Research article

Airborne pollen and fungi indoors: Evidence from primary schools in Lithuania

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ABSTRACT

The number of children suffering from respiratory allergies and asthma has been increasing worldwide and, hence, it is crucial to understand the burden of inhalant biological particles present in school facilities, where children spend one third of their life. From the perspective of indoor air quality, while there are numerous studies on outdoor bioaerosol exposure, there are still uncertainties regarding the diversity and deposition of airborne pollen and fungi indoors. When it comes to schools, there is limited research as to the potential bioaerosol exposure. Here we studied the indoor environment of public schools aiming to reveal whether primary schools of different sizes and at localities of different levels of urbanization may exhibit a variability in the biodiversity and abundance of particles of biological origin, which could pose a risk to child health. To achieve this, 11 schools were selected, located in a variety of environments, from downtown, to city centre-periphery, and to the suburbs. Fungal and pollen samples were collected from various surfaces in school classrooms and corridors, using passive air sampling and swab sampling. We demonstrated that fungi and pollen are detected in school premises during and after the vegetation season. The highest diversity of bioaerosols was found on the top of cabinets and windowsills, with *Penicillium*, *Cladosporium* and *Acremonium* being the most abundant indoors. The levels of fungi were higher in schools with more students. The diversity and amount of pollen in the spring were significantly higher than in samples collected in autumn. Our findings complemented existing evidence that bioaerosol measurements in schools (including kindergartens or informal education facilities) are vital. Hence, we here suggest that, in addition to monitoring air quality and bacterial levels indoors, fungi and pollen measurements have to be integrated in the existing regular biomonitoring campaigns so as to prevent exposure, increase awareness and manage efficiently allergic symptomatology.

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1. Introduction

Air quality in a primary school classroom is one of the key environmental factors affecting children's health [1,2], their learning outcomes [3] and behaviour [4,5]. Innovative learning tools and other attractive technologies for indoor activities reduce motivation to be outdoors. Children spend 90% of their time indoors with a large portion at school (65% of the indoor time is spent at home) [6]. That is just one of the reasons why ensuring the air quality of school premises is a task that requires a modern approach. Indoor air quality can be measured by different types and combinations of numerous pollutants originating from a wide spectrum of pollution sources, but usually, focus on gases, particulate matter, formaldehyde, and volatile organic compounds. Following the current scientific evidence, the World Health Organization (WHO) emphasizes the worldwide need to reduce the anthropogenic load of particulate matter, ozone, nitrogen dioxide, sulphur dioxide, and carbon monoxide in both outdoor and indoor environments [7]. Controlling the growth of air pollutants concentration indirectly contributes to reducing the burden of bioaerosol, such as bacterial endotoxins and allergens of pollen, mites, cockroach, and fungi [8–10]. The fungal spores and anemophilous plant pollen contain proteins (allergens) and in specific circumstances exude them to air. Inhalation of air containing allergenic proteins worsens the symptoms of allergic rhinitis and asthma in susceptible individuals [11,12]. Children are significantly affected by the impact of the environment due to biological immaturity, and ongoing prenatal and postnatal lung development, hence, health professionals should increase their role in managing the exposure of children [13]. There is clear evidence that sensitization does not occur, or symptoms are significantly reduced with the absence of exposure to pollen or spore allergens [14]. The main source of pollen and fungal spores is outdoor environment, and their concentration has strong relationship with local vegetation season. The presence of ornamental vegetation in the area surrounding the building affects the indoor pollen levels [15]. It is recommended to regulate the diversity and abundance of allergenic plants in public green spaces to reduce the number of plants that spread allergenic pollen [16,17].

Staying indoors is one of the several remedies offered [18] to reduce the exposure of inhaled seasonal allergens but does not grant the absence of pollen or the total avoidance of symptoms [19]. Numerous studies have shown that both pollen and moulds are found in living, working, or school environments. Indoor particles' concentration commonly is lower than outdoors [20]. For the fungal spores, indoor load, age of buildings, area of classrooms, temperature, humidity are highly important [21]. Exposure during the plant flowering period was usually at a level that barely induced a reaction even in the most sensitive persons [22]. The main entrance points of airborne biological particles to the indoor environment are mainly considered to be the doors and the windows, as well as the air-conditioning system [15]. Using DNA metabarcoding, it was shown that substantial amounts of pollen produced in summer enter buildings and stay there throughout the year [23]. The main indoor air pollutant sources in school classrooms are related to occupancy and settled dust [24]. Prior exposure to home indoor allergens may exacerbate existing asthma [25], and activities of asthmatic children could be further complicated in the allergen-rich school premises. Indoor bioaerosol studies of different contexts and obtained data evaluation still do not provide answers to issues related to the role of allergenic pollen in indoor health, nor specific measures to reduce indoor pollen levels [10].
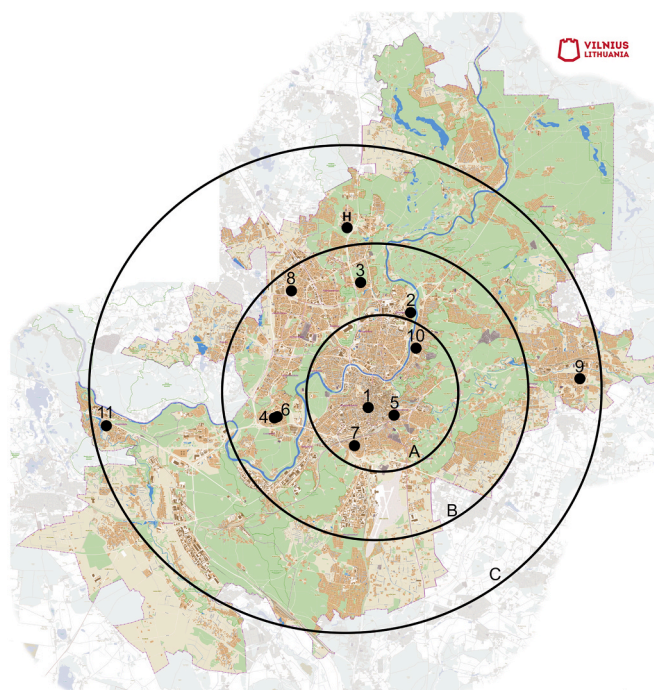

Fig. 1. Map of Vilnius with the indicated indoor and outdoor sampling points. The circles separate areas according to the level of urbanization: A – downtown, B – centre-periphery, C – suburb. Schools are marked with numbers. H – Hirst-type pollen trap for pollen observation in ambient air.

We studied the indoor environment of primary schools aiming to (1) assess airborne fungi and pollen diversity, (2) identify places in schools with the most abundant accumulation of airborne fungi and pollen, (3) comprehend issues related to the dynamics of airborne fungi and pollen indoors, potentially setting the basis for the definition of appropriate exposure criteria in the future. Such comprehensive information on the abundance and seasonality characteristics of pollen and fungal exposure levels in school buildings may be useful for health professionals in the maintenance of indoor air quality in public institutions. In terms of urban planning and greenery, we also expect that such information could be a useful guideline for landscape architects and ornamental gardeners when designing and deciding the plant species to be included (or not) in public spaces. The integration of relevant information as well as the cooperation among policy and decision makers across different scientific disciplines will make feasible the standardisation, evaluation and development of related guidelines for a healthier school environment, indoors and outdoors.

