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Microbiological Air Contamination in Hospital

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***Abstract* – The aim of this study was to assess the microbiological contamination levels of indoor pollution in General hospital in Doboj-Department of Microbiology and Patoanatomy and Department of Microbiology with Parasitology of the Republic of Srpska (Bosnia and Herzegovina). Concentration of bacteria and fungi was determined in order to evaluate the microbiological quality of the air in the subject Hospital. Furthermore, correlation of airborne bacteria and fungi with environmental parameters (temperature, relative humidity) was investigated. Air samples were collected during the winter season (February, 2017) at nine different locations. Variation of microorganism in air samples was discussed, through experimental measuring. The paper presents the average values of bacteria and fungi (in CFU/m^3). Maximum, minimum and other statistical values are in correlation with microclimatic parameters. Variations are directly connected to relative humidity. The results of this study show the high level of microbiological contamination of the air. Increased ventilation, alongside with HVAC system and other hygiene measures in the subject hospital would significantly contribute to the improvement microbiological quality of the indoor air.**

***Keywords* – Pollution; Monitoring; Bacteria; Fungi; Aerosol.**

I. INTRODUCTION

The effects of indoor air pollution are different for healthy people and people with chronic heart or lung disease. Indoor air quality (IAQ) is a concern in buildings but it is especially important in hospitals and other healthcare facilities. IAQ affects a wide range of factors: quality of outdoor air, building construction and materials, HVAC system, temperature, humidity, contaminant sources, occupants, and possible pollutant pathways as the basic factors that influence indoor air quality [Jaakkola et al., 1991]. This group additionally concluded that the literature indicates that in buildings with air-conditioning systems there may be an increased risk of SBS symptoms compared with naturally or mechanically ventilated buildings, and that improper maintenance, design, and functioning of air-conditioning systems contributes to the increased prevalence of SBS symptoms [Wargocki et al., 2002]. Multiple deficiencies in HVAC system design, construction, operation or maintenance, including some that cause pollutant emissions from HVAC systems, may contribute to the increases in symptom prevalence [Seppänen &Fisk, 2002].

Indoor microbial air quality could be affected by several factors: ambient air, soil, microclimatic factors, geographic location, every day human activity and ventilation. Microbial damage in indoor areas is most frequently caused by fungi and bacteria. In the environment, spores of bacteria and fungi may become airborne and are therefore ubiquitous. Airborne microorganisms may cause various negative effects, especially infectious, allergenic and immunotoxin diseases. Their accumulation can have a significantly negative effect on health, so the objective of this study was to investigate airborne fungi and bacteria collected in indoor environment. Polluted indoor air is a significant risk factor for serious illnesses and sometimes life-threatening health effects. [Božić et al., 2012].

Some people are more sensitive to indoor air pollution than others, especially_children, old-aged persons, people with chronic illnesses as well as people who are exposed to constant stress and a have weak immune system. All healthcare institutions have to pay particular care and attention to indoor air. Microclimate of the hospital premises is determined by thermal state of the environment and depends on temperature, humidity, air mobility, temperature of the surrounding surfaces and thermal radiation [Božić, 2010; Božić et al., 2012] Indoor climate in hospitals is usually determined by heating and ventilation, air conditioning units in separate rooms or central air conditioning.

There are a number of factors that may be related to airborne microorganisms and other sources of contamination in hospitals. As the exposure to fungi can cause adverse health effects in immune-compromised people [Park et al., 2013], it is essential to maintain the lowest possible airborne microorganism levels in hospitals. For this, it is crucial to identify the factors influencing these levels.

The state of indoor air monitoring in the Republic of Srpska and Bosnia and Herzegovina is at a very low level and it is necessary to improve it. There are significant researches in the field of air pollution in environmental, specially chemical parameters [Ilić & Janjuš, 2008, Ilić & Preradović, 2009, Lammel et al., 2010, Lammel et al., 2010, Gasic et al., 2010, Lammel et al., 2011], but there are no published studies on the bacteria and fungi in the indoor and outdoor air.

II. Aim Of The Paper

The aim of this paper was to determine the number of microorganisms, i.e. level of airborne bacterial and fungal contamination of the various indoor environments, in the General hospital St. Apostle Luke, in the City of Doboj (Republic of Srpska, Bosnia and Herzegovina). To this purpose concentration of bacteria and fungi population was measured in the sampling period, directly related to relevant microclimatic parameters (temperature and relative humidity).

