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Concentration of air-borne microorganisms in sport facilities

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Abstract. This paper is focused on the microclimatic research in several buildings and rooms used for sport at the University. The attention is paid mainly to the problems of dimensions of space, capacity and activity of sportsmen, and influence of space ventilation. The air samples for microbiological analyses were taken by the microbial air sampler Merck Mas-100 Eco and cultivated by potato-dextrose agar and nutrient agar. Captured microorganisms, are expressed as colony forming units per m^3 (CFU m^{-3}). Measurement results showed that bacteria average quantity was statistically significantly less without students (562 CFU m^{-3}) than with students (1,024 CFU m^{-3}). The students inside the rooms increased the bacteria concentration. From this point of view the ventilation is not adequate for the removal of bacteria from ventilated spaces. From the results we can conclude that the great importance on the air quality in terms of a specific bacteria concentration has the specific volume of the room per one athlete. The worst situation is in rooms with the smallest volume, which has the largest biological load of the space. The lowest quantity of bacteria was in the swimming pool all year round (152 to 300 CFU m^{-3}). The opposite situation was in average quantity of filamentous fungi, which was with students and ventilation (57 CFU m^{-3}) and without students but without ventilation (109 CFU m^{-3}). The pollution of air by fungi was higher without ventilation.

Key words: air, gyms, indoor environment, pollution, swimming pool, ventilation.

INTRODUCTION

This paper is focused on the microclimatic research in different rooms at Czech University of Life Sciences campus. The microbial contamination (bacteria and filamentous fungi including presence of allergenic genus *Cladosporium* and *Aspergillus*) and also main microclimatic parameters of the air (temperature and relative humidity of air) were measured and evaluated in the relation to different performance conditions of tested rooms. The attention is paid to purpose of the rooms, ventilation or air-conditioning.

Many scientific and professional papers emphasize the influence of indoor climate on the human health (Gorny & Dutkiewicz, 2002; Karwowska, 2003; Fisk at al., 2007) and on the comfortable feeling of inhabitant inside the different buildings and rooms (Seppänen at al., 2006).

One of the frequent sources of microorganisms in the air is dust. It is usually also an accompanying sign of higher aerosol contamination by microorganisms. The attention to microbiological pollution is paid in some research works e.g. Bouillard et al. (2005), Orru et al. (2011), Brodka et al. (2012). The methodology and the results of measurements correspond to the research topic, especially to factors that are specific to studied space, e.g. Kic & Růžek (2014).

Results of large research work focused on the problems of seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate is described in the publication Frankel et al. (2012). Indoor fungi peaked in summer (median 235 CFU m^{-3}) and were lowest in winter (median 26 CFU m^{-3}). Indoor bacteria peaked in spring (median 2,165 CFU m^{-3}) and were lowest in summer (median 240 CFU m^{-3}). According to the research findings and measurement results Kic et al. (2014), people are the source of bacterial and fungal contamination inside the rooms.

As the university students as well as the staff should be active not only in the study or research but also in the sport activities, it is important to know what the situation inside the sport facilities is. Due to the fact that during sport activities people breathe very intensively, the air inside the rooms should be very clean. The aim of this paper is to present results of microclimatic research focused on the microbiological pollution, biological load by people and influence of ventilation in several rooms used for physical education and sport activities at the Czech University of Life Sciences Prague.

MATERIALS AND METHODS

This research work and measurements of the actual values were carried out in buildings and rooms of Department of Physical Education at the Czech University of Life Sciences Prague. All rooms are situated in two buildings, three of them in the same building (two conventional gyms GA, GB and one fitness centre GC). The first gym GA has the following dimensions: floor area about 540 m^2, volume 4,320 m^3 and it is used mainly for different ball games. The second gym GB has the following dimensions: floor area about 216 m^2, volume 1,728 m^3 and it is used mainly for sports games, floor exercise, aerobics, table tennis, etc. The third gym GC has the following dimensions: floor area about 92 m^2, volume 240 m^3 and it is used as a fitness centre. The last building is swimming pool centre SP (pool is 25 m long), which has the following dimensions: floor area about 640 m^2, volume 4,018 m^3.

