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MICROBIOLOGICAL INDOOR AIR QUALITY IN FACULTY'S ROOMS: RISKS ON STUDENTS' HEALTH

Olgica D. Stefanović*, Jelena D. Radosavljević, Marijana M. Kosanić

University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia
*Corresponding author; E-mail: olgica.stefanovic@pmf.kg.ac.rs

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ABSTRACT. This study deals with a quantitative and qualitative analyze of indoor airborne microbiota and estimation of microbiological quality of indoor air in faculty's rooms during the summer semester of 2017/18 school year. The concentration of bacteria was significantly higher than the concentration of fungi. The species that belong to human skin microbiota or of environmental origin were identified. According to indoor air quality breakpoints, low to medium/high level of bacterial and fungal air contaminations was noticed.

Keywords: air pollution, air quality, environmental monitoring, public health.

INTRODUCTION

Indoor air constantly contains a certain concentration of microorganisms. In the air, the microorganisms (bacteria, archaea, fungi, protists, and algae) as well as viruses, commonly exist alone or adsorbed on dust particles or saliva/water droplets forming bioaerosols. Microorganisms' concentration depends on several factors such as: temperature and relative air humidity, the number of occupants and their activities in rooms, hygiene condition, and ventilation, even on the size of the bioaerosols and the speed of sedimentation (HAAS *et al*., 2013).

The most of microorganisms present in the indoor air do not cause adverse health effects. However, some airborne fungi, bacteria, as well as viruses are recognized as the causative agents of allergies, asthma, and infections (WANNER and GRAVESEN, 1993; DOUWES *et al*., 2003; HEEDERIK and VON MUTIUS, 2012). Health risks appear when the concentrations of these infectious agents become very high. It is established that some viable bioaerosols cause infectious human diseases, such as tuberculosis, Legionnaire's disease and various forms of bacterial and viral pneumonia, influenza, measles, and gastrointestinal illness (BOUILLARD *et al*., 2005; TSAI and MASHER, 2005). In addition, endotoxin, a component of the outer membrane of Gram-negative bacteria, has been associated with asthma severity (BOUILLARD *et al*., 2005). On the other side, molds produce antigenic proteins that may cause an allergic reaction in sensitized people, including asthma, rhinitis, and conjunctivitis

(JAAKKOLA *et al*., 2010; KARVALA *et al*., 2010). Opportunistic infections caused by some molds are generally limited to people with immunosuppressed immune system. Moreover, molds produce volatile organic compounds that cause the "musty" smell and can be irritating to the human mucous membranes (NEVALAINEN, 2015). World Health Organization (WHO) guidelines for indoor air quality: dampness and mold (WHO, 2009) has recognized connection between poor indoor air quality and certain health problems (irritation of mucous membranes, skin and eyes, fatigue, headache, malaise, lethargy, difficulty concentrating, sensitivity to odors and flu-like symptoms). These symptoms are known as Sick Building Syndrome (SBS). The causative agent has not been identified, moreover, the cumulative effects of numerous factors (presence of microorganisms and their metabolites, volatile organic compounds, humidity) and their excess level in the air have affected human health.

Microbiological air quality is an important criterion that must be considered when indoor workplaces are designed to provide a safe environment. Air quality of indoor environments is one of the main factors affecting health, well-being, and productivity of people. The indoor air quality depends on variety physical, chemical, and microbiological parameters (BASIŃSKA *et al*., 2019). Recent studies have shown that there is an increase in a number of allergic reactions to microbial spores in young people (WHO, 2009; BRĄGOSZEWSKA *et al*., 2018) as well as potential risks of infections and contaminations. Analysis of indoor airborne microbiota represents an important parameter in characterizing specific environments, identifying possible biological risks, and acquiring information for prevention of health hazard. Therefore, there is need for regular monitoring of indoor air quality.

Monitoring data on microbiological quality of indoor air of educational buildings (faculties) in Serbia are unreachable. Students spend much of their working time indoors so poor indoor air could affect their health. Therefore, the aim of this study was to quantitative and qualitative analyze airborne microbiota and estimate the microbiological quality of indoor air in faculty's rooms.