III. Materials And Methods

Subject of this study is testing the presence of bacteria and fungi and microclimatic parameters (temperature and relative humidity) in the General hospital St. Apostle Luke in Doboj-Department of Microbiology and Patoanatomy and Department of Microbiology with Parasitology.

For this research, used a portable bio impactor Air sampler Sampl'air™ Lite, was used Manufactured by BioMérieux S.A, for microbial air monitoring based on the principle of air impaction. Collection of microorganisms is by air aspiration, through a grid which is situated a few millimetres above a Petri dish. The viable organisms in the air are impacted on the agar. Air flow was adjusted at 200 l/min and directed over the surface of a petri dish containing appropriate solid culture media (Trypcase Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) (Fig. 1).

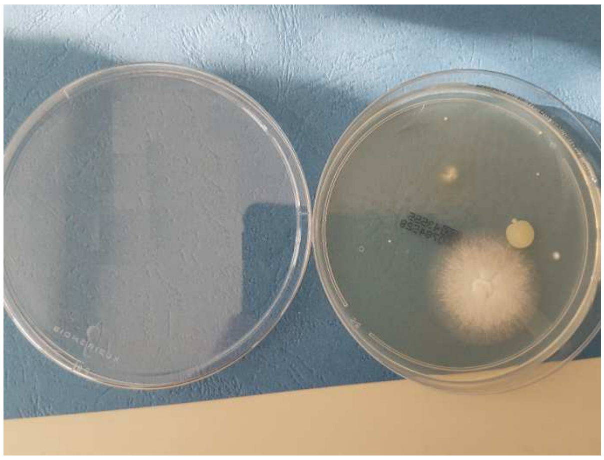


Fig. 1. Agar medium (Petri dish)

Before each sampling session, the head of the air sampler was properly sterilized as a control. The samples were obtained during 1 day (August 23, 2017).

Microbial concentration for each temporal series was expressed as mean values of colony-forming units (CFU) per m^3 of air analysed. The most probable number is assumed, using the table of correspondence. In parallel, a correlation was established between the values found and the microclimatic parameters - temperature and relative humidity, using portable multifunctional device Metrel MI 6401 Poly.

Estimation of airborne fungi was carried out by growth on agar medium. Sampled plates were incubated at 30-35 $^{\circ}$C for 48 h in the case of bacterial analysis and at 28-32 $^{\circ}$C for 72 h in the case of fungal sampling (Fig. 2).

Fig. 2. Incubation of the samples

After the incubation period, colony counting is done with EasyCount 2 colony counter with supporting software and the plates were examined for CFU counting (Fig. 3).

Fig. 3. Colony counting

Statistical data processing while determining interdependence and relationship between bacteria and fungi and microclimatic parameters (temperature and relative humidity), were calculated and displayed with frequency polygon chart and boxplot. Descriptive statistical parameters like mean, standard deviation (SD), median, minimum (min) and maximum (max) were applied to the data. To evaluate the relationship between the concentration of airborne fungi (as CFU/m^3) and microclimatic parameters (relative humidity and temperature), Bivariate Correlations study (Spearman's, Pearson's and Kendall's Correlation Coefficient test) was performed. A significance level of p value $< .05$, $p < .01$ and $p < .001$ was used.

IV. Results And Discussion

Fungal and bacterial flora can be hazardous for health, particularly in rooms with heating, ventilation and air-conditioning and HVAC systems and can breed allergies, SBS symptoms ("sick building syndrome") [Božić et al., 2017] causing irritation of mucous membranes, bad physical condition, tiredness, headaches, vertigo, decrease of concentration, memory and intellectual work ability, dermatosis, respiratory diseases (including asthma) and cancer [Stryjakowska-Sekulska et al., 2007]. Fungi, or their spores, are a frequent cause of asthma, allergic alveolitis, vasomotor rhinitis and urticaria [Ilić, 2015]. Indoor airborne bacteria and fungi levels can be selected as indicators of a healthy indoor environment [Wong et al., 2008], and are usually classified as natural sources of pollution [Ilić et al., 2012b]. Measured values of the number of bacteria and fungi (in CFU/m^3) in the hospital rooms are shown in Table 1, together with microclimatic parameters (temperature and relative humidity).