A ventilation device is relatively outdated. There are not sensors for measurement and control of temperature, humidity or CO_2. There are only two limit states manually operated by the person: on or off. For technical reasons, it is not possible to measure the ventilation rate of air inlet or outlet in individual rooms. The long air-ducts are located in inaccessible places under the ceiling (height approximately 8 m) and air is distributed air to several rooms at the same time.

The measurements were carried out first when all rooms were empty, without students and without ventilation, several days without cleaning the floors. The same measurements were carried out during the normal function of rooms, with students and with standard ventilation calculated and designed by empirical method according to the ventilation rate per one person (30 m^3 h^{-1} $person^{-1}$). There were following number of persons during the measurement inside the rooms: GA 20 students play floorball, GB 27 students doing aerobic exercise, GC 18 students in fitness training, SP 20 students swim.

Rather important is specific biological load of the sport rooms calculated from the previous data. The summarised values of specific volume of space per one person in the rooms are following: gym GA has 216 m^3 per person, GB has 64 m^3 per person, fitness GC has only 13 m^3 per person and swimming pool SP has 201 m^3 per person.

The air samples for microbiological analyses were taken by the instrument Merck Mas-100 Eco, microbial air sampler with volume 0.200 m^3 air, and cultivated by PDA (potato-dextrose agar OXOID CM 139; 100%) and NA (nutrient agar OXOID CM 003; 100%). Colonies of microorganisms were evaluated after 5 days of incubation. Yeast and filamentous fungi were cultivated at 22 °C, bacteria at 29 °C. Captured microorganisms, which on the culture media formed colonies, were expressed as colony forming units per m^3 (CFU m^{-3}). Measuring devices and equipment technology environment continues to improve and provide a larger volume and more accurate results. New studies are constantly providing fresh information, but there are still many uncertainties. Maybe, new and more precise ideas about the influence on the human health can be discovered. Recommended air pollution limits of the indoor environment by a mixed bacteria population and a mixed filamentous fungi population according to the EUR 14988 EN are presented in the Table 1.

The obtained results of air-borne microorganisms measurements were processed by Excel software and verified by statistical software Statistica 12 *(ANOVA* and *TUKEY HSD Test*) to recognise the difference between the results in different rooms of sport centre during the summer and winter measurements. Different superscript letters (a, b, c) in common are significantly different from each other in the columns of the tables *(ANOVA; Tukey HSD Test;* $p \leq 0.05$*)*, e.g. if there are the same superscript letters in the column (rooms GA, GB, GC, SP) it means the differences between the values in rooms or between different states and conditions of the same rom are not statistically significant at the significance level of *0.05*.

Table 1. Air pollution limits of the indoor environment (living rooms) by a mixed bacteria population and a mixed filamentous fungi population (EUR 14988 EN)

Pollution category	Bacteria CFU m^{-3}	Filamentous fungi CFU m^{-3}
Very low	< 50	< 25
Low	< 100	< 100
Medium	< 500	< 500
High	< 2,000	< 2,000
Very high	> 2,000	> 2,000

The temperature and humidity of surrounding air were measured by sensor FH A646–2 including temperature sensor NTC type N with operative range from –30 to +100 °C with accuracy ± 0.1 K, and air humidity by capacitive sensor with operative range from 5 to 98% with accuracy ± 2%. Furthermore the concentration of CO_2 was measured by the sensor FY A600 with operative range 0–0.5% and accuracy ± 0.01%. The sensors were connected to the data logger ALMEMO 2690–8.

RESULTS AND DISCUSSION

Average values and standard deviations of external temperatures t_e and relative humidity RH_e, internal temperature t_i, relative humidity RH_i and concentration of carbon dioxide in the rooms without students and without ventilation during the summer period are summarized in the Table 2.

Some small differences between the measured values in the investigated areas correspond to slight changes in outdoor air during the measurement and different room dimensions as well as to the different thermal and technical characteristics and properties of these rooms.

