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MONITORING OF MICROCLIMATIC CONDITIONS AND THE OCCURRENCE OF MICROMYCETES IN CRAWL SPACE

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ABSTRACT

The risk of creating suitable microclimatic conditions for the growth and development of micromycetes in a crawl space is relatively high. Spores of micromycetes can infiltrate the living space through leaks in ceiling construction of the crawl space due to pressure conditions. The study is focused on monitoring microclimatic conditions and the occurrence of micromycetes in the crawl space in the Czech Republic. Samples were taken from the crawl space structures (ceiling structure and foundation walls) using sponges. Additionally, sedimentation methods were used to monitor the indoor microclimate (in the crawl space) and the outdoor microclimate. In the crawl space, spores of micromycetes of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Didymella*, *Epicoccum*, *Sarocladium*, *Sordaria* and *Penicillium* were detected. The relative humidity in the crawl space ranged from 50% to 95% during the model year. In total, 6,659 hours were spent in the crawl space with a relative humidity of about 75%.

Keywords: airborne fungi; crawl space; relative humidity; micromycetes.

INTRODUCTION

Crawl space foundation is based on the foundation of the building above an air cavity. The floor of the first floor is placed over a cavity, which is usually ventilated by outside air. The advantages of the crawl space include easier access to the plumbing and sanitary installations or the possibility to keep timber elements protected from splashing water. Additionally, crawl space also accommodates construction in floodplains (Werther and Winter, 2009). Despite its advantages, crawl space poses potential risks.The risks are related to thermal and humidity behaviour of the internal microclimate of the crawl space. The source of moisture can be incoming outdoor air or rising ground moisture in the crawl space (Viitanen *et al.*, 2010). Condensation can occur on the cooler surfaces of the crawl space structure especially during summer months when warmer outdoor air enters the crawl space. If the temperature difference between the exterior air and the crawl space (air, surface) is high, the crawl space becomes more problematic in terms of moisture conditions. A time lag is caused by the specific heat capacity of the foundations and soil, as temperatures slowly equalize between the outside and inside air in the crawl space (Matilainen and Kurnitski, 2003; Laukkarinen and Vinha, 2017; Airaksinen *et al.*, 2020).

Crawl space can be ventilated naturally or with a ventilation system. The ventilation system can be depressurised or a pressurised. The pressure difference between the indoor living space and the crawl space is usually up to 10 Pa (Keskikuru *et al.*, 2018; Salo *et al.*,

2018). The depressurization system is based on the principle of creating a slight depressurization in the crawl space compared to the outside environment. The depressurization of the crawl space causes increased flow of radon, flow of outside air or flow of ground moisture into the crawl space. Crawl space depressurization can reduce indoor radon from 70% to 96%. The pressurization system is based on the principle of drawing outside air into the crawl space. The flow of radon is restricted by pressurising the crawl space There is a risk of air infiltration from the crawl space through leaks in the ceiling structure into the living space with a pressurization system (Henschel, 1992; Keskikuru *et al*., 2018). Microbial contamination can enter the living space through leaks in the ceiling structure of the crawl space. These are, for example, spores of filamentous micromycetes (moulds) that are present in the air, in water, in soil or on the surfaces of animals. The study (Airaksinen *et al*., 2004a) focused on laboratory measurements of the transport of mould spores through a timber crawl space floor structure. The number of spores in the air is influenced by seasonality, with the highest incidence from May to September. Maximum exposure to fungal spores is observed in the summer and autumn in regions with a moderate climate. Airborne spores of *Alternaria*, *Cladosporium* and *Helminthosporium* are mainly observed in dry and summer days. Conidia of ascomycetes or basidiospores are released after rain and at night (Malíř and Ostrý, 2003). *Aspergillus*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Penicillium*, *Mucor*, *Rhizopus* and *Syncephalastrum* are commonly observed genera in the air (Yates *et al.*, 2016).

Air infiltration from the crawl space into the living space is influenced by the pressure conditions of the crawl and living spaces, the outdoor climatic conditions (wind speed, outdoor air temperature), and the airtightness of the ceiling structure. If the living space is ventilated by a mechanical unbalanced system (exhaust air is greater than supply air), a higher positive pressure differential across the crawl space ceiling structure can occur, i.e., depressurization of the living space takes place. The implication is an increased flow of microbial contaminants into the living space (Domhagen *et al*., 2021). The pressure difference can be high, from 5 to 10 Pa. The pressure difference between the living space and the crawl space is not as high (from 0 to 2 Pa) when the living space is ventilated with a mechanical balanced system (Airaksinen *et al*., 2004b). The issue of infiltration of contaminated air was discussed in a recent study (Domhagen *et al*., 2021), that investigated the concentration of microbial contaminants in school classrooms. The concentration of contaminants was highest in the class with low outside winds and moderate outside temperatures.

Some micromycetes can have a negative impact on human health, causing mycotic infections (mycoses), allergic reactions (mycoallergies), or mycotoxicosis. Mycotoxins, produced by certain fungi, can lead to a range of adverse health effects and pose a serious health risk (Kraft *et al*., 2021). Respiratory illnesses, conjunctivitis, or asthma can occur in humans in a mould contaminated environment (Kalhotka, 2014). Respiratory tract problems are often referred to as Sick Building Syndrome (*SBS*) (Crook and Burton, 2010). Volatile organic compounds (*VOCs*) can be produced by the decomposition of moulds. *VOC* serves as an indicator of mould activity and growth. *Aspergillus*, *Cladosporium* and *Penicillium* are strong *VOC* producers. High concentrations of VOCs (250 $\mu g/m^3$) can cause irritation of mucous membranes, eyes, ears, or headaches in humans. These substances can act as allergens. In addition to affecting human health, the presence of mould in the indoor environment also has a negative impact on building structures. Moulds can be involved in the biocorrosion of materials. Moulds also produce organic acids (e.g. oxalic acid, acetic acid, etc.) which can then react with the components of the building material and decompose them (Mihinová and Piecková, 2007; Kalhotka, 2014).