Table 1. Concentration of the bacteria and fungi and microclimatic parameters

	Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Total (CFU/m^3)	Temperature T (^{0}C)	Relative humidity RH (%)
1.	290	235	525	26.4	59
2.	395	390	785	24.8	57.2
3.	865	115	980	23.1	57.9
4.	655	400	1055	24.8	57.0
5.	1480	335	1815	24.9	57.3
6.	1740	305	2045	24.6	57.6
7.	6295	350	6645	24.8	57.3
8.	555	105	660	26.0	61.0
9.	455	380	835	25.1	58.0

Airborne microorganisms originate not only from people (including patients), but also are spawned by various indoor hospital characteristics and outdoor environmental sources [Park et al., 2013]. As represented in the Tables 1 and 2, the concentrations of bacteria varied and ranged from 290 CFU/m^3 to 6,295 CFU/m^3. Maximum fungal concentration was 400 CFU/m^3 and the minimum was 105 CFU/m^3. The average levels of bacteria (1,414 CFU/m^3) and fungi (290 CFU/m^3) indicated that all hospital rooms were generally contaminated.

Table 2. Statistical summary of bacteria and fungi and microclimatic parameters

	Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Total microorganism (CFU/m^3)	Temperature T ($^\circ$C)	Relative humidity RH (%)
Mean	1,414	290.6	1705	24.94	58.03
Median	655.0	335.0	980.0	24.80	57.60
Std. Deviation	1,896	114.0	1,922	0.9248	1.264
Minimum	290.0	105.0	525.0	23.10	57.00
Maximum	6,295	400.0	6645	26.40	61.00

Quantitative standards/guidelines for fungi range from less than 100 CFU/m^3 to more than 1,000 CFU/m^3 as the upper limit for non-contaminated indoor environments, but during the study was determined value is exceeded in most measurement points, and has exceeded the average value [Rao et al., 2006].

The degree of contamination by total fungi in the hospital environment may increase dramatically in combination with various factors, such as the presence of construction activity and a favourable microclimate [Perdelli et al., 2006]. The results of the present study reveal various degrees of contamination in all of the environments examined. European Commission Report had decided following limits for bioaerosol: 0 undetectable, 1-499 CFU/m^3 low, 500-999 CFU/m^3 medium and > 1000 CFU/m^3 high [CEC, 1994] and average values of bacteria in research 1,414 CFU/m^3 indicate high level of contamination, with the high measured value on a large number of measuring points. WHO has set the guidelines of bioaerosol counts at 500 CFU/m^3 [WHO, 1990] and American Conference of Governmental Industrial Hygienists (ACGIH) with the cultivable counts for total bacteria not to exceed 500 cfu/m^3 [Jensen & Schafer 1998, Katiyar, 2013], which confirms the thesis that there is a high level of contamination in the Hospital. Microbial contamination including fungi may threaten human health and the quality of life, and indoor contamination should be reduced to safe levels [Bamba et al., 2014]. It is necessary to take urgent measures in order to improve air quality and in that way, to protect health of people [Ilić et al., 2012a].