2. Materials and methods

2.1. Study area

Samples of airborne fungi and pollen were collected in 11 primary schools (Fig. 1) of Vilnius, Lithuania.

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The schools [26] differ in the number of students, location in the city and composition of the surrounding greenery. For investigation purposes, schools were divided into three groups depending on the urbanity in the surrounding area in Vilnius: downtown (A) – located in the city centre, centre-periphery (B) – located on the outskirts of the city, suburb (C) – located outside the city. The second classification criterion is the number of students in the school: <250 students, 250–400 students and >400 students.

According to the number of students, schools were grouped into small (schools indicated in Fig. 1 as 6, 7, 11), medium (2, 4, 5, 8 and 9) and large (1, 3 and 10). During the air sampling period, the same students studied in the classroom, but students of different classrooms walked the corridors. The age of children studying in the classrooms was from 7 to 11 years old. Schools were built more than 30 years ago, but most have been renovated, except second and fourth. All schools have replaced old wooden windows with plastic ones. Artificial ventilation systems were not operating within classrooms where samples were collected. The rooms were naturally ventilated by opening the windows in any period of the year. The maintenance and cleaning of schools took place in the usual mode throughout the study period. After school hours, the school facilities are cleaned (dust removed, floors washed daily), and general cleaning and disinfection are performed twice a year.

An overview of greenery was carried out in the environment of the schools selected for the study. This information is used for the causality analysis of indoor pollen morphotypes and abundance. Because in aerobiological studies pollen is traditionally identified by genus, the vegetation around schools has been registered at the same taxonomic level. In the greenery of school territory, the dominant plant was identified for the aim to perform statistical analysis between dominant (>50% tree coverage in greenery) plants and pollen diversity indoors. More data and information about diversity and dominant plants in the environment of schools are included in Supplement Table S1.

2.2. Sampling of airborne fungi and pollen

The sampling campaign was continuous – without any gaps – and took place in November–December 2020 (hereafter indicated as the autumn-winter period) and May–June 2021 (hereafter indicated as the spring period), given also the unavoidable limitations because of the COVID-19 pandemic and the continuous lockdown over 2020, as well as the closure of schools during summers. Both fungi and pollen samples were collected on various surfaces (Table 1) using two different sampling methods: passive air sampling (gravimetrical) and swab (dust) sampling.

In this study, we evaluated 1935 samples. Data on mould diversity was obtained by analysing 366 passive air samples and 738 swab

Table 1

Surfaces of objects from where samples of fungi and pollen were collected. Collection places are marked with squares: ■ – classroom, □ – corridor. No labelling signifies that no samples were collected.

Surface	November–December 2020				May–June 2021			
	Fungi		Pollen		Fungi		Pollen	
	Passive	Swab	Passive	Swab	Passive	Swab	Passive	Swab
Bench					□	□		□
Desks (teacher's)	■	■	■	■	■	■	■	■
Desks (student's)	■				■			
Floor	□	■ □		■ □	□	■ □		■ □
Table	□	□			□	□		□
Top of cabinets	■ □	■ □	■	■ □	■ □	■ □	■	■ □
Ventilation		■ □	■			■ □		■ □
Windowsills	■ □	■ □	■	■ □	■ □	□	■	□

samples. Data on pollen diversity were registered after analysis of 179 passive air samples and 652 swab samples.

2.2.1. Sampling of airborne fungi

Samples for indoor environment contamination with airborne fungi were collected and analysed in accordance with the requirements and recommendations of the European standard EN 17141:2020 [27]. The passive sampling was realised by the settle plate method. 90 mm Petri dishes containing culture medium (MERCK KGaA, Germany) of potato dextrose agar (PDA) with citric acid and Triton X additive were open for 10 min. Collection of airborne fungi on cultivation media was implemented in (1) classrooms placing Petri dishes on the floor, teacher table, children's table, and on the top of the cabinet and (2) corridor: on the floor, table, windowsill, and on the top of the cabinet. In total 1104 samples were analysed in this study.

The sterile pre-moistened cotton-tipped sticks were applied to collect samples from different surfaces. After swabbing 10 cm^2 of the selected target place, immediately inoculated on Petri dishes containing PDA media with citric acid and Triton X additive. Using swabs, samples were collected in classrooms from the floor, teacher table, top of the cabinet, ventilation hole and corridors from the floor, table or bench, windowsill, and top of the cabinet. Petri dishes with samples were taped and stored at 4 °C (using a portable box) when transported to a laboratory. The Petri dishes were incubated at 23 ± 1 °C for 4–7 days. To ascertain that the medium was not contaminated during the preparation process, at least one Petri dish per school was unexposed and used as a control. Results of the settle plate method were expressed as the number of colony-formed units (CFU) per plate per hour, abbreviated as CFU/dm^2/h: CFU = (number of colony forming units per plate/fungi deposited per unit area of medium, dm^2)/plate exposure time, h.

Surface contamination by fungi was assessed by counting up to 150 colony-forming units in a Petri dish. If more colonies were present, the result is reported >150 CFU on a plate. Fungal isolates were analysed using a light microscope. Genera were determined microscopically based on morphological characteristics of fungi using mycological taxonomy manuals [28]. Attention was paid especially to the potential prevalence of toxigenic genera.

2.2.2. Sampling of airborne pollen

Airborne pollen samples were taken in schools at the same places as for the fungi using the passive air sampling method (Table 1). We collected pollen employing passive particle deposition on a microscope slide (76 × 26 mm). The surface was coated with a layer of wax to trap pollen falling. Microscope slides were exposed at the same position for 7 days. At the end of exposure time, slides were covered with a cover slip and were transported to the laboratory for analysis. The whole slide surface was examined under the microscope. The swab sampling method was used to extract pollen from dust. Using sterile cotton swabs, samples were collected once a month from surfaces in classrooms and corridors at the same points as for fungi. The processing area was also the same - 10 cm^2. The swab with the trapped dust was immediately transferred to a sealed tube for transport to the testing room. In the laboratory, cotton swabs were washed with the sterile distilled water. The liquid fraction was transferred to tubes and centrifuged at 3000 rpm for 2 min to concentrate pollen at the bottom of the tube. We removed the supernatant and added glycerol to the precipitate to help the sample spread smoothly on the microscope slide. Prepared samples were analysed using a microscope. In total 831 samples were analysed.

Aiming to analyse the ambient air pollen situation in Vilnius, we also included aerobiological data. The 7-day Hirst-type volumetric pollen trap [29] was in the northern part of the city (Fig. 1) at an altitude of 18 m and represents the concentration of airborne pollen within a radius of 50 km. This study employed airborne pollen data collected during the whole observation period of 2020, and for the 2021 pollen load, we extracted data from the first part of the year (until the end of June). Outdoor pollen samples were analysed using standard aerobiological sampling methods [30,31]. Twelve vertical sweeps of the slides were used for the identification of pollen. Pollen concentration per day was expressed as pollen grains/m^3. The pollen season was calculated using the 95% method [32,33]. Pollen collected in both indoor and outdoor samples were analysed under a light microscope using magnification ×400. Pollen grains were recognized according to distinct morphological characteristics, and at the taxonomic level of plant family or genus.