The infiltration of fungal spores depends on the pressure difference between the crawl space and the living space, on the size of the spores, and the degree of disturbance of the ceiling structure. The spore size of microscopic fungi ranges from 1 μm to 100 μm and depends on the species (Keskikuru *et al.*, 2018). According to Johansson *et al.*, (2005), in terms of risk for indoor environment contamination when pressurization of the crawl space occurs, this is classified in the range between medium and high category. All ventilation systems are included in this category (medium and high risk) because pressure conditions can be affected by wind.

The occurrence and development of microorganisms and the subsequent production of mycotoxins are influenced by environmental conditions such as humidity, temperature, substrate material, oxygen availability, sporulation and microbial interactions. Water is the main factor that influences mould growth. The factor that takes into account the availability of water for the growth of mould in the building material is called water activity a_w (Malíř and Ostrý, 2003; Yates *et al.*, 2016). The water activity can be expressed as equivalent to the equilibrium relative humidity (Yates *et al.*, 2016), (1).

$$a_w = \frac{equilibrium\ relative\ humidity}{100} \tag{1}$$

Equilibrium Relative Humidity (*ERH*), represents the equilibrium between the relative humidity of the surrounding air and the moisture content (*MC*) of a given material (Pasanen *et al.*, 2000). Pasanen *et al.* (2000) found in their study that mould, yeasts, and bacteria require an ERH of at least 90-95% for germination and growth. Regarding microorganism growth, wood is at risk when its moisture content (*MC*) exceeds 20%, and (*ERH*) is between 80-85% (Pasanen *et al.*, 2000). Malíř and Ostrý (2003) found that for optimal mould growth, the water activity (a_w) of the material should be in the range of 0.6 to 0.99, and the relative humidity should be maintained between 80-100%. The ideal air temperature for mould growth falls within the range of 18-28°C. Some moulds can grow even at temperatures ranging from 0°C to 60°C.

The study (Viitanen *et al.*, 2010) determined, that the critical ambient relative humidity for mould is 75% at temperatures between 0°C and 50°C. The critical relative humidity is 75-80% for wood and wood based materials. The critical relative humidity is 90-95% for concrete (Johansson *et al.*, 2005). Mould spore germination can take place at a relative humidity of 60%. Nevertheless, active growth is observed at relative humidity of 75-80%, accompanied by the production of CO_2. Peak mould growth occurs at relative humidity approaching 100%. However, growth typically decreases at a relative humidity of 100% due to the formation of a thin layer of water on the material surface, restricting the entry of oxygen, which is essential for mould growth (Balík, 2008).

Importance of Microclimate Stability

The duration of exposure to critical conditions is crucial (Sedlbauer, 2002; Isaksson *et al.*, 2010). Even a brief period of wetting is adequate for mould development and the release of spores. The microclimate layer aids mould in surviving low relative humidity conditions during humidity fluctuations This microclimate layer is located in close proximity to the construction's surface. A stable level of relative humidity is essential for mould development and growth. Mould growth on the surfaces of a structure occurs only when the relative humidity of the air and the relative humidity of the material are in equilibrium (Balík, 2008). The dew point temperature is an important factor in the moisture content of the building material. The dew point temperature can be determined from the Mollier

Hx diagram or by mathematical equations. There are several mathematical equations, for example equation (2) (Lawrence, 2005).

$$t_d = \frac{B_1\left[ln\left(\frac{RH}{100}\right) + \frac{A_1 t}{B_1 + t}\right]}{A_1 - ln\left(\frac{RH}{100}\right) - \frac{A_1 t}{B_1 + t}} \quad (2)$$

Where, *RH* is air relative humidity, factor A_1 = 17.625, B_1 = 243.04°C. Condensation occurs on the surface of a structure when the air temperature falls below the dew point temperature.

MATERIALS AND METHODS

In October 2022, surface and indoor microclimate monitoring of a crawl space was conducted on a timber house located in the Czech Republic. The house is a detached structure with a rectangular plan, measuring 10.680 × 6.740 meters. The crawl space is constructed using concrete blocks (breeze blocks) supported by foundation strips, with a 0.115 W/(m²K) *U*-value (heat thermal transmittance value) of the ceiling structure. The crawl space is divided into two tracts, namely a small tract with a floor area of 23.6 m² and a large tract with a floor area of 36.4 m². Both tracts have a height of 1.2 meters, and the bottom of the crawl space is situated approximately 750 mm below ground level. The bottom of the crawl space is covered with geotextiles and a layer of aggregate (size 16/32 mm). The layer of aggregate is 150 mm thick. Natural ventilation is provided to the crawl space through ventilation holes in the foundation walls, each hole measuring 500 × 250 mm.