The internal temperatures are related to the external mainly in the large gyms GA and GB. The internal temperature is smaller than external temperature in the fitness GC, which is not too large and the massive walls and mass of exercise tools, dumbbells and other equipment manifest as an accumulation mass of energy, so the cold accumulated during the night in this mass decreases slightly during the internal temperature during the day. The internal temperature is higher than the external temperature in the swimming pool SP as it is influenced by the warm water in the pool.

Relative humidity of internal air corresponds to temperature and rather high humidity ratio of external air in the summer. As there is not ventilation, the concentration of CO_2 is slightly higher, which is a rest of polluted air from the previous periods with some people activities.

Table 2. External temperature t_e and relative humidity RH_e, internal temperature t_i, relative humidity RH_i and concentration of carbon dioxide CO_2 in the rooms without students and without ventilation during the summer period

Room	t_e	RH_e	t_i	RH_i	CO_2
	°C ± SD	% ± SD	°C ± SD	% ± SD	ppm ± SD
GA	25.2 ± 0.2	51.8 ± 0.8	25.9 ± 0.1	56.2 ± 0.4	568 ± 11
GB	25.9 ± 0.2	48.5 ± 1.7	25.9 ± 0	55.8 ± 0.4	518 ± 10
GC	26.5 ± 0.1	44.4 ± 0.3	26.1 ± 0.1	57.2 ± 0.6	654 ± 20
SP	26.9 ± 0.1	42.6 ± 0.5	28.9 ± 0.4	53.4 ± 3.0	512 ± 41

SD – Standard deviation.

Table 3. External temperature t_e and relative humidity RH_e, internal temperature t_i, relative humidity RH_i and concentration of carbon dioxide CO_2 in the rooms with students and with ventilation during the winter period

Room	t_e	RH_e	t_i	RH_i	CO_2
	°C ± SD	% ± SD	°C ± SD	% ± SD	ppm ± SD
GA	7.2 ± 0.1	74.1 ± 0.2	22.7 ± 0.1	41.3 ± 3.0	740 ± 10
GB	7.4 ± 0.1	74.4 ± 0.6	21.6 ± 0.1	41.7 ± 1.0	689 ± 56
GC	7.3 ± 0.4	74.3 ± 0.1	22.4 ± 0.1	42.3 ± 0.4	771 ± 8
SP	7.1 ± 0.1	74.8 ± 0.2	27.2 ± 0.2	33.3 ± 0.7	350 ± 0

SD – Standard deviation.

The same external and internal quantities measured in the rooms with students and with ventilation during the winter period are presented in the Table 3. The results show the influence of intensive heating and ventilation in the swimming pool, which caused the highest air temperatures but also the lowest relative humidity and lowest CO_2 concentration.

The internal temperatures, relative humidity and CO_2 concentration in all gyms are in suitable range. Temperatures are increased by the heating system of the rooms. Relative humidity is lower than in the summer as the humidity ratio of external air is rather low (see low external temperature). The internal production of water vapour produced by respiration is not influencing the indoor humidity negatively thanks to the ventilation.

Table 4. Results of measurement of microbiological contamination by air-borne microorganisms in summer and winter. Different superscript letters (a, b, c) are the sign of the high significant difference between the rooms GA, GB, GC and SP (*ANOVA*; *Tukey HSD Test*; $p \le 0.05$)

Room	Bacteria		Filamentous fungi	
	Summer	Winter	Summer	Winter
	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD
GA	850 ± 137^{a}	$1{,}065 \pm 170^{a}$	$118 \pm 12^{a,b}$	45 ± 3^{a}
GB	467 ± 39^{b}	$1{,}433 \pm 179^{a}$	$115 \pm 20^{a,b}$	58 ± 28^{a}
GC	778 ± 54^{a}	$1{,}297 \pm 84^{a}$	133 ± 16^{a}	70 ± 7^{a}
SP	152 ± 39^{c}	300 ± 223^{b}	70 ± 20^{b}	55 ± 3^{a}

SD – Standard deviation.