2.3. Data analysis

Analysis of airborne fungi and pollen data was performed using R [34] and RStudio [35] software. The ggplot2 package [36] was used for results' visualization. Data were processed using the dplyr package [37]. The data transformation functions of the package made it possible to systematize the results. The fmsb package tools [38] were used to create the radar charts to evaluate airborne fungi distribution on different surfaces in schools. As datasets were not normally distributed, data normalization was performed by using fungi data and applying linear scaling. Normalized quantitative fungal data were used to illustrate the whole spectrum of airborne fungi on various surfaces like furniture, ventilation system or floor.

Linear regression analysis was used to determine the relationship between the mean concentrations of fungi found in classrooms and school corridors. For airborne pollen data visualization, we used Box-Whiskers plots to show the differences in outdoor airborne pollen concentration. The results are displayed in the Supplementary Material.

Integrated heatmap with dendrograms of fungi and pollen diversity on different surfaces in schools were created by using ComplexHeatmap [39], circlize [40], and dendextend [41] packages. Hierarchical clustering of averaged data was performed according to Euclidean distance method.

A nonparametric Kruskal-Wallis (H) test was performed to evaluate the amount of indoor airborne fungi and pollen and determine whether there is a statistically significant difference between the sampling collection and environmental conditions. Sampling method, season of sampling, sampling location and surface, as well as school size and urbanity in the school's surroundings, were set as independent parameters for probable variation of indoor airborne fungi and pollen. We analysed statistically significant cases when the H value had $p < 0.05$.

3. Results

Investigations of indoor bioaerosol in schools during the autumn-winter and spring periods showed quite remarkable diversity of airborne particles. Sampled data reveals that indoor air bioaerosol consisted of fungi belonging to 11 genera (Fig. 2) and pollen belonging to 24 plant taxa (Fig. 6). The diversity of airborne fungi and pollen varies in teaching classrooms and school corridors. Qualitative and quantitative evaluations of indoor identified fungi and pollen presented comparing of the obtained results in the frame of vegetation season and school distance from the Vilnius centre. According to the data collecting periods and the school's distance from the city centre, the biodiversity and abundance of fungi and pollen identified in classrooms and corridors were evaluated.

3.1. Diversity of airborne fungi

The results of indoor fungal diversity are presented in Figs. 2 and 3. The average units of fungi are displayed by sampling method, seasonality, and the school's location in the city. Analysis of samples collected in 11 schools revealed that *Acremonium* (Fig. 2) was found mostly in passive air samplings and *Cladosporium, Penicillium* (Fig. 3) in swabs samplings.

The quantitative evaluation of the passive air samples showed (Fig. 2) that a relatively higher abundance of fungi was detected indoors in the downtown schools during spring. However, following the school distance from the city centre, the biodiversity of fungi did not differ considerably. The absence of *Stachybotrys* in downtown schools and *Ulocladium* in the schools of the suburban areas could be mentioned as the noticeable difference of observation. In the samples collected during the autumn-winter period, the differences in fungal diversity in urban and suburban schools were more considerable than in the spring samples. In suburban schools, the indoor passive air samples revealed a lower amount and diversity of fungi, while in centre-periphery schools, richer fungal diversity was observed (8 genera from 11). Downtown schools were characterised by higher amounts of *Penicillium*.

The quantity and quality of fungi in swab samples are different from those found in passive air samples (Fig. 3). Comparing the gravitational sampling with the swabbing, *Acremonium* was less frequently detected in dust samples, independently of the school examined. The opposite was true for *Penicillium* and *Cladosporium*, which in dust were more abundant than in the air. *Penicillium* dominated indoor swab samples, regardless of the sample collection season or school location. *Acremonium, Alternaria* and *Mucor* were detected in small quantities, regardless of the season or school. *Trichoderma* was found only in spring samples in centre-periphery schools.

Simultaneously, an analysis of the diversity and abundance of fungi in terms of sample collection time and school size (Supplement Figure S1,S2) was performed. We found that more fungi were in gravitationally collected samples in large schools (>400 students) comparing to schools with fewer students. Swabbing data evaluation results differ. In the dust samples, the highest number of fungi was found in small schools in autumn-winter and medium-size schools in springtime.

The concentration of fungi in schools (Fig. 4) was evaluated in classrooms and corridors.

Scatter of fungi concentration in a different area of schools allows evaluating the abundance of microbial contamination. Statistical analysis of the results revealed that the concentration of fungi was relatively higher in the samples collected in the spring than in the autumn-winter period. Comparing the concentrations of airborne fungi in the corridors and classrooms it was found that there are more fungi in the air of the classrooms than in the corridors ($r = 0.78$, $p = 0.005$).

In Supplement Figure S3, results of airborne fungal diversity by location (desks, cabinets, windowsills, floor, or ventilation) in school are presented. Fig. 5 demonstrates that more fungi were deposited on Petri dishes placed on the floor in the passively collected samples than on other surfaces. Here, *Mucor, Alternaria* and *Cladosporium* were especially common. *Acremonium* was more prevalent in the middle level, which includes windowsills, student and teacher tables. *Stachybotrys* and *Penicillium* dominated in the samples taken from the top of cabinets. Dust samples taken from ventilation holes were particularly abundant in *Penicillium* and *Cladosporium*. It should be emphasized that dust samples collected from any of the tested surfaces most frequently contain these two morphotypes.

Detailed results of fungal diversity on different indoors surfaces of schools are provided in Supplement Figure S4-S7. Results of the passive air sampling showed that *Acremonium, Cladosporium,* and *Penicillium* were the most abundant on the student's desk with fewer
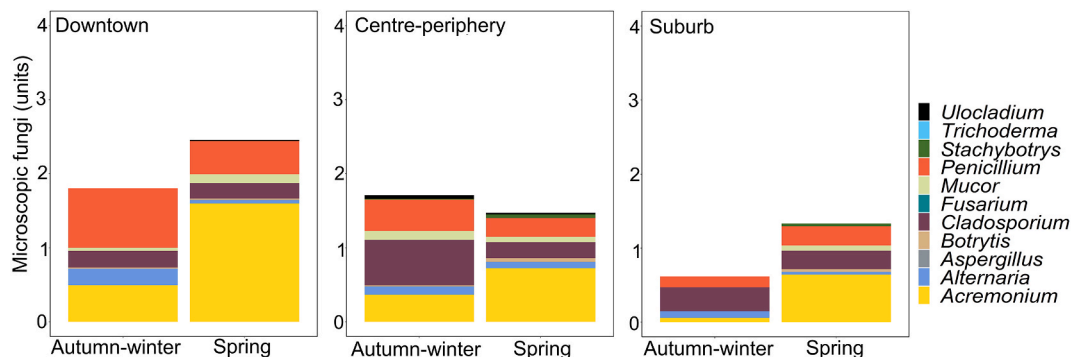