Monitoring Parameters

Various parameters were continuously monitored at 15-minute intervals, including air temperature, relative humidity, airflow velocity, the surface temperature of the ceiling structure, and surface temperature of the crawl space bottom (approximately 50 mm below the bottom surface). For these measurements, Omega PLTH temperature and humidity sensors, as well as Omega PLTT temperature Almemo FVAD 35 hot-wire thermoanemometers, were employed in the crawl space. The outdoor climatic conditions were measured at approximately 700 m aerial distance from the monitored house by Mobile Alerts sensors. The outdoor temperature and humidity sensor was placed 1.25 m above the ground. Around the sensor was natural terrain - grass. The sensor was not exposed to direct sunlight. Small variations in outdoor conditions are expected, due to the location.

Microbial Analysis of Crawl Space Microclimate

The sedimentation method was used for the qualitative and quantitative determination of airborne microorganisms in the crawl space. This technique is based on the natural tendency of microorganisms to settle (sediment) on solid surfaces (Klánová, 2001; Klánová and Vrkoslavová, 2021). Petri dishes containing Plate Count Agar (PCA BIOKAR Diagnostics, France) and Chloramphenicol Glucose Agar (BIOKAR Diagnostics, France) were strategically placed within the crawl space tracts, ensuring a minimum distance of 100 mm and a maximum of 300 mm between the dishes Fig. 1. In the crawl space, the petri dishes were placed in the centre of the small tract (sample SP1) and in the centre of the large tract (sample SP2). Outdoors, the Petri dishes were placed approximately 2 m from the monitored house (sample EX). The duration of exposure in the absence of human presence was set at 1 × 30 minutes. Sampling was carried out on 10 October 2022. All

samples were placed inside a refrigerated box and transported to a laboratory (within 24 hours), maintaining a temperature range of 1-8°C. Petri dishes with PCA were incubated at 30°C for 72 hours. Petri dishes with Chloramphenicol Glucose Agar were incubated at 25°C for 5 days. The growth of microorganisms in the crawl space and in the outdoor environment was then visually compared.

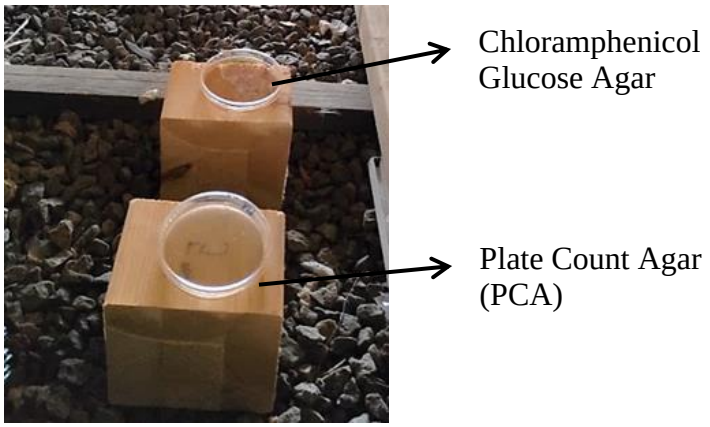


Fig. 1 The sedimentation method in the crawl space.

Microbial Analysis of Crawl Space Structures

The investigation of crawl space surfaces was carried out in accordance with ČSN EN ISO 18593 (2019). A systematic sampling network was established within the crawl space, with each sampling point covering an area of 100 cm². Six samples were collected on the crawl space ceiling structure made from fibreboard (samples b1 to b6) and one sample on the foundation concrete wall (sample c1). Sampling was performed using sponges moistened with a buffered peptone water solution (BIOING, SR18-10BPW-G). Fig. **2** to enhance adhesion to the surfaces to be analysed and to ensure optimal conditions for microbial detection. Samples were also taken on 10 October 2022.

a) b) c)

Fig. 2 a) Sponge, b) crawl space ceiling sampling template, c) crawl space foundation concrete wall.

These sponges were handled aseptically when removed from their protective packaging. The surface was sampled horizontally and vertically simultaneously with alternating sides of the sponge. Surface sampling was conducted by applying moderate pressure, and to prevent contamination, the protective bags were securely sealed. All samples were placed inside a refrigerated box and transported to a laboratory (within 24 hours), maintaining a temperature range of 1-8 °C.

The swab sponges were shaken on a STOMACHER homogeniser in sterile polyethylene bags with 40 ml sterile saline solution for 1 minute. Subsequently, 1 ml and 0.1 ml were inoculated onto the surface of Chloramphenicol Glucose Agar for the selective detection of yeasts and moulds and PCA for the enumeration of live, culturable heterotrophic microorganisms including bacteria, yeasts and moulds. Petri dishes with a diameter of 9 cm were inoculated in two replicates and then incubated at 30 °C for 72 h (Plate Count Agar) and at 25°C for 5 days (Chloramphenicol Glucose Agar). The number of microorganisms was expressed as colony forming units per 100 cm^2 (CFU/100 cm^2). For identification, morphologically distinguishable colonies of filamentous fungi were isolated and subcultured on Chloramphenicol Glucose Agar at 25°C to obtain an axenic culture.

Identification of fungal isolates

Representatives of visually distinct groups of filamentous fungi were selected for identification. Determination to the genus level was based upon macroscopic and microscopic morphological characteristics. Micromorphology was examined using the slide culture technique (Riddell, 1950) from seven-day-old cultures grown on Malt Extract Agar (MEA, Oxoid^{TM}) in the dark at 25 °C. Lactic acid was used as an observation medium. The microscopic slides were examined with an Olympus BX 53 light microscope and microphotographs were taken with an Olympus DP74 digital camera. Non-sporulating isolates producing only mycelium were cultivated on Soil Extract Agar, SEA (Crous, 2009) at 20°C, 12 h under near-UV light and 12 h in the dark for two weeks to stimulated sporulation. Macromorphology, which encompasses colony texture and pigmentation, type of aerial mycelium and presence of fungal structures such as ascomata, pycnidia, sclerotia or sporodochia, was assessed on seven-day-old cultures growing on Sabouraud Dextrose Agar (Oxoid^{TM}) at 25°C in the dark. A scheme of microbial analyses is shown in Fig. 3.