MATERIALS AND METHODS

Sampling

Sampling area included four faculty's rooms with natural ventilation at the Faculty of Science, University of Kragujevac, Serbia, as follows: 1. Lecture room A-I-1 (surface area of 117.5 m^2), 2. Lecture room A-I-28 (surface area of 54.8 m^2), 3. Student laboratory A-II-2 (surface area of 48 m^2) and Reading room A-0-1 (surface area of 55.6 m^2). Sampling was carried out during the summer semester of 2017/18 school year, from March to May, once a week, including two sampling period, in the morning (9 a. m.) and in the afternoon (4 p. m.).

Determination of the total number of bacteria and fungi

The total number of mesophilic aerobic bacteria and fungi was determined by passive air sampling technique - the settle plate method using 9 cm-diameter Petri dishes. Bacteria and viable fungal propagules were collected on Nutrient agar (Torlak, Serbia) with amphotericin B (Sigma-Aldrich, USA) and Sabouraud dextrose agar (Torlak, Serbia) with amoxicillin (Sigma-Aldrich, USA), respectively. Three sampling points were set in each sampling room. The Petri dishes were placed on the center of the studied rooms at a height of about 1 m above the floor. The sampling time was 30 minutes. After exposure, the samples were incubated at 25 °C for 7 days. Colony forming units (cfu)/m^3 were estimated by the Omeliansky formula (BORREGO *et al*., 2010) (1):

$$N = 5 \times 10000 \times a/(p \times t) \quad (1)$$

where N = cfu/m^3 of indoor air; a = number of colonies per Petri dish; p = Petri dish surface (cm^2); t = exposure time (min).

Determination of the index of microbial air contamination

The index of microbiological air contamination (IMA) was determined as described (PASQUARELLA *et al*., 2000). A Petri dishes, 9 cm in diameter, containing Nutrient agar (Torlak, Serbia) were exposed to air according to the 1/1/1 scheme (for 1 h, 1 m from the floor, at least 1 m away from walls or any relevant physical obstacle). After 48 h incubation at 37 °C for the isolation of human pathogenic bacteria, the grown colonies were counted. The number of colonies was the IMA. Five classes of IMA were devised: 0-5 very good; 6-25 good; 26-50 fair; 51-75 poor; >76 very poor quality of air. The values of IMA were established related to different infection or contamination risks.

Identification of isolates of bacteria and fungi

After the sampling and determination of the total number of bacteria and fungi, every morphological different colony of bacteria or fungi were re-inoculated on a nutrient medium (Nutrient agar for bacteria and Sabouraud dextrose agar for fungi). The established collection of isolates was maintained at 4°C until the time of identification. Bacterial isolates were characterized by microscopic examination and identified further by biochemical tests (HOLT *et al*., 1994). The fungal isolates were identified by colonial appearance, microscopic examination of the spore and hyphal characteristics (BARNETT and HUNTER, 1998).

Statistical analysis

Statistical analysis was conducted using SPSS 20.0 software. Student's *t*-test was used to test the significance between two groups. One-way analysis of variance (ANOVA) was used to test the significance among different groups. $P<0.05$ was considered to indicate statistically significant differences.

RESULTS AND DISCUSSION METHODS

Total number of bacteria and fungi

The total number of bacteria, the total number of fungi and IMA indices are shown in Tables 1-6. The parameters were monitored during the summer semester, once a week, in the morning and in the afternoon. Passive sampling of airborne microorganisms by the settle plate method provides a valid health risk assessment as it detects larger bioaerosols which under the effect of gravity falls onto a critical surface, such as a working area in breathing zone (NAPOLI *et al*., 2012).

The total number of mesophilic aerobic bacteria, for all research period, was from 26 to 2117 cfu/m^3 while the total number of fungi was from 21 to 749 cfu/m^3. The concentration of bacteria was significantly higher than the concentration of fungi ($P=0.0005$). The statistically significant difference in the total number of bacteria and fungi between sampling months (March-May) was notice ($P=0.0005$). It was observed that the concentration of bacteria and fungi was in increase level. The concentration of bacteria was significantly higher in April and May than in March while the concentration of fungi was constantly increased. On the other side, the total number of bacteria and fungi in the morning was not significantly differed than in the afternoon ($P>0.05$). Furthermore, the concentration of

microorganisms was not statistically significant influenced by the surface area of sampling rooms and the number of students.