Fig. 2. Abundance of airborne fungi in samples gathered using the passive (gravitational) collection method. The histogram shows the average amount of fungi in the respective group. The legend lists all the identified fungi genera.
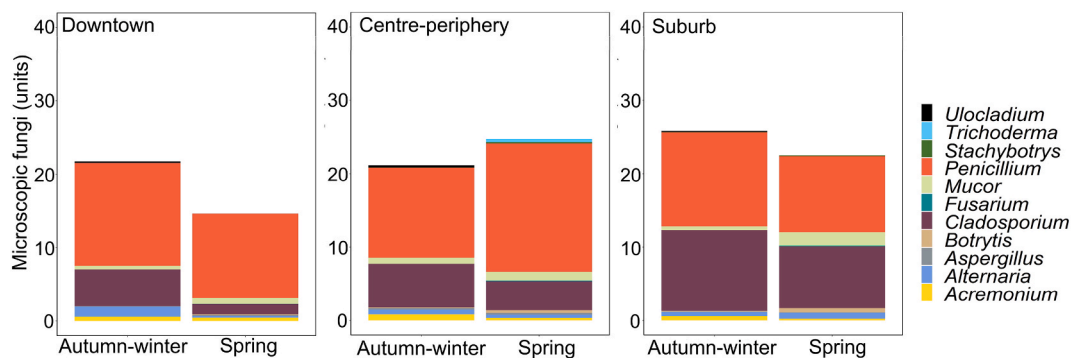
Fig. 3. Abundance of fungi gathered from dust samples. The histogram shows the average amount of fungi in the respective group. The legend lists all the identified fungi genera.
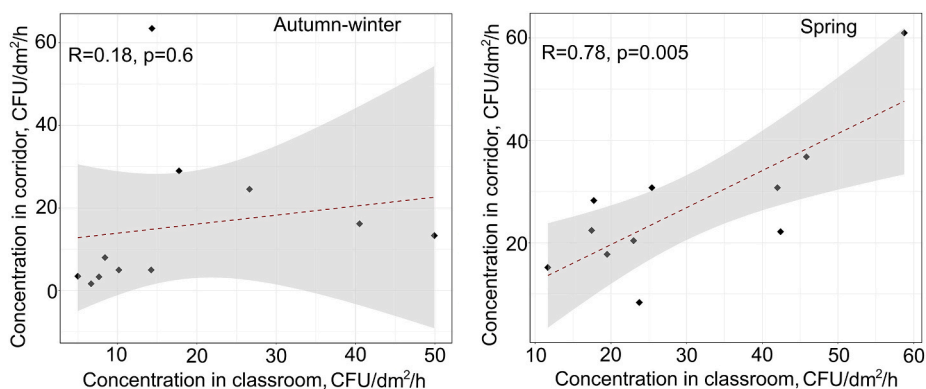

Fig. 4. Averaged concentration of airborne fungi in classrooms and corridors of tested schools.

amounts of *Mucor*, *Alternaria* and *Botrytis*, whereas *Ulocladium* and *Stachobotrytis* were only rarely found there. In the corridors, *Acremonium* and *Penicillium* were observed mainly on the windowsills. *Ulocladium* was detected on the floor and top of the cabinet. The evaluation of fungal data obtained from swab samples taken in the classrooms revealed a higher biodiversity of fungi in ventilation. In the study, of the total of 11 genera of fungi that we identified, 10 were found in this location. *Fusarium* was observed on the teacher's desk and the floor. The dust samples taken in the corridors showed the highest diversity of fungi on the floor and the windowsill.

According to results (Table 2) of one-way analysis of variance, significant ($p < 0.05$) difference was observed between the sampling method, sampling on different surfaces and airborne levels of fungi. The results indicate that fungal abundance as found in samples was mainly determined by the collection method and the sample collection place. School size and the urbanity in the school surroundings are also important variables for the indoor amount of airborne fungi. In this study, no relationship was found between the amount of fungi and the time of year when the samples were collected and the collection location.

3.2. Diversity of airborne pollen indoors

In parallel to the indoor fungi study, airborne pollen were also sampled in classrooms and corridors of schools participating in the study. Results of airborne pollen samples analysis demonstrated a wide pollen diversity indoors (Fig. 6: 24 taxa comprising both woody and herbaceous plants). Regardless of whether the samples were taken by passive or swab method, only a few pollen types dominate according to the abundance in samples. In any case and independently of how far the schools were from the city centre, *Betula* and *Pinus* pollen predominated in samples. It should be noted that *Aesculus*, *Picea* and *Quercus* pollen was found in larger quantities in the suburb schools. *Picea* pollen was abundantly found in downtown schools in springtime.

Results differ between the samples collected during autumn-winter (Figs. 6 and 1) and spring (Figs. 6 and 2) periods. In the autumn-winter samples, indoor pollen diversity was lower, and pollen was observed in small quantities, i.e., an average of few pollen per sample. We expected to find that a small amount of pollen remains indoors at the end of the vegetation season.

The diversity of pollen was not large and mostly represented by plant taxa with high pollen production. In analysing period, *Alnus*, *Artemisia*, *Betula*, *Picea*, *Pinus*, *Ulmus* pollen were deposited in 7-day passive air samples, although the plants (except *Artemisia*) flowering in the first half of the year. In outdoor samples, a large amount of *Betulaceae* pollen was observed in spring of 2020 (Supplement Figure S8). This supports the presumption that a small amount of allergenic pollen could stay indoors after the vegetation season. In dust samples, a greater variety of pollen types was recorded. It is worth noting that in samples of accumulated dust, pollen of
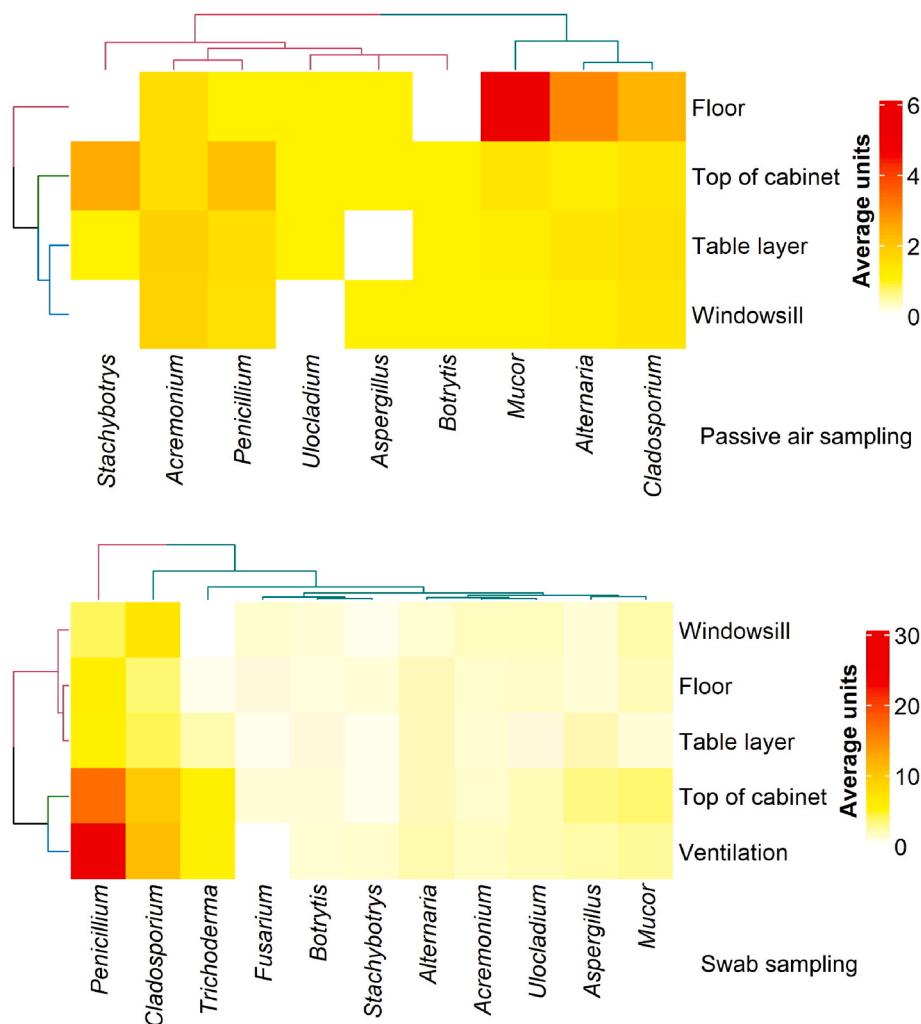
Fig. 5. The abundance of fungi indoors on different surfaces in primary schools. The average quantity is indicated in colour and presented as a heatmap. Dendrograms demonstrate the relationship between clusters of fungi genera detected on various surfaces.