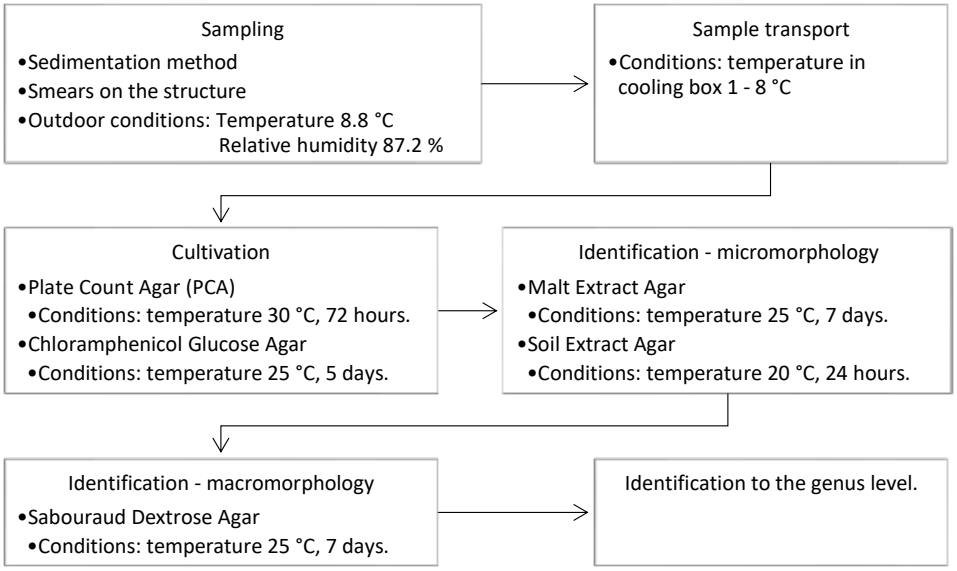


Fig. 3 Chart of microbial analysis of crawl space environment.

RESULTS AND DISCUSSION

A total of six sensors were employed to monitor temperature and relative humidity throughout the model year, spanning from April 2022 to April 2023, within the crawl space. The sensors were located on the ceiling structure of the crawl space. These sensors were

strategically placed on the ceiling structure of the crawl space, with sensors labeled 11, 12, and 13 situated in the small tract, and sensors 14, 15, and 16 positioned in the large tract. 24-hour averages of temperature and relative humidity were determined. 24-hour average temperature and relative humidity values were computed for each tract, revealing differences between the two. The following table, Tab. 1, presents the average values of air temperature and relative humidity within the crawl space during the model year where it is clear that the average temperature was lower in the small tract in contrast to the relative humidity which was higher.

Tab. 1 Avarage temperature a relative humidity in the crawl space for the model year.

	Spring		Summer		Autumn		Winter		Spring	
Start of season	20. 3. 2022		21.6.2022		23. 9. 2022		21. 12. 2022		20. 3. 2023	
	Date from 5.4.2022								Date to 30. 4.2023	
Tract	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
T (°C)	13.28	13.76	18.35	18.85	7.41	7.80	3.17	3.50	7.63	8.00
RH (%)	68.0	64.67	71.22	67.37	83.10	79.66	82.18	78.85	76.05	71.73
Exterior										
T (°C)	13.30		18.40		5.80		2.29		7.39	
RH (%)	73.07		76.31		91.69		86.78		79.02	

Air temperatures within both the small tract (sensors 11, 12, 13) and the large tract (sensors 14, 15, 16) exhibited minimal variance, as shown in Fig. 4. Across all sensors, the standard deviation of air temperature remained within 0.4 °C. Throughout the model year, the average air temperature was 10.5 °C (average value determined from all sensors).

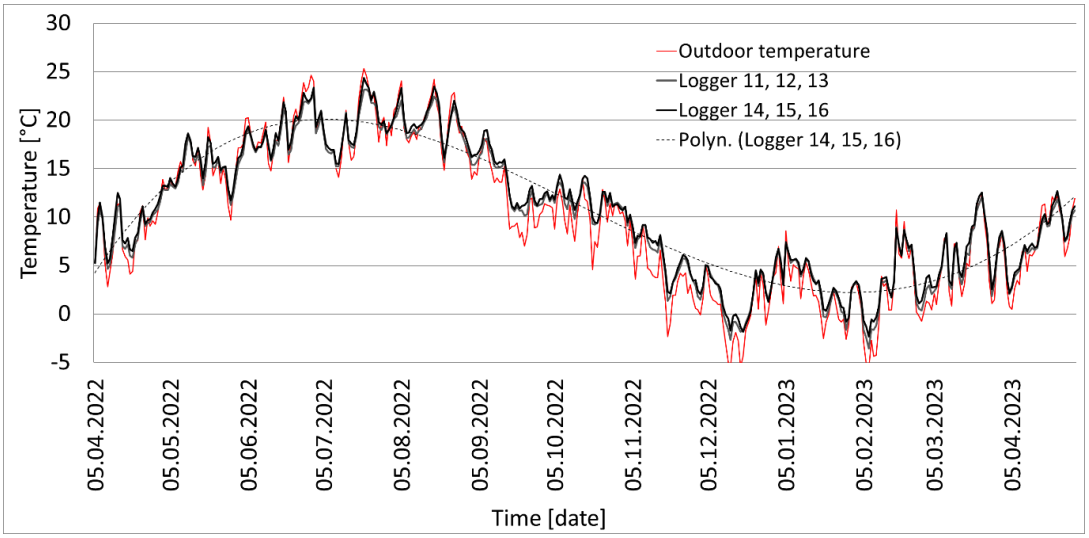


Fig. 4 Temperature in the crawl space, (24-hour average).