In March, the total number of mesophilic aerobic bacteria was in the range from 35 to 828 cfu/m^3, while the number of fungi was from 21 to 201 cfu/m^3 (Tab. 1). The number of microorganisms varied during the month of March depending on activities at Faculty. The second week of March was the exam week (no lectures), which is clearly affected the number of microorganisms in lecture rooms, it was the lowest or not detected. In this period, the highest number of bacteria was noticed in the reading room. When the lectures were continuing, in the last two weeks of March, the number of bacteria and fungi was noticeably increased. The IMA indicated a low to moderate level of contamination (very good-fair air quality) (Tab. 2).

Table 1. The total number of bacteria/fungi (cfu/m^3) in indoor air in faculty's room during the month of March.

Room		**Sampling period**							
		1. week		**2. week**		**3. week**		**4. week**	
		a.m.	***p.m.***	***a.m.***	***p.m.***	***a.m.***	***p.m.***	***a.m.***	***p.m.***
A-I-1	**bacteria**	42	47	116	-	233	106	289	88
	fungi	35	33	-	-	153	169	167	35
A-I-28	**bacteria**	42	42	42	-	106	276	124	70
	fungi	-	23	21	-	63	201	124	61
A-II-2	**bacteria**	84	63	116	-	201	254	376	105
	fungi	-	82	42	-	138	127	49	74
A-0-1	**bacteria**	35	48	828	-	55	191	270	366
	fungi	-	21	42	-	76	118	148	54

– not detected.

Table 2. The index of microbiological air contamination during the month of March.

Room	**Sampling period**							
	1. week		**2. week**		**3. week**		**4. week**	
	№ of colonies	**Level**	**№ of colonies**	**Level**	**№ of colonies**	**Level**	**№ of colonies**	**Level**
A-I-1	14	good	-	-	3	very good	3	very good
A-I-28	17	good	2	very good	9	good	28	fair
A-II-2	44	fair	2	very good	22	good	4	very good
A-0-1	23	good	20	good	25	good	11	good

– not detected.

In April, the total number of mesophilic aerobic bacteria was in the range from 82 to 2117 cfu/m^3, while the number of fungi was from 47 to 476 cfu/m^3 (Tab. 3). The number of microorganisms during the month of April was in increase, and the number of bacteria was greater than fungi. The highest concentration was observed in the first and the second week of sampling. Regular teaching process, students attending lectures obviously contributed to the increased number of microorganisms. According to the IMA a low to medium degree of contamination was noticed (Tab. 4).

Table 3. The total number of bacteria/fungi (cfu/m^3) in indoor air in faculty's room during the month of April.

Room		Sampling period							
		1. week		2. week		3. week		4. week	
		a.m.	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*
A-I-1	**bacteria**	446	662	258	2117	247	690	482	-
	fungi	241	155	99	476	203	52	64	-
A-I-28	**bacteria**	812	775	2062	368	146	258	319	258
	fungi	330	199	250	188	148	104	78	68
A-II-2	**bacteria**	310	164	94	893	174	141	352	82
	fungi	58	117	47	209	-	-	70	78
A-0-1	**bacteria**	487	723	509	265	188	282	470	941
	fungi	202	68	358	191	117	54	120	148

– not detected.

Table 4. The index of microbiological air contamination during the month of April.

Room	Sampling period							
	1. week		2. week		3. week		4. week	
	№ of colonies	Level	№ of colonies	Level	№ of colonies	Level	№ of colonies	Level
A-I-1	39	fair	10	good	9	good	-	-
A-I-28	14	good	1	very good	5	very good	2	very good
A-II-2	3	very good	34	fair	8	good	1	very good
A-0-1	11	good	14	good	12	good	22	good

– not detected.

In May, the total number of mesophilic aerobic bacteria was in the range from 26 to 838 cfu/m^3, while the number of fungi was from 60 to 749 cfu/m^3 (Tab. 5). The concentration of fungi during the month of May was in increase probably due to warmer weather, prolonged natural ventilation and thus greater contact with the outside air. It is known that the number of fungi indoors is depended on the number and diversity of "outdoors" fungi. The IMA demonstrated a low level of contamination (Tab. 6).