Table 4 shows that the air pollution by biological contaminants is comparable in gym GA and fitness GC, which is according to EUR 14988 EN high (500-2000 CFU m^{-3}) in the case of bacteria during the summer. Different superscript letters (a, b, c) are the sign of the high significant difference between the rooms. The same letter (e.g. a) in lines of the same columns means, that there is not significant difference between values which were measured in different rooms.

The concentration of bacteria in gym GB and swimming pool SP is medium. The lowest quantity of bacteria is in the swimming pool SP.

It is also medium pollution in the case of filamentous fungi (100-500 CFU m^{-3}) in summer. The concentration of filamentous fungi was low (25–100 CFU m^{-3}) in all sport rooms during the winter. The lowest quantity of fungi was also in the swimming pool SP. The presence of conidia of filamentous fungi in the indoor environment is due to their presence in the incoming outdoor air. The inlet air is less polluted by the conidia of filamentous fungi in the winter. Their main source of filamentous fungi are necrotizing parts of plants, and those are few in the winter.

Prussin & Linsey (2015) identified eight major sources of airborne microorganisms in the built environment: humans, pets, plants, plumbing systems, heating, ventilation, and airconditioning systems, mold, dust resuspension and the outdoor environment.

Khan & Karuppayil (2012) recognized different sources of air-borne microorganisms in indoor environment. Our research carried out in the sport facilities eliminated other indoor factors. The arrangement of equipment was the same in summer (without students) and in winter (with students). The number of bacteria increases with the number of people present in both gyms GA and GB, in the fitness GC as well as in the swimming pool SP. Summer is a period without education, with a minimum number of people using gyms or fitness, but in winter it's the opposite. Many students and other clients come to gyms especially during the winter to strengthen their health and increase resistance to the expected respiratory problems. However, during sports activities, the air is heavily polluted with the finest dust particles (PM_1 and $PM_{2.5}$), which can penetrate into the lungs. The finest dust can contain biological contaminants.

The cleanest environment in terms of air pollution by biological contaminants has a swimming pool all year round. It can be caused by chlorine, which is released in a negligible amount from the water, and also by the ventilation. The right side of the pool with windows and ventilation system had during the whole year significantly lower values of biological contaminants than the left side without windows. The described situation well reflects the standard deviation, especially in case of bacteria in winter.

To study the influence of ventilation on the occurrence of microorganisms in the rooms there are statistically evaluated the differences between the ventilated and not ventilated spaces. The comparison of bacteria concentration and fungi concentration between the room without ventilation in summer and the same room with ventilation in winter are presented in the Table 5. The same letter (e.g. a) in both columns of the same line means, that there is not significant difference between both values. E.g. there is not significant difference between the bacteria concentrations in GA in summer and winter measurements. The difference of filamentous fungi concentration in GA in summer and winter measurements are significant.

Table 5. Results of measurement of microbiological contamination by air-borne microorganisms in summer and winter. Different superscript letters (a, b) are the sign of the high significant difference between the results measured in the not-ventilated (Summer) and ventilated (Winter) of the same room (*ANOVA*; *Tukey HSD Test*; $p \leq 0.05$)

Room	Bacteria		Filamentous fungi	
	Summer	Winter	Summer	Winter
	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD
GA	850 ± 137^{a}	$1,065 \pm 170^{a}$	118 ± 12^{a}	45 ± 3^{b}
GB	467 ± 39^{a}	$1,433 \pm 179^{b}$	115 ± 20^{a}	58 ± 28^{a}
GC	778 ± 54^{a}	$1,297 \pm 84^{b}$	133 ± 16^{a}	70 ± 7^{b}
SP	152 ± 39^{a}	300 ± 223^{a}	70 ± 20^{a}	55 ± 3^{a}

SD – Standard deviation.

As already mentioned, people are the source of bacterial and fungal contamination inside the rooms. The results in the Table 5 show that regardless of ventilation, during the winter with the presence of people in all spaces, the concentration of bacteria was higher than in the summer, without people. From this point of view the ventilation is not adequate for the removal of bacteria from ventilated spaces. From the results we can conclude that the great importance on the air quality in terms of a specific bacteria concentration has the specific volume of the room per one athlete. The worst situation is in rooms with the smallest volume, which has the largest biological load of the space.