In contrast, relative humidity showed considerable fluctuations between the small and large tracts, as depicted in Fig. 5. Despite these variations, the standard deviation of relative humidity across all sensors remained within 4%. Sensors 12 and 15 were compared, showing a standard deviation of 5%. The relative humidity averaged 74.5% (average value determined from all sensors) within the crawl space.

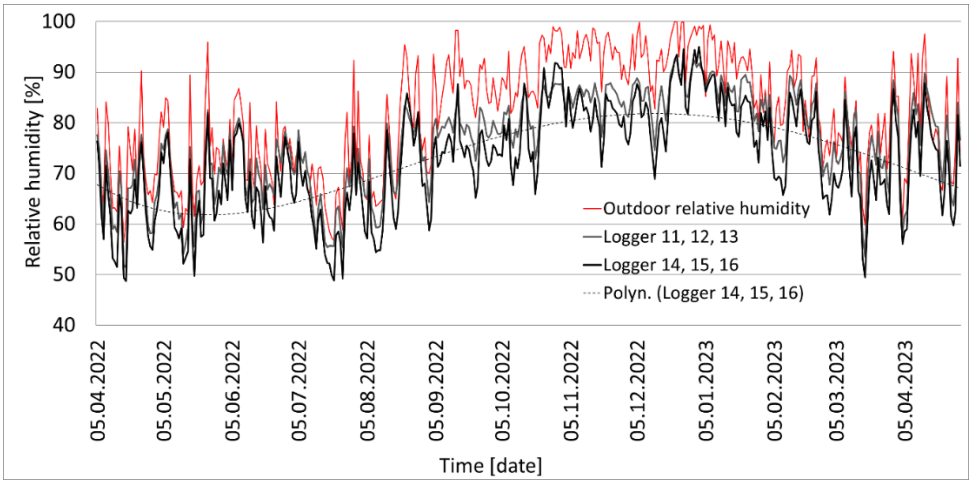


Fig. 5 Relative humidity in the crawl space, (24-hour average).

A comprehensive study conducted by Laukkarinen and Vinha (2017) focused on eleven temperature-related indicators describing microclimatic conditions in crawl spaces. These indicators included data such as the number of hours during which relative humidity exceeded 80% or 90% in the crawl space and the annual average temperature difference between outdoor air and crawl space air. The data from these indicators are summarized in Tab. 2. It is important to note that, as indicated by (Viitanen *et al.*, 2010) a critical threshold for mould growth is a relative humidity of 75%. Therefore, this value was taken into account and assessed. In total, 6,659 hours were spent in the crawl space with a relative humidity of about 75%. This was assessed using data from central sensors 12 and 15. Additionally, during the model year, the average crawl space temperature was 0.9°C higher than the average outdoor temperature.

Tab. 2 Microclimatic indicators and relative humidity in the crawl space during the model year.

	Number of hours in model year		Difference
	Small tract Logger 12	Large tract Logger 15	
$RH \geq 75\%$	**1,855**	1,550	305
$RH \geq 80\%$	**4,330**	2,097	2,233
$RH \geq 90\%$	305	**474**	169

These findings underscore the significant influence of relative humidity on crawl space conditions. Specifically, the crawl space frequently experienced relative humidity levels above 75%, providing favorable conditions for mould growth, as suggested by (Viitanen *et al.*, 2010). An interesting study by Risberger and Westerlund (2020) looked at monitoring the microclimate in a subarctic climate using a dehumidifier. There was no risk of mould growth when using a dehumidifier.

Dewpoint Temperature and Condensation Risk

The dewpoint temperature was calculated according to equation (*2*) and compared with the surface temperature of the crawl space ceiling structure, as illustrated in Fig. 6. While the dewpoint temperature remained below the surface temperature of the ceiling structure, it's important to note that these measurements were conducted in the large tract of the crawl space. Variations may exist in the smaller tract. Of particular concern was the month of January 2023, during which the air temperature was typically higher than the

dewpoint temperature in most measurements across the entire crawl space. Only one day in October 2022 and January 2023 witnessed the dewpoint temperature being higher than the air temperature in the crawl space, indicating a potential risk of condensation.

