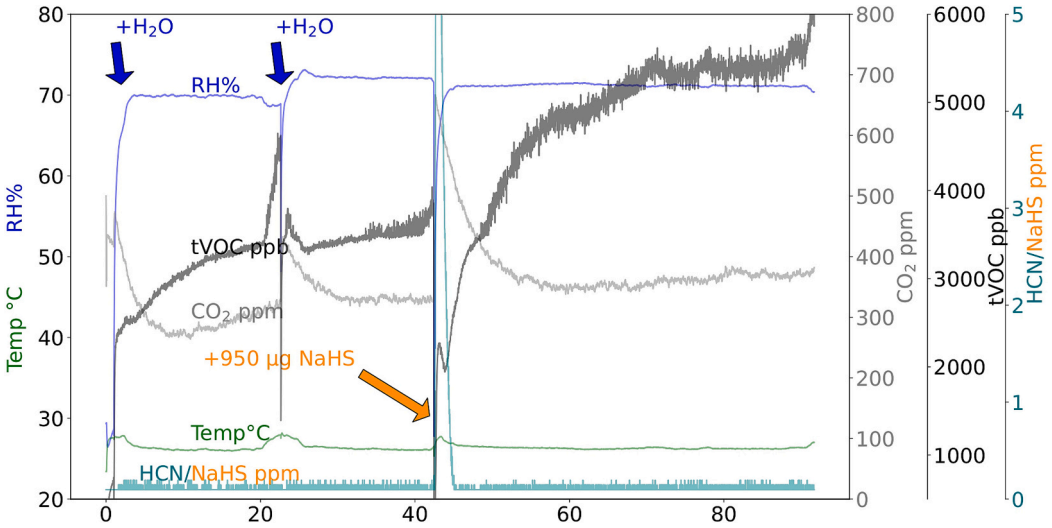


Fig. 4. The experimental data of H2 mortar sample from woodwork corridor emitted over 6000 ppb of VOC and non-volatiles. H2 mortar sample (5 g) at the very beginning of the measurement, RH% was ambient (30%). When the H2 mortar sample (5 g) was moistened with 1.0 mL of H_2O, the VOC and non-volatiles emission increased from 2600 to 6000 ppb. The temporary decline in airborne CO_2 was due to absorption by the alkalinity of the moistened mortar. The VOC and non-volatiles emission from mortar H2 was insensitive to added sulphide (950 µg/9.5 µL) but in another experiment, emissions were totally inhibited by cyanide (1900 µg/19 µL, not presented).

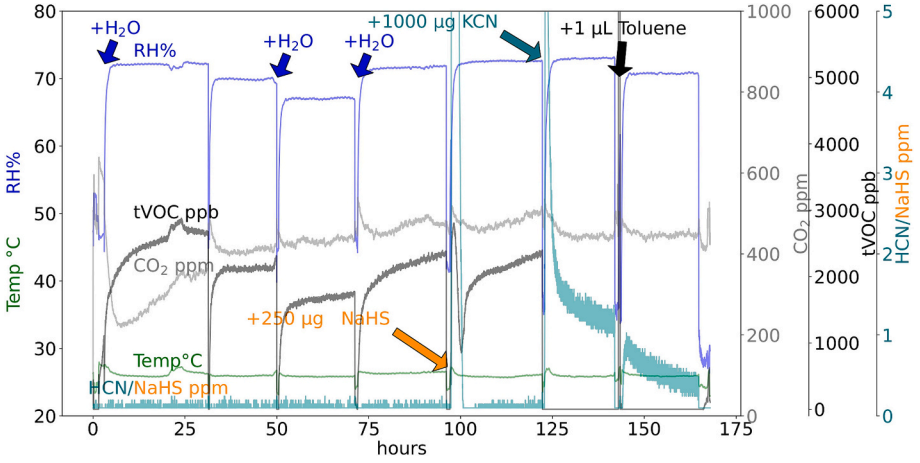


Fig. 5. The emission of VOC and non-volatiles from the H3 mortar followed relative humidity (RH %). The H3 mortar was sampled closest to the damaged site in the school building. Water (1.0–1.5 mL) was added repeatedly to restore the ventilation-related loss of moisture (3–50-72 h). VOC and non-volatiles emissions reflected the upward, downward and upward changes in RH %. H3 continued emitting in the presence of NaHS but this activity was inhibited by KCN (for the last 48 h). The VOC sensor was tested and confirmed by 1 µL of toluene.

