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THE INFLUENCE OF UV-LED LAMPS RADIATION ON INDICATORS OF MICROFLORA IN UNIVERSITY AUDITORIUMS

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Introduction. There is a constant presence of a certain amount of bacteria, fungi and viruses in air and on surfaces of premises where a man spend a significant part of time today, that requires preventive measures. Recently, bactericidal ozone-free light-emitting diode (LED) sources of UV radiation have been introduced to disinfect the air in premises of various purposes, that requires researches of their effectiveness and hygienic regulation.

The aim of the research is to determine the effectiveness of the use of open-type bactericidal ultraviolet monochrome LED lamps for disinfection of work surfaces in auditoriums of a higher educational institution.

Materials and methods of the research. The studies of the bactericidal efficiency of open-type LED irradiators were carried out in three auditoriums of the Kiev National University of Civil Engineering and Architecture (KNUCEA) of the Ministry of Education and Science of Ukraine. LED UVC T5-5W-275NM lamps with a wavelength of 280 nm were installed in two auditoriums and their efficiency was assessed. Bactericidal air recirculators were additionally installed in one auditorium together with LED UVC T5-5W-275NM lamps and their mutual impact on the quantity of CFUs in this premise was evaluated. The duration of use of the bactericidal equipment was 3 months. The contamination of work tables in the auditoriums by an amount of colony-forming units (CFU) per 1 dm^2 on surface area before and after exposure was studied. The swabbing method was used to determine the quantity of CFUs. The assessment of microbial contamination of indoor spaces was carried out in accordance with the recommendations of the SBM-2015 standard (Germany).

Results. The most contamination of the surfaces with mold fungi – up to 120 CFU/dm^2 is observed in the points furthest from the entrance to the premises. The total microbial count in the center of individual classrooms reaches 194 CFU/dm^2. Also, the microbiological studies indicated a small amount (1–7 CFU/dm^2) of *Staphylococcus aureus* on table surfaces among 33–44 % of the samples taken. There is a weak negative correlation between the number of CFU of mold fungi and the total microbial amount: before exposure, the Spearman correlation coefficient $r = -0.314$, after three months of UV exposure $r = -0.463$. There is a noticeble decrease in the quantity of CFU microorganisms on work surfaces ($p < 0.05$) when using open-type UV-irradiators, while in a premises without such equipment the quantitative indicators of microflora practically did not change ($p > 0.05$).

Conclusions. In university auditiriums on the surfaces of tables where students study, microbial contamination is detected from «light» (< 20 CFU/dm^2) to «extreme anomaly» (> 100 CFU/dm^2) degree according to the criteria of the SBM 2015 Guidelines for biological assessment of buildings (Germany). The use of LED UVC T5-5W-275NM bactericidal lamps of the open type in the presence of people leads to a decrease in microbial contamination of surfaces in all places of research by 2.8 times ($p < 0.05$) or by 1–2 degrees, according to the criteria of the SBM 2015 Guidelines. The simultaneous use of UV LED lamps and air recirculators allows to reduce the amount of colony-forming units (CFU) of mold fungi in the auditoriums by 20 times ($p < 0.05$). The introduction of modern energy-saving LED sources of bactericidal UV radiation is a promising direction for indoor air improvement. At the same

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time, there is a need to develop appropriate hygienic regulations for their use, taking into account the requirements of biological safety in accordance with the Order of the Ministry of Health of May 6, 2021 No. 882 and DSTU EN 62471:2017 «Safety of lamps and lamp systems photobiological (EN 62471:2008, IDT; IES 62471:2006, MOD)».
Key words: indicators of microflora, bactericidal ultraviolet light-emitting lamps, disinfection of indoor surfaces

Introduction

There is a constant presence of a certain amount of bacteria, fungi and viruses in the air and on surfaces in every industrial, office or household premises, that requires certain preventive measures. Microorganisms brought in by people from the street on clothes, with their breath, are added to their total quantity in this premises. These microorganisms are either attached to dust particles or are in particles of microbial droplet aerosol formed during coughing or sneezing [1]. The microflora of the human upper respiratory tract is represented by various types of streptococci (in particular, pneumococci), diphtheroids, staphylococci, neisseria, peptococci, moraxella, and pseudomonads. At the same time, up to 30 % of people are carriers of *Staphylococcus aureus* [2].

According to the literature data, the main components of the microbial community or «microbiome» in the premises where people spend a significant part of his time are representatives of the following types of microorganisms: *Actinomycetales*, *Lactobacillales*, *Bacillales*, *Sphingobacteriales*, *Rhizobiales*, *Burkholderiales*, *Chroococcales*, *Pseudomonadales* [3]. Literature data show that unique microbiomes found on different parts of the human body can be transferred to work surfaces after contact with a human [3, 4]. These bacterial «traces» can be detected in all places in the room [5]. They have a significant impact on the structure of the microbial community in the room and on the dispersion of microorganisms in the air [6].