Literature search aimed on microbiological indoor air quality of faculties' rooms have showed similar results. Other researchers have noticed a higher number of bacteria than fungi. GUAN *et al*. (2015) in the reading rooms of faculty in China determined the total number of bacteria between 209 and 838 cfu/m^3. The total number of bacteria and fungi in the reading rooms at the University of Ethiopia was between 367 and 2595 cfu/m^3 and 524 and 1992 cfu/m^3, respectively (HAYLEEYESUS and MANAYE, 2014). The highest total mean bacterial load was 2826.35 cfu/m^3 in the morning and 4514.63 cfu/m^3 in the afternoon in classrooms of primary schools determined by settle plate method (ANDUALEM *et al*., 2019). Two-years research (STRYJAKOWSKA-SEKULSKA *et al*., 2007) was carried out at the University in Poland. During the first year, the number of bacteria was 120 to 2300 cfu/m^3 and fungi from 130 to 1100 cfu/m^3. During the second year, the number of bacteria was 110 to 3300 cfu/m^3, while the number of fungi was 90 to 800 cfu/m^3.

Table 5. The total number of bacteria/fungi (cfu/m^3) in indoor air in faculty's room during the month of May.

Room		Sampling period							
		1. week		2. week		3. week		4. week	
		a.m.	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*
A-I-1	**bacteria**	304	146	173	112	52	363	550	68
	fungi	279	268	439	749	163	131	334	170
A-I-28	**bacteria**	225	104	146	269	183	26	802	262
	fungi	356	146	289	165	356	111	348	243
A-II-2	**bacteria**	235	799	323	149	183	52	550	537
	fungi	111	183	222	211	196	104	483	484
A-0-1	**bacteria**	373	437	138	173	94	68	838	225
	fungi	343	277	340	374	199	60	138	235

Table 6. The index of microbiological air contamination during the month of May.

Room	Sampling period							
	1. week		2. week		3. week		4. week	
	№ of colonies	Level	№ of colonies	Level	№ of colonies	Level	№ of colonies	Level
A-I-1	1	very good	-	-	1	very good	3	very good
A-I-28	8	good	-	-	3	very good	2	very good
A-II-2	4	very good	-	-	-	good	17	good
A-0-1	5	good	-	-	13	good	21	good

– not detected.

Identification of bacterial and fungal microbiota

Among the bacterial isolates collected, the most numerous were Gram-positive cocci (16 isolates), then Gram-positive rod-shaped bacteria (5 isolates) and, finally, Gram-negative rod-shaped bacteria (6 isolates). The most dominant were bacterial species from the genera: *Micrococcus*, *Staphylococcus*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Pseudomonas* (Fig. 1).

Quality characteristics of mycobiota isolated from the air in faculty's rooms showed dominating contributions of the genera: *Aspergillus* (13 isolates), *Cladosporium* (4 isolates), *Penicillium* (2 isolates), *Fusarium* (2 isolates), *Candida* (2 isolates), *Botrytis* (1 isolate), and *Trichoderma* (1 isolate) (Fig. 2). The genus *Aspergillus* has contained four identified species, *A. fumigatus*, *A. flavus*, *A. versicolor* and *A. niger*, the genus *Cladosporium*, two identified species *C. cladosporioides* and *C. spaerospermum*, the genus *Penicillium* with one identified species, *P. expansum* and the genus *Fusarium* with two identified species, *F. oxysporum* and *F. solani*.

In addition to the total number of microorganisms, the identification of isolated air microbiota is important for estimation of air quality and potential risk to public health because of eventually detection of some pathogenic microorganisms. This research showed that the taxonomic profile of the air microbiota isolated from faculty's rooms consisted of human-associated and environmental taxa. It was observed that Gram-positive bacteria were more abundant. They possess better adaptation (thick cell walls, spores) and tolerance to dehydra-

tion and adverse environmental conditions than Gram-negative bacteria. The most isolated bacteria were Gram-positive cocci (*Micrococcus*, *Staphylococcus*), which are, generally, associated to human skin and mucosa, thereby suggesting that the main bacterial contamination suspended in the indoor air derives from human presence. The other isolates were from genus *Bacillus* originated from environmental habitats (soils).