cells, causing 91% and 10% cell death, the first sample was one week younger than the second. Consistent with this, Andersson et al. reported that fresh mould growth is more toxic than dormant or desiccated growth (Andersson et al., 2020). Furthermore, moulds excrete surfactants and enzymes which are needed for lowering the surface tension of water, for penetration through materials (Andersson et al., 2020). Interestingly freshly produced exudates have been reported to contain some 100 times more mycotoxins than the spores (Gareis and Gareis, 2007), even 20,000 times according to Salo et al. (2019). Actually, already in 2002 Gorny et al. reported that fungal fragments released from several well-known moulds were higher in numbers than spores (Gorny et al., 2002).

H1 culture emitted no droplets, it had been sampled from a site most distant from the structural damage in the building corridor (Fig. 2). H2 was taken from the middle of the corridor, H3 was closest to the damage site. As further evidence, in our earlier publication, a swab sample taken from the top of the piano—located at the damaged H3 site—was found to be the most cytotoxic to BJ cells (Hyvonen et al., 2020). The piano, schoolbooks and desks were later moved to the hut school (§6), where the students experienced symptoms (Table 1). It is important to show high level of secondary metabolites of mould exudates, but the final potential is evaluation of the cytotoxicity on human cells. In our recent publication of the same school (§1), the 50 µL droplet of the H3 sample from MA + B culture killed 75% of the BJ cells, emitting VOC and non-volatiles humid-dependently (Vaali et al., 2025). However, there is still some research puzzle in this area, since lower RH % has shown to be associated with higher concentrations of airborne fungal particles when mould cultures are exposed to airflow (Madsen, 2012). Other studies indicate that under wet conditions, fungal particles exhibit a higher total inflammatory potential than those released under dry conditions (Frankel et al., 2014).

Many compounds are in indoor air, but they are not detected. The content of the H2 droplet was not toxic according to cell tests but contained secondary metabolites according to LC-MS/MS analysis. We should understand that there can be a lot of various compounds in the air that do not necessarily have any impact on us. Water vapour mobilized

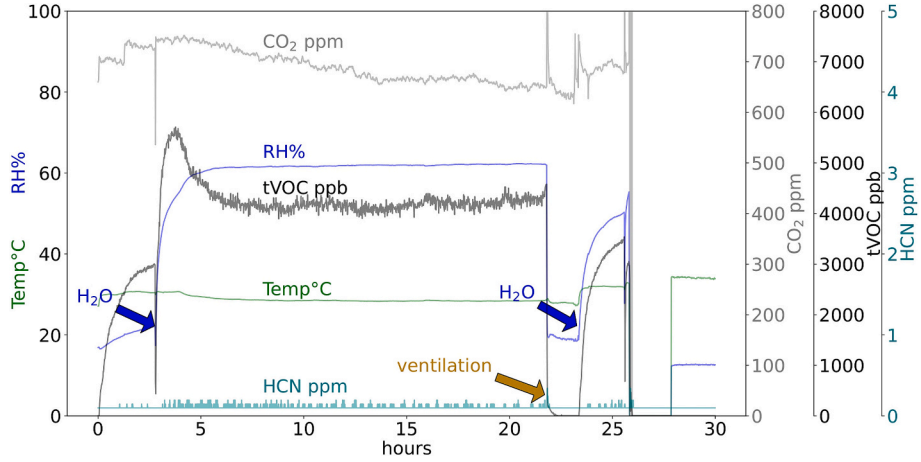


Fig. 6. H2-produced a droplet, which mobilized VOC and non-volatiles depending on RH %. The mortar sample H2 cultivated on malt agar containing boric acid (MA + B, 2000 mg/L), emitted a droplet. At 0 h, 0.5 mL H_2O was pipetted on one petri dish, and the 9 μL droplet of H2 on another in the same chamber. Water vapour (RH 60%) carried VOC and non-volatiles from the droplet into the chamber air. At 21 h, the chamber was ventilated for 1 h to allow the aerosolised emission to be released, and 0.5 mL of water was pipetted onto the bottom of the chamber. After closing the chamber, emissions from the droplet resumed until the recording ended. This experiment mimics a situation when a classroom's ventilation is interrupted during nights/weekends, deposition of VOC and non-volatiles on surfaces increases, and restarting ventilation releases a bolus dose of VOC and non-volatiles into air.