woody and herbaceous plants was deposited. Schools in the suburbs had more grass and weed pollen than schools close to downtown.

In the spring samples, the diversity of indoor pollen was significantly higher. In schools, we observed 21 different pollen types. *Betula*, *Picea* and *Pinus* led the list of pollen load independently of the indoor pollen collection method. Spring measurements were performed during the flowering season. Betulaceae and Pinaceae plants usually spread the highest amount of pollen in the area (Supplement Figures S8, S9). Samples taken from suburban schools had the lowest pollen diversity. Compared to downtown schools, those locations had up to 35% fewer morphotypes found in passively collected air samples and 20% fewer in swab samples. Downtown schools exhibited the highest variety of pollen types independently of the sampling method. Furthermore, herbaceous plant pollen act as an important factor in indoor air in the evaluation results of the measurements made in the spring. In each air sample, 2 to 5 pollen types belonged to grasses or weeds. The indoor air of schools located downtown in this study was characterized by a higher variety of herbaceous plant pollen, accounting for at least 4 and 6 types in passive or swab samples accordingly. *Artemisia* pollen (plants flower in the second part of summer) was found in air samples collected by the swabbing method.

The essential impact on pollen concentration and diversity makes the local environment. An analysis of the greenery surrounding the schools was performed to identify pollen sources. The details in Supplement Table S1 show the diversity and abundance of woody vegetation in the environment of the schools selected for the study. From the variety of growing plants, the 1–2 most represented taxa were selected into the dominant group. 5 Plant genera were identified as the most abundant, namely *Acer*, *Betula*, *Populus*, *Thuja* and *Tilia*, and this is how 8 groups of greenery in school environments were composed. According to the greenery results, the airborne pollen data were divided into 8 groups and the pollen biodiversity in each group was assessed. (Supplement Figures S11, S12). Generalised results revealed that *Betula* pollen was found in schools with birch-dominated greenery, but *Betula* pollen was also found in schools where no birches were among the predominant plants. *Acer* pollen was observed in air samples where *Acer* and *Thuja* plants predominated in school surroundings. *Populus* pollen was low indoors, although *Populus* was the dominant plant around some schools.
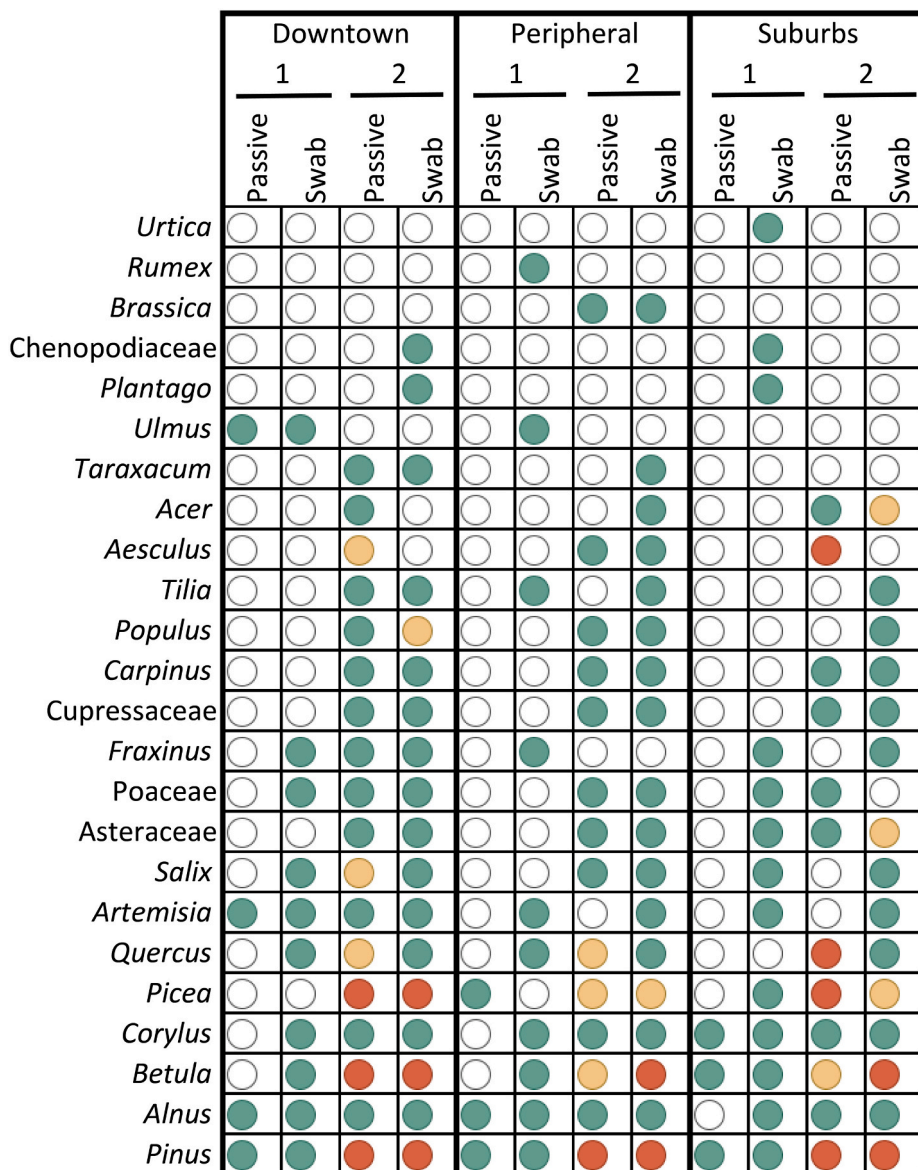
Fig. 6. The average amount of indoor pollen observed in schools located in different parts of the city (downtown, centre-periphery, suburbs). The number in the table indicates the sample collection period: 1 – samples collected during autumn-winter time; 2 – during spring. The amount of pollen is represented by coloured circles: colourless - no pollen was observed; green – 0–1 pollen; yellow – 1–10 pollen; red – > 10 pollen in total per school type.