Statistical evaluation showed that significantly higher concentrations of bacteria are only in the smaller GB gym and in the fitness GC. Higher concentrations of bacteria in the large gym GA and in pool SP were not statistically confirmed. This result can be explained by the positive effect of ventilation.

Another is the situation in these rooms with regard to the occurrence of a fungi. In all rooms the concentration of fungi has decreased in the winter, which can be explained by the positive influence of ventilation, the supply of fresh clean air and the removal of contaminated air out.

It can be concluded from this comparison that intensive ventilation has a positive effect on reduction of the microorganisms' concentration of in space. Intensive ventilation is necessary, especially in smaller rooms, with higher numbers of actively moving people.

Table 6 is focus on the presence of allergenic filamentous fungi (*Cladosporium* and *Aspergillus*). Different superscript letters (a, b, c) are the sign of the high significant difference between the rooms. The same letter (e.g. a) in lines of the same columns means, that there is not significant difference between values which were measured in different rooms. *Cladosporium* reached nearly 30% of all isolated filamentous fungi in the gym GA, surprisingly in winter. In other cases, its representation was lower (12–17%). However, its presence in the indoor air of the sports facilities may be potentially dangerous for students and other sportsmen. *Cladosporium* presents was negligible (< 4%) in both gyms (GA, GB) and fitness GC in the summer. Only in swimming pool SP *Cladosporium* occurred almost in 24% of filamentous fungi.

Table 6. Results of measurement of microbiological contamination by allergenic filamentous fungi *Cladosporium* and *Aspergillus* in summer and winter. Different superscript letters (a, b) are the sign of the high significant difference between rooms (*ANOVA*; *Tukey HSD Test*; $p \leq 0.05$)

Room	*Cladosporium*		*Aspergillus*	
	Summer	Winter	Summer	Winter
	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD	CFU m^{-3} ± SD
GA	0.0 ± 0.0^{a}	8.3 ± 4.4^{a}	18.3 ± 5.6^{a}	1.7 ± 2.2^{a}
GB	$1.7 \pm 2.2^{a,b}$	13.3 ± 7.8^{a}	3.3 ± 4.4^{b}	0.0 ± 0.0^{a}
GC	$5.0 \pm 6.7^{a,b}$	10.3 ± 6.7^{a}	3.3 ± 2.2^{b}	1.7 ± 2.2^{a}
SP	16.7 ± 5.6^{b}	8.3 ± 5.6^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{a}

SD – Standard deviation.

The genus *Aspergillus* was found only in gyms (GA, GB) and fitness (GC), not in the swimming pool SP in summer. Attention should be paid to his 16% presence in the gym GA, in other sports facilities (GB and GC), its share was only 2–3%. In winter the genus *Aspergillus* was not present at gym GB and swimming pool SP. The source in gyms GA and fitness GC was probably not in the air drawn from the outside.

Large differences between the sampling points (see SD) mean that there is no relation to the ventilation and outside air.

CONCLUSIONS

The results of measurements in the University sport facilities showed that:

- The average pollution by bacteria in all gyms was rather high during the summer without students, without ventilation and without cleaning of the rooms. The presents of students even increased the bacteria concentration several times despite intensive ventilation during the winter period.
- The lowest quantity of bacteria was in the swimming pool all year round.

- Very big influence on the indoor air cleanness and reduction of air pollution has the intensive ventilation. Intensive ventilation is more important if the room has small dimensions and number of sportsmen is high.
- The pollution of air by fungi was higher during the summer period without students but without cleaning and without ventilation. Sufficient ventilation by fresh and clean air caused lower pollution by fungi in all rooms during the winter period in spite of students' activities.

The pollution by air-born microorganisms in sports facilities can be considered as significant. It is necessary to continue in this research and focus attention mainly on filamentous fungi. From the practical point of view it is possible to recommend an improvement of the air-conditioning system function, which should be equipped with automatic control and sensors for measurement of temperature, relative humidity and CO_2.

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