VOCs and non-volatiles from the H2 exudate droplet as far as 150 mm upwards (Fig. 6), in a humidity-dependent manner and without air turbulence. While the sensors are not molecule-specific, their readings are informative when combined with LC-MS/MS data. When the H2 droplet (9 μL) was dispensed into the chamber, this generated a plateau release of VOC and non-volatiles. If there is a problem with IAQ, the toxicity is collected to floors and tables over nights and weekends when the ventilation is halted. Restarting it in the mornings when people enter the building, will remobilise most of the toxic emission by moist air to inhalation. This school (§1) has since been demolished.

4.5. *Many toxic substances cannot be measured by gas chromatographical methods from indoor air*

The studied damaged school (§1) had previously been investigated by utilizing gas chromatographic methods, (according to the guidelines of the Finnish authorities, ISO16000-6, using TENAX TA adsorbent) (Standardization IOf, 2004). The assayed 2-ethyl-hexanol (2-EH) levels were 0.2–7.5 μg/m^3, while the action limit is 10 μg/m^3, and the highest measured tVOC concentration in one classroom was 200 μg/m^3, did not exceed 400 μg/m^3, the action limit specified by Finnish and German authorities (Fromme et al., 2019). Results of 9789 public buildings in Finland indicated that a mere 1% from the samples collected exceed this tVOC limit (Wallenius et al., 2022). It is worth noting that the US Model State Indoor Air Quality Act does not mandate precise levels for tVOC, as it is a challenging task to assess indoor air toxicity or safety (Gostin et al., 2023).

Most organic compounds cannot be in the gas phase due to being non-volatile at room temperature and because of their low vapour pressure. This fact is totally forgotten in most of the published studies concerning IAQ. Air samples are often collected with air pumps to resin-containing columns. These resins absorb liquids, to prevent liquid contamination of the gas chromatographic equipment. When followed by extraction for gas chromatography analyses, it is only the gaseous compounds which can become analysed, and therefore the compounds that are not in gas phase cannot be detected. In the present study, we used VOC sensors which may detect liquid emissions from non-harmful materials, which produce methodological difference between the sensors and gas chromatographic assays. We could not collect a sample inside the glass chamber into a gas column by a pump. To avoid external VOC sources, we used isolated, autoclaved and sealed chambers to avoid sources of errors in studies of VOC and non-volatiles, but the emissions could contain liquids, and the sensor are not able to differentiate toxic VOC from unharmful VOCs like from coniferous extracts.

In 2011, a humidifier disinfectant lung injury and high mortality rate after a chemical exposure occurred in South Korea (Lee et al., 2012). Polyhexamethylene guanidine chloride (PHMG, synthesized as a hyperdispersed mixture of polymers with molecular weights ranging from 500 to 6000 g/mol), and its derivative oligo(2-(2-ethoxy)-ethoxyethyl guanidine chloride (PGH), had been present in homes for several months in the humidifiers, with intention to prevent microbial growth. PHMG was not believed to enter to gas phase at room temperature due to the low volatility, so it's use in humidifiers was not prohibited. From another group of disinfection chemicals, a review described the incidence of lung disease caused by humidifier disinfectants, isothiazolinones (Song et al., 2022). We propose that the humidifiers generate water clusters, enabling the transfer of toxic chemicals into indoor air by water vapour. PHMG has been used in Finland especially for washing mouldy buildings, and although officially restricted by EU in 2013, these disinfectants can still be found in shops.

In our earlier publication four different compounds were shown to be found in water vapour when studied in glass chambers. In that study the RH % was increased up to 70–80% for longer time. The studied compounds were disinfectants (like PHMG, DDMAC), surfactant Genapol X080. Genapol is used to decrease the water tension, to help the wet concrete to dry, although the building is covered with tiles. Furthermore, a model compound for mycotoxins, 1-octen-3-ol was studied. All these pure compounds were shown to become airborne as the RH % increased. We believe that when the RH % becomes >70%, and as the time goes by, the clustered water can no longer lift heavier molecules up into air (Selkäinaho et al., 2018).