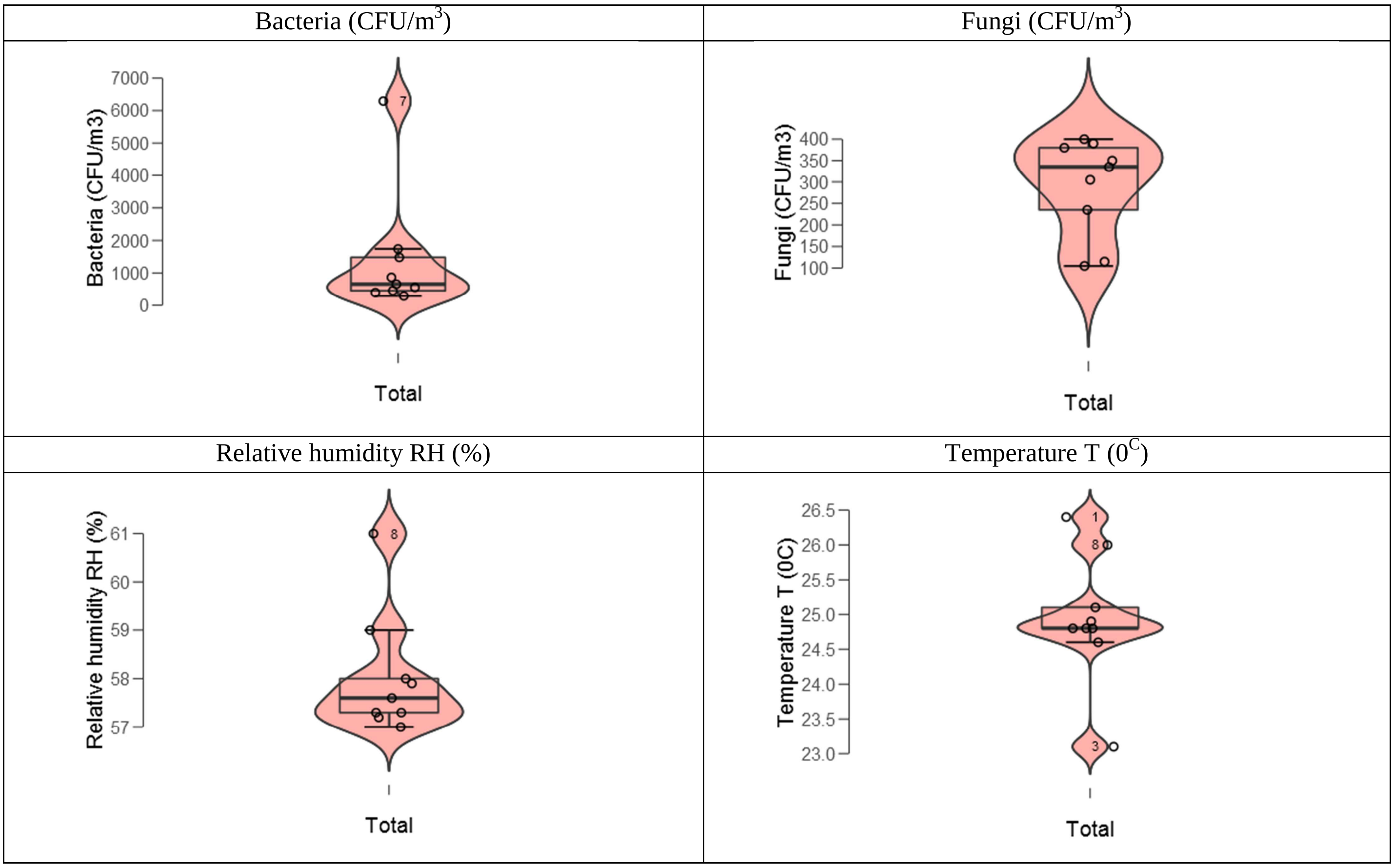
The Graph 1. shows boxplot for the values of bacteria, fungi, relative humidity and temperature. The boxplot is displaying the distribution of data based on the five number summaries: minimum, first quartile, median, third quartile, and maximum.

Hospital buildings may be regarded as dynamic environments affected by season, weather conditions, indoor ventilation system design and operation, intrusion of moisture, outdoor microbial load, the number of occupants and visitors, and by human activities. These factors can be related to a condition for microbial growth. The relationship between bacteria and fungi concentrations (CFU/m^3) and relative humidity in the study area was analysed using correlation analysis. The results are shown in Table 2. The results of the correlation analysis between bacteria and fungi concentration and relative humidity are shown for the level of significance $p < .05$, $p < .01$ and $p < .001$ (Table 2, Graph 2).

Concentration of fungal spores in indoor and outdoor air is strongly related to meteorological factors such as temperature and relative humidity [Reanprayoon et al., 2005]. Using Pearson Correlation Coefficient, there is a negative correlation ($r = -0.748$) between the fungi concentrations and relative humidity. However, no such correlation was discovered between fungi and relative humidity in researches by other authors [Aydogdu et al., 2005], which is in contradiction with the research in air-conditioned rooms [Wong et al., 2008], while in the investigated areas there is no air conditioning. Negative correlation may indicate the presence of a specific species of fungi. According to research in Thailand Pearson correlation coefficient (r) between relative humidity parameters was found to be positively with *Alternaria* ($r = 0.824$, $P<0.05$), while *Aspergillus* was detected to be negatively correlated (r

= -0.849, P<0.05) [Reanprayoon et al., 2005] and in accordance with the results it should be continue researches of the presence of certain types of microorganisms.