Table 2
The difference in fungi amount according to sampling method, indoor and outdoor environmental conditions. Results were obtained by using the Kruskal-Wallis test ($n = 10,660$).

Independent variable	H	df	p
Sampling method: swab and passive	640	1	<0.01
Season of sampling: autumn-winter and spring	0.195	1	0.66
Sampling location: classroom and corridor	0.0465	1	0.83
Sampling on different surface	388	9	<0.01
School size: number of students	8.77	2	0.01
Urbanity in the schools surrounding area: downtown, centre-periphery, suburb	8.41	2	0.01

According to airborne pollen data (Supplement Figure S8-S10), *Populus* pollen concentrations were low outdoors during indoor sampling. *Pinus* pollen was found in small numbers in the swab samples, but an exceptionally high pollen number was registered in the passive air samples.

Pinus and *Betula* pollen amounts were the highest in comparison to other pollen types found indoors. Therefore, we performed a separate assessment to determine how this abundance varies both in terms of the study surface and in terms of the location of the school in the city. For this purpose, cases consisting of passive air sampling events with an amount of more than 50 pollen per event were separated. Fig. 7 demonstrates the distribution of selected events. The results showed an interesting tendency that *Betula* pollen prevails on all tested surfaces in schools located downtown. With increasing distance from the city centre, the case of the high amount of *Pinus* pollen becomes more common, especially in schools located in the centre-periphery. In suburbs, unlike other schools, *Pinus* is more often found on windowsills than on other surfaces.

To determine which pollen taxon accumulated on which surfaces the most, we performed an analysis of the distribution of the averaged pollen amount. The relative values of the distribution are shown in Fig. 8.

Pinus was one of the most abundant pollen types, and was observed year-round (Fig. 8). Abundance obviously depends on the vegetation season (ambient pollen concentration in Supplement FiguresS8-S10), but *Pinus* is the pollen morphotype that has been present continuously. In a similar position is the pollen of the Betulaceae family. *Betula* predominates in spring, but in autumn-winter samples, *Alnus* appears as a morphotype detected more frequently than other genera of the family Betulaceae (Fig. 8). In spring, especially the surfaces of the corridor are covered with quite a large amount of *Picea* pollen, however, the pollen concentration in the ambient air is not high.

Pollen deposition on surfaces (Fig. 9) varied according to the sampling method. The pollen data gathered by the passive air sampling method group in two clusters; the results of the first cover data from the windowsill samples and the second - from the table layer and the top of the cabinet. In dust samples, the table layer pollen data formed a separate independent cluster. The surfaces that make up the second cluster in this group are further divided into two specific clusters, one of which covers data from the floor layer and ventilation and the other comprises results obtained from the top of the cabinet and windowsills.

By comparing the results (Table 3) of the samples collected in corridors and classrooms during the spring, it was found that the diversity on top of the cabinet in the corridor is richer than in the classrooms in general. In the autumn-winter results, the diversity of pollen was greater on top of the cabinets in the classrooms as well. The lowest pollen diversity was found in the school corridor during the autumn-winter period. The differences in indoor airborne pollen amount relate to the sampling method (H = 8.4, $p < 0.05$). Whether swab or passive air sampling were used, pollen amounts varied.

Season of sampling is important factor for airborne pollen (Table 2), whereas the pollination of plants determines the presence of pollen in the air. Significant differences with other independent variables were not observed except the urbanity of the school surrounding ($H = 14.3, p < 0.05$). Fig. 7 illustrates that schools in the suburbs had a high amount of *Pinus* pollen indoors, and *Betula* pollen was abundantly observed in downtown schools.

4. Discussion

Our study on indoor bioaerosol revealed the features of airborne fungi and pollen dispersion within primary schools. We identified

Fig. 7. Distribution of the *Betula* and *Pinus* (the most abundant pollen taxa found indoors) cases with registered of more than 50 pollen per passive air sample on the tested indoor surfaces according to schools' location.
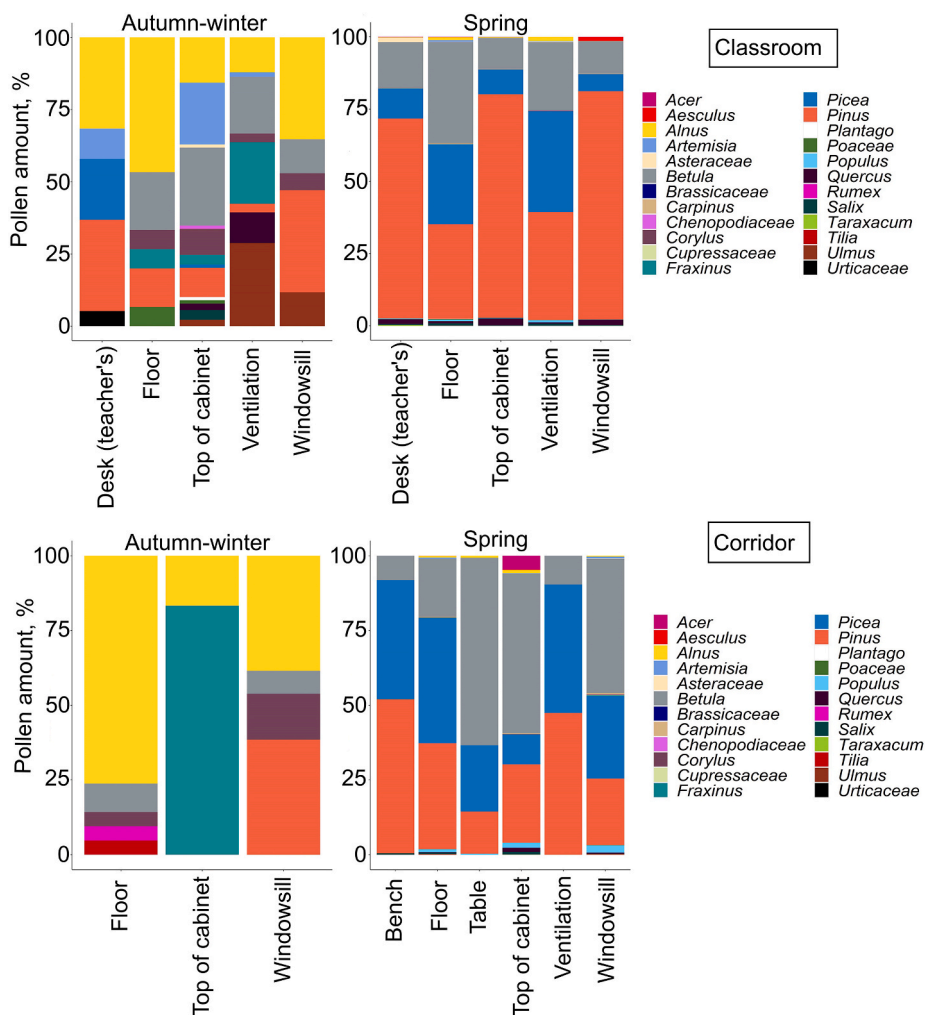
Fig. 8. Abundance of airborne pollen on different sampling surfaces in classrooms and corridors. The legend contains the most abundant pollen types (identified pollen with relatively low abundances do not appear on the graphs, in favour of clarity).