4.6. *Why settled dust samples are not sufficient for studies of IAQ*

In the school (§1), the numbers of several fungal species in the settled dust were low, ≤1 colony forming units (cfu), except for the wall material-derived *Aspergillus, Eurotium* species group (110 cfu/g) (Hyvonen et al., 2020). However, there are no official health-related threshold values for material-derived mould growths. If samples of settled dust after seven days culture are assayed in cfu, are not purified by re-culture, then quickly growing non-toxic fungi (five mitochondrial complexes) can overgrow the slowly growing *aox*-fungi (three mitochondrial complexes). It is important to note that the total number of cfu does not reveal cytotoxicity toward human cells, and we find that

calculation of the colony numbers will produce bias results. This is why high cfu results of the harmful moulds are typically missing from results, when the so called "indicator" moulds are hunted. Culture of such settled dust in commercial laboratories is typically performed in seven days, and commercial laboratories do not have time or capacity to isolate each primary cfu. It seems that the cytotoxicity, penetration capacity, and the freshness of the exudates (Andersson et al., 2020) are more important than the number of spores, suggesting that the function of spores is rather for spreading of the moulds.

Salin et al. published symptoms reported by teachers in 15 Finnish schools, when the cultured moulds were assayed from wiped indoor dust or by using microbial biomass, cultured on fallout plates (the settled dust method). The toxicity was measured using sperm motility inhibition assay, which correlated to symptom severity. The difficulty in these studies is that the samples needed to be taken from above lamps and cabinets that had not been cleaned 8–12 months earlier to be able to get enough material. Moreover, the settled dust needed to be cultured for 4–6 weeks (Salin et al., 2017). In this article when the samples EC_{50} toxicity result was $\leq$6 μg/ml, the number of building-related symptoms of the teachers was 2.8-fold higher, when compared to the results of $EC_{50} > 50$ μg/mL ($p < 0.001$). In a later article Salin et al. reported of symptoms, which were not earlier reported by others (Salin et al., 2021a). These correlated to the mould EC_{50} toxicity findings of the buildings, such as wet eyes (12.7), hypersensitivity to sound (7.9), difficulty falling asleep (7.6) and increased need for sleep (7.7) when evaluated with adjusted odds ratio values (Salin et al., 2021b). Our results from adults also support that the IAQ symptoms should not be limited to airways (Vaali et al., 2023, 2022b, 2022c). We suggest it is the genetics of the exposed which will importantly manifest the individual symptoms.

4.7. Limitations of the study

As a weakness, the number of samples in this retrospective study was limited. We could not receive water vapour samples from other schools than from the school (§1), so the IAQ of the other schools was based on students' reports. Due to the retrospective nature of the study, we could not repeat chamber experiments nor collect more exudate droplets. As the strength of our study, we could confirm various clinical symptoms in students from the same school building. However, although this report is limited in its control results, we have results from a lot of controls in our earlier studies from 688 buildings, 385 (e.g. 65% of the studied building samples) were without cytotoxicity, based on water vapour samples which were evaluated with the THP-1 method (Mannerstrom et al., 2020). Additionally, we could show that when water vapour was sampled in a classroom from incoming ventilation, it was non-toxic to THP-1 cells. However, water vapour was cytotoxic when sampled at the area of outgoing air, indicating that the room itself produced the cytotoxicity. Also at the same timepoint samples taken outdoors of the building were non-toxic (Vaali et al., 2023). We applied several in vitro methods, using cultured moulds from wall samples and we used glass chambers to measure emissions from the school mortar samples. We utilized an accurate, sensitive and highly selective LC-MS/MS-based analytical approach to confirm that the secondary metabolites of mould exudates could migrate into air and showed that the emitted droplets were cytotoxic to human BJ cells.