This is a moderate negative correlation, which means there is a tendency for high X variable scores (fungi) to go with low Y variable scores (relative humidity), and vice versa. The value of r^2, the coefficient of determination, is 0.560. The value of R is -0.795 and the fungi and relative humidity value of p is 0.010 (Spearman's Rank Correlation).



Graph 1. Box plot for bacteria, fungi, relative humidity and temperature

By normal standards, the association between fungi and relative humidity would be considered statistically significant. Correlation was also confirmed by the Kendall rank correlation coefficient, for level of significance $p < .01$ (Table 3, Graph 2).

Considering that there is a negative correlation between fungi and relative humidity, the need for further investigation on the effect of relative humidity in indoor environment in the Hospital is suggested.

Table 3. Correlation between bacteria and fungi concentrations (CFU/m3) and relative humidity

	Correlation tests and coefficients	Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Total microorganism (CFU/m^3)	Temperature (^{0}C)	Relative humidity (%)
Bacteria (CFU/m^3)	Pearson's r	—				
	p-value	—				
	Spearman's rho	—				
	p-value	—				

	Correlation tests and coefficients	Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Total microorganism (CFU/m^3)	Temperature (^{0}C)	Relative humidity (%)
	Kendall's tau B	—				
	p-value	—				
Fungi (CFU/m^3)	Pearson's r	0.200	—			
	p-value	0.606	—			
	Spearman's rho	-0.050	—			
	p-value	0.912	—			
	Kendall's tau B	0.000	—			
	p-value	1.000	—			
Total microorganism (CFU/m^3)	Pearson's r	0.998 ***	0.257	—		
	p-value	< .001	0.505	—		
	Spearman's rho	0.933 ***	0.283	—		
	p-value	< .001	0.463	—		
	Kendall's tau B	0.833 ***	0.167	—		
	p-value	< .001	0.612	—		
Temperature (^{0}C)	Pearson's r	-0.160	0.018	-0.157	—	
	p-value	0.681	0.963	0.687	—	
	Spearman's rho	-0.593	-0.153	-0.627	—	
	p-value	0.092	0.695	0.071	—	
	Kendall's tau B	-0.435	-0.087	-0.493	—	
	p-value	0.110	0.750	0.070	—	
Relative humidity (%)	Pearson's r	-0.295	-0.748 *	-0.336	0.571	—
	p-value	0.440	0.020	0.377	0.109	—
	Spearman's rho	-0.318	-0.795 *	-0.552	0.528	—
	p-value	0.404	0.010	0.123	0.144	—
	Kendall's tau B	-0.254	-0.704 **	-0.423	0.353	—
	p-value	0.345	0.009	0.116	0.199	—
* p < .05, ** p < .01, *** p < .001						