UV radiation of the «C» range is widely used to disinfect air and work surfaces indoors [7, 8]. UV radiation destroys the DNA of microorganisms, rendering them harmless [9–10]. Pathogenetic mechanisms consist in the creation of pyrimidine dimers in the DNA molecule, which leads to the inability of the organism to replicate or its death [11].

The standard source of bactericidal radiation in commercial systems are low-pressure mercury lamps that emit maximum UV energy at a wavelength of 253.7 nm [9]. However, in recent years, energy-efficient ozone-free LED sources of UV radiation have been actively implemented for air disinfection in rooms of various purposes [12].

It should be noted that the effectiveness of the bactericidal effect depends, first of all, on the dose of UV radiation and the sensitivity of microorganisms [13]. The survival fraction (S) of the microbial population exposed to UV is an exponential function of the dose:

$$S = e^{-kDUV}, \quad (1)$$

where k is the species-dependent inactivation rate constant, ($cm^2/\mu J$), DUV – dose of UV radiation (J/m^2).

The choice of the k-value is particularly difficult for heterogeneous microbial populations.

The main condition for the use of open sources of UV radiation in the presence of people is that the effective bactericidal dose should not exceed the effective exposure limit value (ELV) for human skin and eyes according to DSTU EN 62471:2017 [12]. At the same time, the exposure of UV-irradiation should be sufficient to reduce the number of microorganisms in the air and on surfaces to the required premises cleanliness criteria.

Thus, the problem of microbial contamination of non-medical premises, where there is a gathering of people, needs its own rational solution [14].

In addition, there are currently no microbiological pollution standards for offices, many types of industrial premises, and educational institutions in Ukraine.

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Instead, the German standard SBM-2003 provides the following criteria for microbial contamination of surfaces that must be regularly cleaned indoors: < 20 CFU/dm^2 – «no anomaly», 20–50 CFU/dm^2 – «light anomaly», 50–100 CFU/dm^2 – «severy anomaly», > 100 CFU/dm^2 – «extreme anomaly» [15].

Hygienists pay significant attention to the creation of healthy conditions for students in educational institutions [16, 17]. The use of bactericidal sources of UV radiation is proposed to reduce the microbial load on the human body and prevent diseases that are transmitted by airborne droplets [18]. The disadvantages of the proposed method include the use of low-pressure mercury lamps and the need to control the exposure regime, which creates obvious risks for the body of adolescents in educational institutions.

Closed-type UV irradiators (recirculators) show high microbiological efficiency and good biological safety when used in the presence of people [19].

The aim of the research is to determine the effectiveness of the use of open-type bactericidal ultraviolet monochrome light-emitting diode (LED) irradiators for disinfection of work surfaces in the classrooms of a higher educational institution.

Materials and methods of the research

The study of the bactericidal efficiency of the open-type LED irradiator UVC T5-5W-275NM manufactured by LED Azimut LLC (Kamyanske, Ukraine) was carried out in three auditoriums of the Kyiv National University of Civil Engineering and Architecture of the Ministry of Education and Culture of Ukraine.

The nominal power of the UVC LED lamps was 5 W, the radiation wavelength λ_{max} = 278,6 nm. The UVC LED lamps were attached to the ceiling, the height of which was 3.3 m above the floor. Ultraviolet radiometers UV-C «Argus-06», UV-B «Argus-05», UV-A «Argus-04» were used to measure the physical parameters of UV radiation. In accordance with biological safety requirements for exposure to human eyes and skin according to DSTU EN 62471:2017 [20] and Directive 2006/25/EC [21], the level of UV radiation at wavelength of 280 nm on a table surface at a height of 1 m from the floor was 1 mW/m^2. The duration of the study was 3 months.

Based on the literature [22], it was evaluated the doses of UV radiation necessary to achieve a bactericidal efficiency of 90 % for the possible and most common microorganisms in the premises: *Streptococcus green*, *Escherichia coli*, *Diphtheria bacillus*, *Enterococcus*, *Staphylococcus aureus*, *Candida mycetes*, mold fungi.

The object of the study is contamination of work surfaces (tables) in auditoriums – colony-forming units (CFU)/dm^2.

The general assessment of microbial contamination of indoor spaces was carried out in accordance with the recommendations of the SBM-2015 standard: up to 20 CFU/dm^2 – «absent», 20–50 CFU/dm^2 – «light», 50–100 CFU/dm^2 – «strong», > 100 CFU/dm^2 – «extreme anomaly» [15].

Statistical data processing was carried out using standard Microsoft Office Excel 2007/Windows 8 (00250-40835-58924-AAOEM) and STATISTICA 7.0 programs installed as personal computer software. The reliability ($p < 0.05$) of the results and their group differences were analyzed by statistical reliability criteria: Sign test (p–level) and Spearman Rank Order Correlations (r).