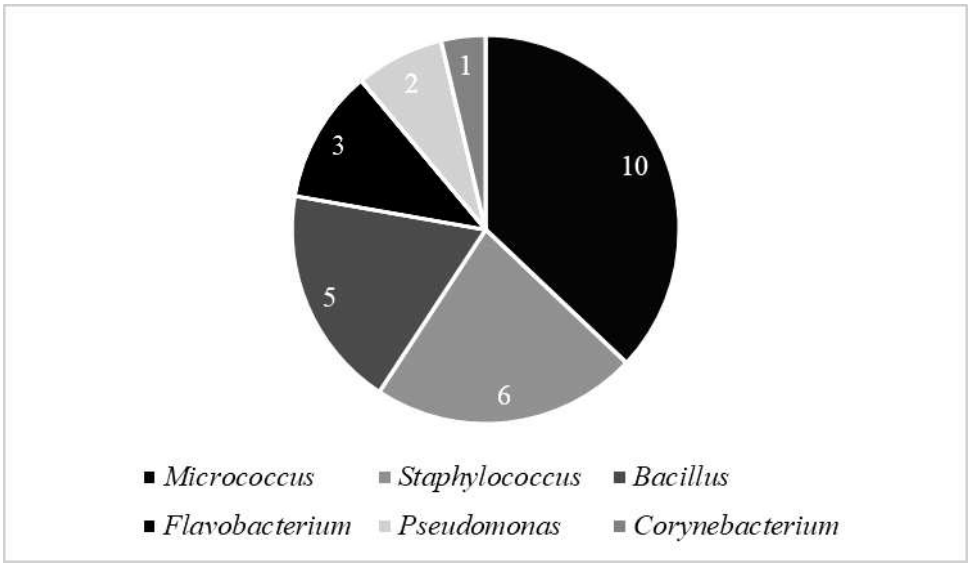

Figure 1. The isolated genera of bacteria and the number of strains.

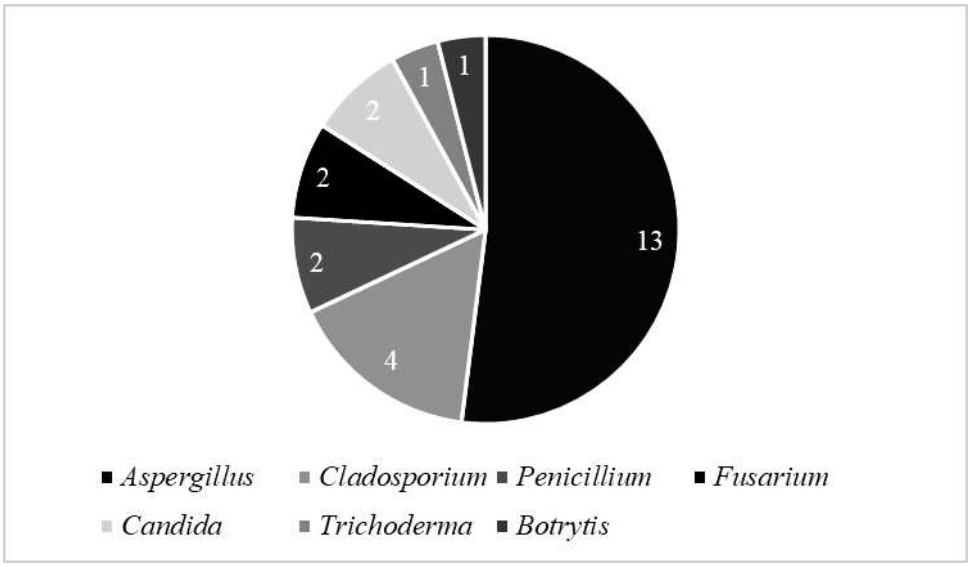

Figure 2. The isolated genera of fungi and the number of strains.