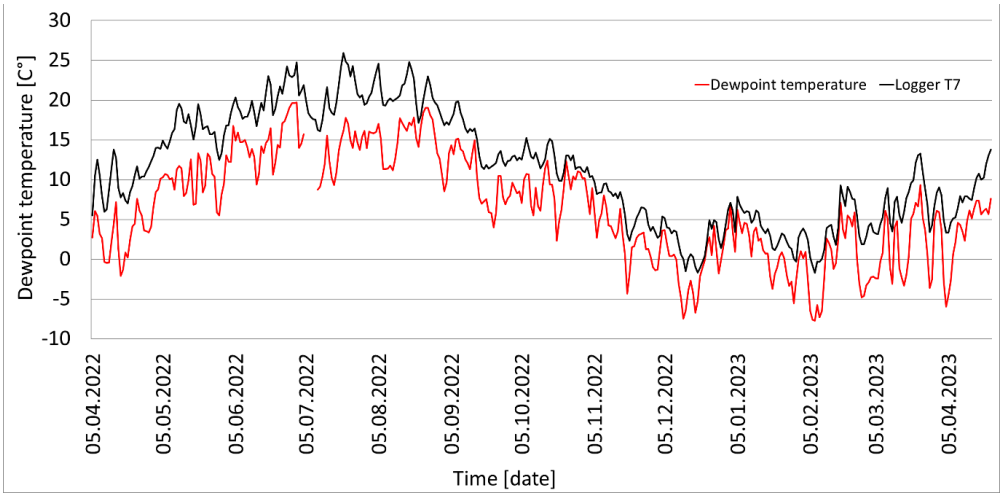


Fig. 6 Dewpoint outdoor temperature and surface temperature of the board sensor.

Microbial Analyses of Crawl Space Environment

The sedimentation method was employed to collect mould spores from the crawl space environment. Both the outdoor microclimate (EX) and the microclimate of the crawl space (SP1, SP2) showed a notable occurrence of mould spores (Fig. 7), which can be attributed to the crawl space's ventilation with untreated outside air. Consequently, the occurrence of mould spores in the exterior microclimate was comparable to that within the crawl space.

No visible growth of filamentous micromycetes was observed on the surfaces of the walls and ceiling structure of the crawl space. The number of moulds on the ceiling structure (fibreboard) was lower than on the concrete wall constructed from breeze blocks (sample c1). The greater porosity of concrete blocks, relative to fibreboards, provides a higher likelihood of spore entrapment. The highest number of mould spores was found in the corners of the crawl space ceiling, as shown in samples b1 and b6 (Fig. 7).

The enumeration results of the microorganisms from the swabs of crawl space (Tab. **3**) show that the highest number of microorganisms was found in sample b6, specifically 818 CFU/100 cm^2. The highest mould count of 109 CFU/100 cm^2 was found in samples b1 and b6, both of which located in the corners of the crawl space. It was also found that, the mould occurrence was higher in the small tract than in the large tract. No yeasts were detected in any sample.

Tab. 3 Numbers of colony forming units (CFU/100 cm^2) of bacteria and moulds isolated from the crawl spaces swabs cultivated on Plate Count Agar.

Marking of sampling points in the crawl space	CFU/100 cm^2		
	Bacteria	Moulds	Total
b1	400	109	509
b2	520	80	600
b3	20	40	60
b4	419	36	455
b5	180	60	240
b6	709	109	818

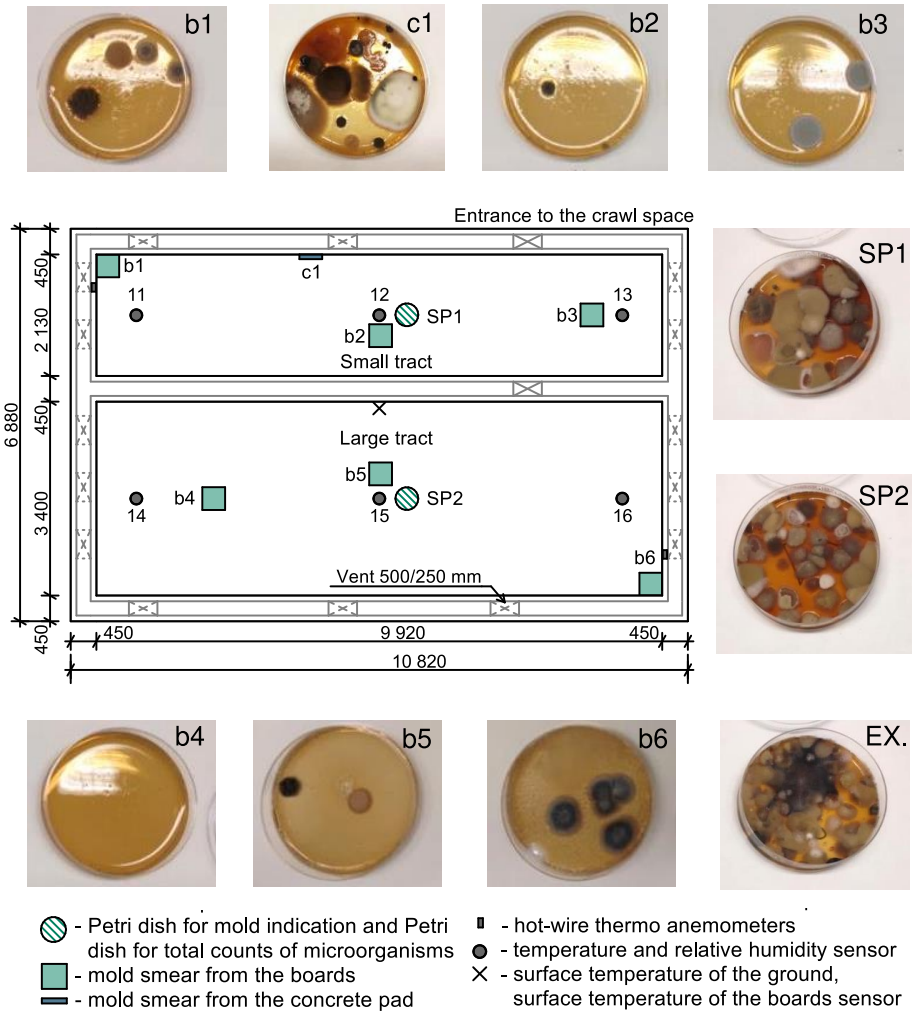


Fig. 7 Results of isolation of fungal community from the crawl space environment, medium Chloramphenicol Glucose Agar.