pollen of 24 plant taxa and fungi belonging to 11 genera on different surfaces of classrooms and corridors. *Acremonium*, *Cladosporium* and *Penicillium*, which have different health effects, were the most commonly detected in the test samples. The prevalence of *Cladosporium* and *Penicillium* are associated with non-infectious diseases provoked by inhaled allergens [9,42] while *Acremonium* can initiate both infectious and non-infectious health effects [43]. Non-infectious health effects of fungi could also be provoked due to the increased load of inhaled mycotoxins [44]. Mazur et al. [42] demonstrated that mycotoxins are primarily found in fungi such as *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Stachybotrys*, and *Trichoderma*. Evaluating the air quality in school, we found that *Alternaria*, *Aspergillus*, *Fusarium*, *Stachybotrys*, and *Trichoderma* form a lower atmospheric load compared to the quite numerous spores from *Cladosporium* and *Penicillium*. It should be specified that *Acremonium* prevailed in some cases of passive air samples. The number of *Acremonium* was higher during springtime in large schools (Supplement Figure S1). Regarding the distance of schools from the city centre, more fungi were found in schools located downtown. Inside the school a windowsill was the typical place for *Acremonium*. According to the results, we assume that *Acremonium* could come from outside through open windows.

Cladosporium and *Penicillium* are common fungal genera of indoor air [45,46] (and were both observed in studied schools in Vilnius, characterized with humid continental climate). In our study, windowsills are marked as the main location of *Cladosporium*. Accumulation or proliferation of fungi on windowsills is possible for several reasons. *Cladosporium* is likely to enter through open windows because schools do not use automatic ventilation systems and usually open windows in classrooms during the breaks. Fungi are widespread in the natural environment, and annual spore concentration in the atmosphere reaches hundreds of thousands [47]. Therefore, *Cladosporium* can easily enter indoors. Other studies demonstrated that the windowsills are suitable for the proliferation of fungi due to higher moisture accumulation [48]. Using two different methods, in our study it was shown that more *Cladosporium* accumulated on surfaces (especially windowsills) than in indoor air in general (Figs. 2 and 3). The parallel measurement methods approach enabled us also to supplement the knowledge on *Penicillium* dispersion in school classrooms and corridors. This difference could be supported by the pointed-out note of Sousa [49] that *Penicillium* is associated not only with physical characteristics, but also
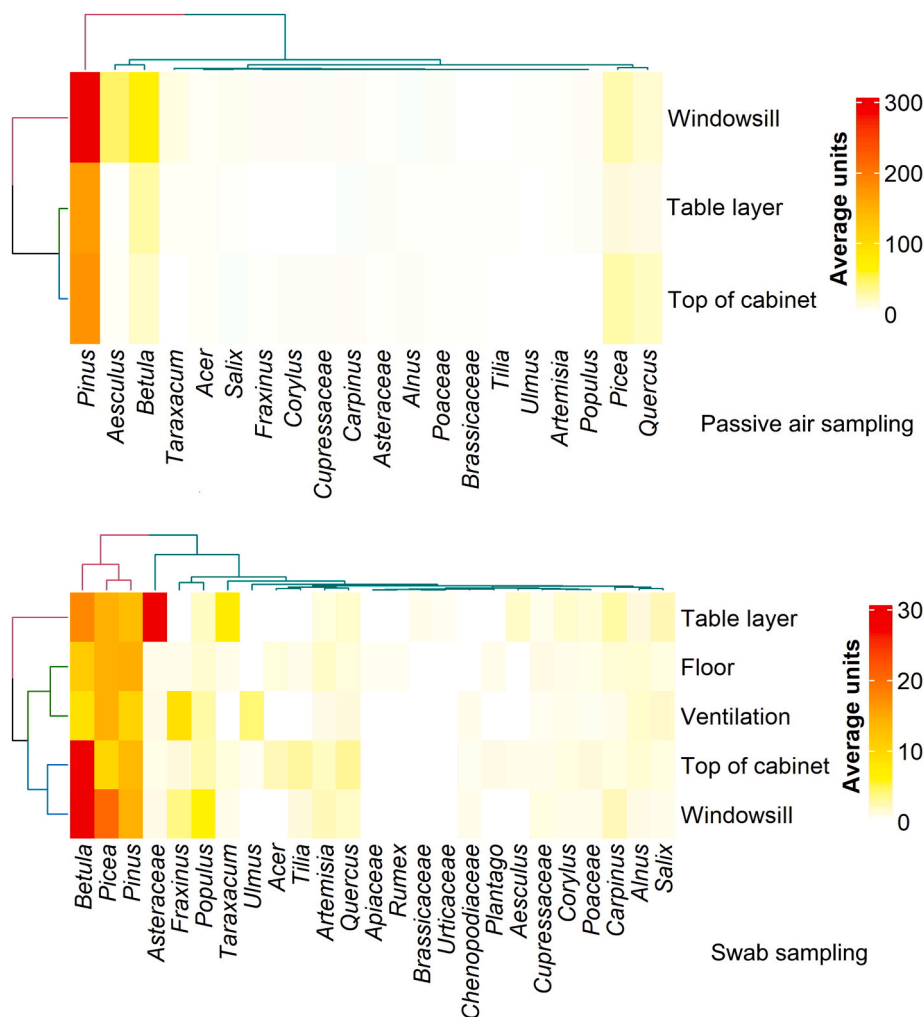
Fig. 9. The abundance of pollen indoors on different surfaces in primary schools. The average quantity is indicated in colour and presented as a heatmap. Dendrograms demonstrate the relationship between clusters of plant genera and families detected on various surfaces.

Table 3
The difference in airborne pollen amount according to sampling method, indoor and outdoor environmental conditions. Results were obtained by using the Kruskal-Wallis test ($n = 11,829$).

Independent variable	H	df	p
Sampling method: swab and passive	8.4	1	0.004
Season of sampling: autumn-winter and spring	586	1	<0.01
Sampling location: classroom and corridor	0.0266	1	0.87
Sampling on different surface	9.19	7	0.24
School size: number of students	3.75	2	0.15
Urbanity in the schools surrounding area: downtown, centre-periphery, suburb	14.3	2	0.001

with the user behaviour in the room. The school activities in classrooms and corridors differ in the more intense movement of students in corridors during breaks. The top of the cabinet was evaluated as the most frequent location to identify *Penicillium* in comparison with other airborne fungi.