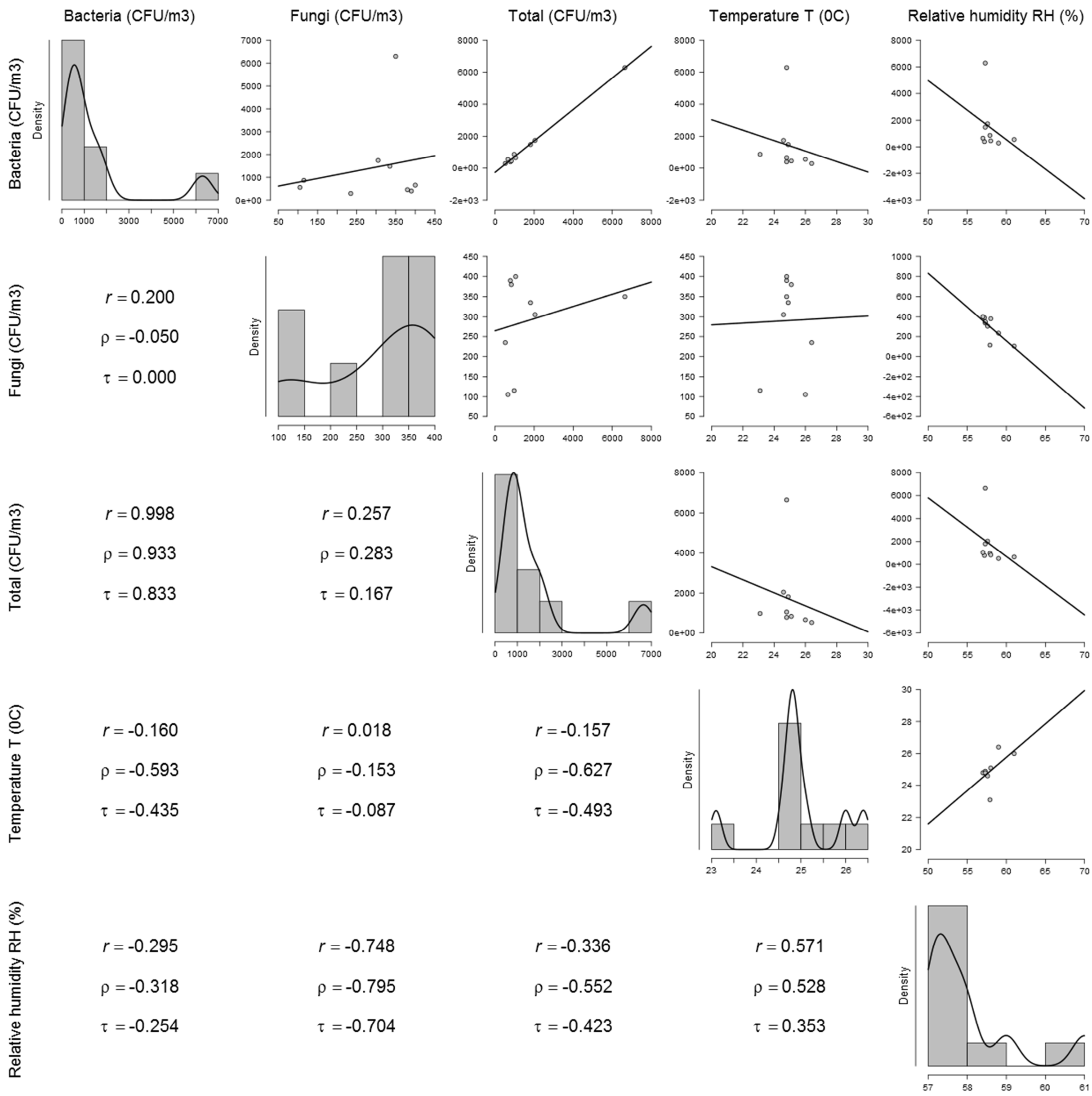
Many publications are studying the effect of environmental parameters (e.g. humidity) on the survival of airborne infectious organisms (bacteria and fungi). Factors such as relative humidity can inactivate free-floating, airborne infectious organisms [Tang, 2009], which was confirmed during the research.

Graph 2. shows a frequency polygon of bacteria, fungi and total microorganism concentrations at the Hospital where the data are binned into regular intervals. Frequency of measured values bacteria, fungi and total microorganism clearly indicates that the largest number of samples has very high value of microorganism [WHO, 1990, CEC, 1994, Jensen and Schafer 1998, Katiyar, 2013].

Analysis shows that relative humidity had a negative impact on the concentration level of airborne fungi and bacteria (Graph 2).

V. Conclusion

This paper presents the results of the measurement of bacteria and fungi and microclimatic parameters in the General hospital St. Apostle Luke, Doboj. The results show a high level of microbiological contamination of the air. Statistical analysis confirms direct connection between the number bacteria and fungi and microclimatic parameters, especially relative humidity.



Graph 2. Correlation between the number of bacteria and fungi and relative humidity and frequency

The ideal approach to preventing the adverse effects of indoor pollution is through a proper ventilation and preventive maintenance of air handling systems. Increased ventilation, alongside with systematic maintenance of HVAC system and other hygiene measures in the subject hospital would significantly contribute to the improvement microbiological quality of the indoor air.

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