A total of 19 isolates of microscopic fungi visually representing different groups, were selected for identification. Of these, 17 isolates of filamentous micromycetes were successfully identified in eight genera (Fig. 8). Two isolates could not be assigned to any genus due to the lack of morphological characters. *Penicillium*, *Alternaria*, and *Aspergillus* appeared as dominant genera both in the microclimate crawl space and in the outdoor environment (Fig. 9). The genus *Penicillium* was observed on the ceiling structure of the crawl space and also on the concrete wall.

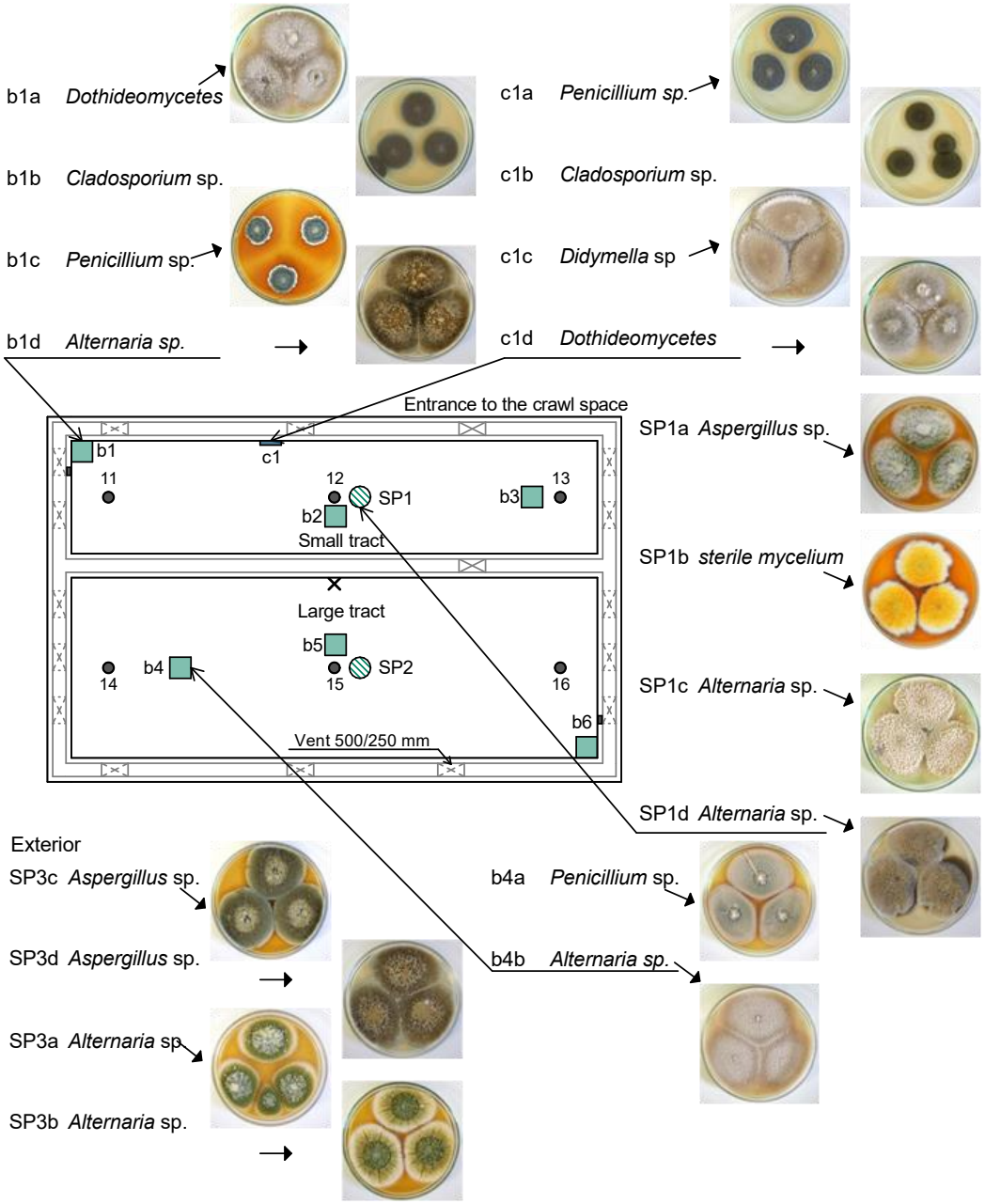
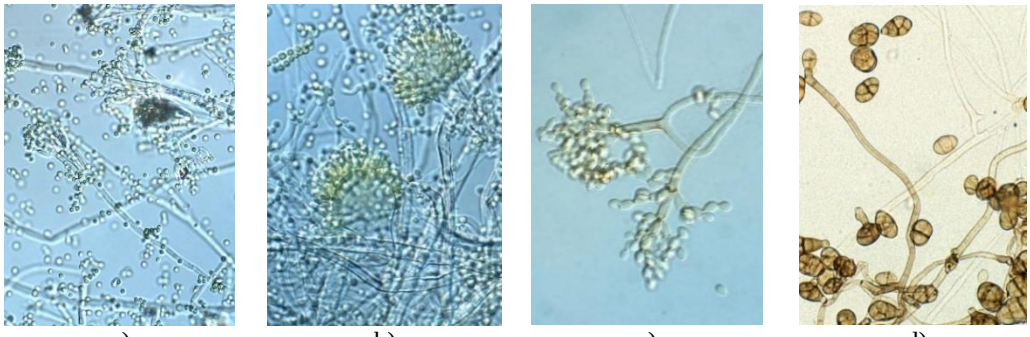