The isolated fungi were from genera *Aspergillus*, *Penicillium*, as well as so-called "outdoor mold" *Cladosporium*, *Alternaria*, *Trichoderma*, *Botrytis*. Moreover, the yeast *Candida*, habitant of human skin, hair, and nails was also detected. Species from the genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichoderma*, *Alternaria* are recognized as species which, in increased concentrations, may contribute to the respiratory problems for asthmatic patients or patients with allergies (SHARPE *et al*., 2015).

The mentioned genera of bacteria and fungi have been shown to be among the most common microorganisms often isolated from the indoor air (GÓRNY and DUTKIEWICZ, 2002;

STRYJAKOWSKA-SEKULSKA *et al*., 2007; GUAN *et al*., 2015; SAVKOVIĆ *et al*., 2021). Owing to sequencing technology, in the last several years, more detailed information of indoor air microbiota is available. HEWITT *et al*. (2012) have, also, concluded that a primary source of the bacteria present in the indoor air was the human body and the microbiota which inhabit the skin, oral cavity, mucous membrane of the nose. So that the isolated species of the genera *Streptococcus, Corynebacterium, Flavimonas, Lactobacillus, Prevotella, Neisseria, Pseudomonas, Actinomyces* are common. Also, the authors have found the species which are associated with the digestive tract of human Bacteroidetes, *Lactobacillus*, and members of the Enterobacteriaceae. Most bacteria were commensals and healthy people do not pose a health problem. The second source of bacteria in the air was the environment itself and thus were in the air present, and species of the genera *Bacillus, Bradyrhizobium, Planomicrobium, Planococcus* and members of the Microbacteriaceae. In addition, more diverse mycobiota was observed by PCR and clone library sequencing (PITKÄRANTA *et al*., 2005). The authors detected yeasts from genera: *Malassezia*, *Candida*, *Cryptococcus*, and *Rhodotorula*, especially in winter samples, as well as filamentous fungi from genera: *Cladosporium*, *Penicillium*, *Acremonium*, *Aspergillus*, *Fusarium*, *Mucor*, *Trichoderma*, *Chetomium*, *Alternaria*, *Leptosphaerulina*, *Macrophoma*, *Ustilago*, and yeast-like fungi *Aureobasidium*, *Filobasidium*, *Rhodosporidium*.

In this study, the microbiological quality of the air in the lecture rooms and reading room of the Faculty of Science, Kragujevac, Serbia was evaluated. As parameters of quality, the total number of bacteria/fungi and identification of collected isolates were determined. The breakpoints recommended by the American Industrial Hygiene Association (AIHA) for fungi in indoor air of business building, the Commission of the European Communities (CEC) for bacteria and the IMA index (indicate the number of opportunistic pathogenic bacteria) were used as breakpoints for evaluation of air quality (WANNER and GRAVESEN, 1993, HUNG *et al*., 2005; STRYJAKOWSKA-SEKULSKA *et al*., 2007).

The CEC recommendations, regarding the total number of bacteria, define several levels of bacteriological air contamination: 1-499 cfu/m^3 – low level, 500-999 cfu/m^3 – medium level, >1. 000 cfu/m^3 – high level, and >2. 000 cfu/m^3 – very high level. Appling these breakpoints, during the sampling months, the April and the May, medium to very high level of bacterial air contaminations was noticed. According to determined IMA index, very good to fair level of air quality was detected during sampling period. The IMA index is a valuable parameter which defines different levels of contamination in places at different biorisk. For example, hospital operation rooms, with very high risk, should have a maximum IMA value of 5. In this study, the IMA values were in the range 1 – 44. This parameter, also, confirmed the fair air quality in April and May.

Regarding the total number of fungi, the AIHA has recommended that the number of fungal spores in business buildings should be less than 250 cfu/m^3. If we use this breakpoint and compare with results of this study, the students, especially in May, were exposed to a higher concentration of fungi.

The obtain results confirm the importance of regular monitoring of microbiological quality of air in educational buildings. As it can be seen, the students have been exposed to higher concentration of bacteria and fungi, in particular periods. Many factors affect microbiological indoor air quality. These factors include poor ventilation, problems controlling temperature, high or low humidity, the number, and activities of occupants. Accordingly, frequent natural ventilation, decrease of humidity and adequate hygiene represents the primary procedures for safe environment.

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