5. Conclusions of the present and previous studies of the school (§1)

The results from the present and previous publications can explicate the sick-building syndrome (SBS): 1) The exposed students ($n = 47$) experienced various severe symptoms in the school (§1). 2) School air was cytotoxic based on the results of water vapour and THP-1 cells and the swab sample taken from top of piano, from the same area as H3 sample, which was cytotoxic to BJ cells (Hyvonen et al., 2020). 3) We showed that exudate from H3 sample, closest to the damaged area, carried cytotoxic VOC and non-volatiles (Vaali et al., 2025). 4) In the present study the results from students ($n = 9$) of the school (§1) suffered from severe symptoms, and their symptoms could attenuate if they entered to schools from which there were no reports of IAQ problems but reappeared, within the same day, if there had been IAQ reports. Also, from the school (§1) we showed 5) evidence that droplets from cultured mortars emitted the same secondary metabolites which were found on top of the colonies and hanging from lids. 6) *aox*-fungi were found in mortar samples of the school (§1). 7) Our earlier studies in aquarium-sized chamber showed how known purified chemicals or mould secondary metabolites are carried by water vapour to the top of the chamber, and of these Ochratoxin A was shown in capillary electrophoretic method (Selkäinaho et al., 2018). 8) Water vapour collected from a campus mouldy building (29/32 symptomatic individuals) and control building (4/18 symptomatic individuals) were evaluated with human cell testing (THP-1) (Vaali et al., 2023) and showed a great difference in cytotoxicity of the water vapour. 9) In another study of six women, all became diseased in three months after moving to the new office. From this office 3/4 water vapour samples were cytotoxic (Vaali et al., 2022a). When put together, all these results support our original hypothesis that cytotoxic substances were carried by water vapour into indoor air (Vaali et al., 2025), representing the culprit for the symptoms. This innovation needed us to combine two unexpected factors together, which are: 1) Clustered water can mobilize compounds with much higher molecular weights than 18 g/mol of water. 2) Even hydrophobic molecules can be carried by water vapour when the hydrogen atoms point outward. Both findings were earlier explained by van Oss et al. (Van Oss et al., 2005).

The above listed factors address that water vapour is the most optimal sampling material for studies of IAQ, but although LC-MS/MS studies could show secondary metabolites, it is the cellular testing which can confirm indoor air cytotoxicity and additive effects of various toxic substances. We conclude that toxic secondary metabolites are transported by water vapour and contribute to the morbidity observed among the students. Given that moisture can act as an effective carrier of airborne substances, assessment of indoor air quality (IAQ) benefits from analyses that include the water vapour phase, combined with human cell viability assays. Hence, this presented approach has a great potential to improve the assessment and understanding of IAQ-related health effects.

This article has been written without the help of AI.

CRediT authorship contribution statement

Kirsi Vaali: Writing – original draft, Conceptualization. **Michael Sulyok:** Validation, Software, Methodology, Investigation, Formal analysis. **Jari Louhelainen:** Visualization, Software. **Marika Mannerström:** Writing – review & editing, Validation, Methodology, Investigation. **Tuula Heinonen:** Supervision, Project administration. **Rudolf Krska:** Writing – review & editing, Supervision, Project administration. **Leena A. Räsänen:** Writing – review & editing, Methodology, Investigation. **Arto Visala:** Resources, Project administration, Funding acquisition. **Panu Harmo:** Visualization, Software, Resources, Methodology. **Mirja Salkinoja-Salonen:** Writing – original draft, Methodology, Conceptualization.

Informed consent statement

All parents and students gave a written informed consent for this study.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board, the Ethical

Committee of Northern Ostrobothnia gave a recommendation to allow the search for biomarkers in mould-related disease among school-children (protocol code EETTMK 10/ 2020) to conduct this study.

Funding

The study was supported by TEKES (Finnish Funding Agency for Technology and Innovation), Finland.

Declaration of competing interest

K.V. is the main owner of SelexLab. One of the expertise of SelexLab is indoor air quality testing. In this study K.V.'s role is coordinating the study and being an author for writing this manuscript. L.A.R. has participated in writing, reviewing and editing of the manuscript, and has later got salary for analysis of water samples when using toxicity test with THP-1 cells. Other authors report no conflict of interests.

Acknowledgments

The authors thank all study participants. Building surveyor Elisa Aattela from SEA-IN, Tampere, Finland, is thanked for her help in the practical arrangements. Vesa Niiranen from Aalto University, Espoo, Finland, created the recording program and provided support with the chamber recordings. Leo Mustonen is thanked for his IT-help. Associate Prof. Tamara Tuuminen, PhD is acknowledged for advice on clinical interpretation of the presented cases. Associate Prof. Jaana Tyynelä, PhD is thanked for comments on the manuscript and Prof. Analytical Chemistry Heli Siren for her knowledge of chemistry. Emeritus lecturer Ewen MacDonald is thanked for his help with the paper's style and grammar. Graphic designer Rik Poppius from Poppius & Co, Helsinki, Finland, is thanked for illustration of the graphical abstract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2026.182020.

Data availability

The authors do not have permission to share data.

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