Fig. 8 Results of identification of selected isolates.



a) b) c) d)

Fig. 9 Microscopic morphology a) *Penicillium* sp. b) *Aspergillus* sp. c) *Cladosporium* sp, d) *Alternaria* sp.

The genus *Penicillium* is a typical representative of the moulds that occur in crawl spaces. Additionally, genera such as *Aspergillus* or *Cladosporium* are present. *Penicillium* and *Aspergillus* produce smaller spores that are more easily released into the air than *Cladosporium*. This is one of the reasons why *Penicillium* is the most common indoor contaminant (Airaksinen *et al*., 2004b). The occurrence of the genus *Penicillium* is also reported in a study (Bok *et al*., 2009), in which 212 crawl spaces were analysed for microorganisms. The dominant genus in these spaces was *Penicillium corylophilum*. Another study (Airaksinen *et al*., 2004b) investigated the contamination of the indoor environment caused by mechanical ventilation of the interior. Species of the genera *Penicillium*, *Aspergillus*, *Cladosporium* and *Acremonium* were most frequently identified. The highest concentration of mould spores was observed in summer (Airaksinen *et al*., 2004, b). The study (Hyvärinen *et al*., 2002) investigated the occurrence of mould on various materials, e.g. fibreboards, wooden boards, plasterboards, ceramic tiles and concrete elements. *Penicillium* was the dominant genus on the observed materials (Hyvärinen *et al*., 2002).

In this study, the high prevalence of *Penicillium* and *Aspergillus* in the crawl space was remarkable. Many species of the genera mentioned are marginally xerophilic or xerophilic, allowing them to thrive in drier conditions. Together with the ability to reproduce rapidly asexually they are more competitive in this environment (Pitt, 1999; Bok *et al*., 2009). The presence of these moulds can be attributed to the relatively dry conditions in the crawl space and the limited competition from other mould species (Bok *et al*., 2009). *Cladosporium* and *Alternaria*, on the other hand, tend to occur on damp cellar walls (Kalhotka, 2014). The ability of moulds to reproduce plays an important role in their spread and colonisation of different substrates, as not all spores released survive and germinate under unfavourable conditions. Some fungi avoid this risk by only producing spores when they have optimal conditions for germination and reproduction. However, other fungi, such as *Penicillium* and *Aspergillus*, produce dormant spores that germinate immediately when environmental conditions become favourable. Increase water availability is often sufficient for the transition of dormant spores to the metabolically active state (Mysyakina *et al*., 2016; Zabel and Morrell, 2020).

Moulds require suitable conditions for their growth and development, with relative humidity and air temperature being crucial factors. Typically, active mould growth occurs at a relative humidity of 75-80%, with an optimal air temperature range of 18-28°C (Malíř and Ostrý, 2003; Balík, 2008). The air temperature at the time of sampling was 8.8°C and relative humidity was 87.2%. Due to the lower-than-optimal temperature, no visible mould growth was observed on the crawl space structures, although mould appeared in the form of spores from various genera. These genera of saprotrophic fungi are commonly found in the air and soil.

Crawl space microclimatic conditions vary considerably throughout the year, impacting mould growth and development. The stability of relative humidity is important for mould production, as growth occurs when equilibrium between the air and material's relative humidity is achieved (Balík, 2008). Mould spores can infiltrate living spaces through windows during ventilation. Changes in pressure conditions within the crawl space, such as overpressure, pose a potential risk of contaminated air entering living spaces. Pressure conditions can be altered by wind flow around the house (Johansson *et al*., 2005). Visible mould growth indicates a higher concentration of spores in the air, potentially increasing the risk of contamination in living spaces.

CONCLUSION

In this study, the occurrence of moulds in the crawl space was closely monitored during the month of October in the Czech Republic. Additionally, the microclimatic conditions both outdoors and in the crawl space were thoroughly documented. Over 474 hours were recorded with relative humidity exceeding 90% in the crawl space. The relative humidity above 75% persisted for a total of 6,659 hours within the crawl space during the measurements. The dewpoint temperature remained lower than the surface temperature of the crawl space ceiling structure, especially in the larger tract.

Mould spores of various genera, including *Alternaria*, *Aspergillus*, *Cladosporium*, *Didymella*, *Epicoccum*, *Sarocladium*, *Sordaria* and *Penicillium* were identified on the surfaces of the crawl space. The genus *Penicillium* was found on both the ceiling structure (fibreboard) and the foundation wall (concrete blocks). No visible mould growth was observed on the surfaces of the walls and ceiling structure in the crawl spaces. *Penicillium*, *Aspergillus*, *Alternaria*, and *Cladosporium* were particularly dominant in the crawl space and outdoor environment, with *Penicillium* and *Aspergillus* being more abundant due to their smaller spores and adaptability to dry conditions. The results of this study highlight the importance of controlling relative humidity in crawl spaces to prevent mould growth and the potential health risks associated with it.

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