Indoor fungi are commonly associated with various factors: vegetation period [50], the outdoor environment around buildings [51], damp or wet building materials [44], heating conditions, ventilation [52] and other aspects, which influence the presence of fungi indoors all year-round. Hence, it is considered rather challenging to manage contamination by fungi, since it is known that all residential surfaces in buildings appear to be passive collectors of airborne fungi [48]. This is also confirmed by the results of our study that reveal that a large number of fungi is found inside school buildings. As a passive collector, the surface of every location investigated is contaminated with common fungi such as *Cladosporium*, *Penicillium*, *Acremonium* and *Alternaria*. From previous bioaerosol

studies, it is suggested to reduce the source of fungi by keeping indoor environments dry, maintaining good hygienic conditions in ventilation systems, as well as applying effective filtration on mechanical supply ventilation [53]. According to our data, natural ventilation without any filtration system was used in schools, which may have been one of the reasons for the diversity of fungi identified.

Flowering plants growing near buildings could determine airborne pollen presence in various types of houses. Our results have confirmed that a significant part of pollen inside schools is related to the greenery and flowering plants in the vicinity to nearby buildings. For example, due to the very high Betulaceae pollen concentration and pollen morphological properties allowing to stay in the atmosphere, the amount of pollen inside schools was also high, not only in swab samples but in passive air samples as well. Increased *Betula* pollen levels are associated with *Betula* plants growing near schools. Knowing that birch pollen is one of the most common aeroallergens in Europe [16,22,33], birch planting in school greenery should be avoided. The study results indicate (Fig. 7) that this knowledge has not yet been applied in practice, and the highest birch pollen load is observed in schools downtown. The management of the school environment plays an essential role in controlling aeroallergens. On the other hand, the theory of existing long-distance transport [54,55] cannot be ruled out. Some pollen can be transported over long distances, and it is difficult to estimate its possible source. Pollen, on the other hand, can be carried from plants far from schools, such as city parks and nearby forests. Our results demonstrated that there was a lot of *Pinus* pollen indoors, although these plants were not present in the school greenery. *Pinus* pollen identified in the samples may have been obtained due to pollen transport from urban parks located short-distance from schools. In Vilnius, one of the most common tree species in parks is *Pinus sylvestris* L [56].

Our study has shown that the diversity of pollen in spring indoor air samples is significantly higher than in those collected in late autumn. Although some pollen may be brought in with schoolchildren's clothing or other items, doors and windows are recognized as the main routes for pollen to enter indoor [6,15,57]. The need to ensure air renewal in the classrooms is not only unequivocal but mandatory [58]. The schools we chose for the study use natural ventilation, which means that during breaks, the windows are opened, allowing pollen to enter the classrooms. Thus, the increased diversity and abundance of pollen indoors in the spring could be the result of inappropriate indoor ventilation methods.

This study showed that pollen is found on all surfaces inside the school. More pollen morphotypes accumulate in the classrooms than in corridors. We found the same tendency as with fungi when in the late autumn, on the whole, the diversity of bioaerosol is lower in corridors than in classrooms. High diversity of pollen on the windowsills in the spring could remain after the recent ventilation through the open windows. The origin of pollen detected in air samples collected inside the school in late autumn remains unresolved. This supports the hypothesis that "pollen background" is still an issue indoors. School administrators should prohibit the decoration of corridors and classrooms with dry plant bouquets, particularly ones containing grasses. Can the responsibility for maintaining classrooms and corridors be transferred to undeveloped algorithms? An additional argument for unsatisfactory management could be the fact that the diversity on the top of the cabinets is the largest both in the samples of fungi (Fig. 5) and in the samples collected for pollen measurements (Fig. 8). It is worth taking this information into account when ensuring the quality of the indoor environment for formal and non-formal learning. A cognitive function could be affected during the pollen season in pollen-allergic children [59], and allergenic particles of biological origin accumulated in the classroom can cause allergy symptoms even after the end of the vegetation season. Our research has supplemented existing evidence that bioaerosol measurements in schools (including kindergartens or informal education facilities) are vital [6,9] and, in addition to monitoring bacterial or fungi, should include at least pollen measurements to prevent allergenic reactions. The evidence on the presumption that the indoor school environment is a significant reservoir of allergens, endotoxin and pollutants [60] is increasing, but there are still gaps in the understanding of indoor bioaerosol exposures on pupils' health, absenteeism, learning achievements, as well the school staff's working conditions and related productivity. Indoor biomonitoring has been continued until today, attempting to comprehend the dynamics of airborne fungi and pollen, towards a more efficient improvement of public health. It would be beneficial to assess the synergistic effects of bioaerosols and pollutants for a more accurate understanding of indoor air quality. In this case, an important field of future study is the viability and allergenicity of indoor bioaerosol particles trapped in dust, as this would significantly contribute to the evaluation of bioaerosol exposure. The sedimentation method used in this study allowed the assessment of particles deposited by gravity but left unanswered questions on the possible presence of bioaerosol particles suspended in indoor air. This limitation should be considered in future studies by supplementing the analysis with a volumetric air sampling method.

5. Conclusions

The present study demonstrated that bioaerosol particles, specifically fungi and pollen, are detected indoors in school during and after the vegetation season. First and foremost, the findings showed that it is important to evaluate the school maintenance system by revising cleaning guidelines for corridors and classrooms. Indoor *Penicillium*, *Cladosporium* and *Acremonium* were found as major fungi, while *Pinus* and *Betula* were the dominant pollen. However, we need to emphasise that the results of pollen diversity are determined by the time of indoor air sampling. Overall, the diversity and amount of pollen in the spring was significantly higher than in samples collected in autumn.

Indoor pollen counts were higher from those plants whose phenological flowering stage coincided with the study period. Here, we get a tendency for higher *Betula* pollen concentrations in schools downtown compared to schools further away from the city centre or in the suburbs. The highest diversity of bioaerosol particles was found on the top of cabinets and windowsills. The study results showed that *Cladosporium* was more concentrated on windowsills and *Penicillium* on cabinets in classrooms and corridors. In the spring, when *Betula* and *Pinus* pollen dominated, a difference in their quantity was found between corridors and classes. Pollen from plants of these genera was mostly found on desks or tables, top of cabinets, and windowsills. The difference is related to the room type: more *Betula*

pollen was detected in classrooms, and more *Pinus* pollen at corridors. Concentrations of fungi were higher in spring than in late autumn, both in classrooms and in corridors. We have noticed a tendency for schools with more students to have higher levels of fungi. Pollen remains indoors in the autumn, but the impact of its health effects should be deeper analysed in the future.

Author contributions

Ingrida Sauliene: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the paper. Arunas Valiulis: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Ilona Keriene: Performed the experiments; Analysed and interpreted the data; Wrote the paper. Laura Sukiene: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the paper. Dovile Dovydaityte: Performed the experiments. Nina Prokopciuk: Performed the experiments; Wrote the paper. Vaidotas Valskys: Performed the experiments; Wrote the paper. Roberta Valskiene: Performed the experiments. Athanasios Damialis: Analysed and interpreted the data; Wrote the paper.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2022.e12668.

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