Results of the research and their discussion

The parameters of the microclimate, lighting, and noise in the auditoriums were within the limits of

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permissible values according to the data of sanitary and hygienic studies.

In auditorium No. 476, No. 478, and No. 482, from 26 to 50 university students attended lectures during 6–8 hours a day. The lecturers keep track of the time of stay and the number of groups. Table 1 presents the technical characteristics of the selected auditoriums and the conditions of the experiment.

As can be seen from the data in the table 1, the area of the classrooms ranged from 33.5 m^2 to 48.6 m^2. At the same time, the area per one person during a lecture ranged from 0.98 m^2 to 1.2 m^2. No air and surface disinfection equipment was installed in auditorium No. 476 (control premises). UV LED lamps only were installed in auditorium No. 482. UV LED lamps and 2 air recirculators using PHILIPS UV ozone-free mercury lamps were installed in auditorium No. 478.

The quantity and technical characteristics of UV radiation sources and air recirculators presented in the Table 2.

LED UVC T5-5W-275NM lamps were placed on the ceiling of the auditoriums in such a way that the intensity of UV radiation during their operation at the table surfaces was equal to 1 mW/m^2. It is amounted to a total UV actinic radiation dose of 25 J/m^2, which was below the exposure limit value (ELV = 30 J/m^2) and met the requirements of biological safety according to DSTU EN 62471:2017 «Safety of lamps and lamp systems, photobiological» for an exposure duration of 8 hours.

Regarding the comparison of the possible effects of bactericidal action, the table 3 presents the calculated values of UV-C doses, which are necessary to achieve a bactericidal efficiency of 90 % for certain microorganisms, taking into account the coefficient of biological activity of radiation at the wavelength of 280 nm (Table 3) [23, 24].

The data in Table 3 show that the dose of UV-C radiation to achieve a 90% bactericidal effect at the wavelength of 280 nm exceeds the ELV (30 J/m^2) for humans in the case of the following microorganisms: *Diphtheria bacillus* (38 J/m^2), *Staphylococcus aureus* (55 J/m^2), *Escherichia coli* (34 J/m^2), *Enterococcus* (41 J/m^2), molds (136–2052 J/m^2), *Candida* (136 J/m^2).

But the ELV is quite sufficient to achieve a 90 % bactericidal effect in order to inhibit such common pathogens as *Streptococcus viridans* (23 J/m^2) and *Escherichia coli* (30 J/m^2). It makes possible to use open sources of monochromatic UV-radiation in the presence of people with taking into account the requirements of DSTU EN 62471:2017.

In Figure presents the sampling points of microbiological material in the auditoriums before the start of UV-irradiation on 09/21/2022 and after three months of operation of the bactericidal equipment on 12/21/2022.

Table 4 presents the averaged data on the quantity of CFUs per 1 dm^2 of mold fungi in individual points of selected audiences.

Table 1

Technical characteristics of auditoriums and experimental conditions

Auditorium No.	Volume, m^3	Height, m	Area, m^2	Number of students	Volume per 1 student, m^3	Area per 1 student, m^2	Conditions of research
476	160	3.3	48.6	50	3.2	0.97	Without air purification
478	102	3.3	31.2	26	3.9	1.2	UV LED lamps (only)
482	110	3.3	33.5	34	3.2	0.98	UV LED lamps + recirculators

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Table 2

Technical characteristics of UV radiation sources and recirculators

Auditorium No.	LED sources of UV radiation			Air recirculators		
	Number of lamps	Power, W	Intensity of UV – irradiation on the surface table, mW/m^2	Number, piece	Power, Wt	Efficiency of air exchange, m^3/h
476	–	–	–	–	–	–
478	5	25	1	2	15	58
482	5	25	1	–	–	–

As can be seen from the data in the Table 4, significant mold contamination is observed at the farthest points from the entrance – near the far wall of the auditorium. At the same time, the combined use of UV LED lamps and air recirculators allows to reduce the amount of mold fungi CFU in the premise by 20 times ($p < 0.01$), despite the sufficiently high dose of direct UV radiation necessary for suppressing mold fungi (136–2052 J/m^2), which significantly exceeds the ELV for human body (30 J/m^2). In this case, we can talk about the high efficiency of the system of open-type recirculators and UV-irradiators for disinfection of spore-forming microorganisms [24]. In the auditorium where was used only UV-irradiators a decrease in the total quantity of CFUs is observed by 2.3 times, and in the auditorium without a cleaning system, there were practically no changes.

Table 5 presents the total microbial quantity (CFU/dm^2) in selected points of the auditoriums.

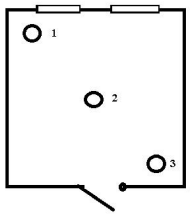

Figure. Selection points of microbiological material in the premises:
p.1 – the farthest point from the entrance near the far wall;
p. 2 – the geometric center of the premise;
p. 3 – the closest to the entrance of the premise

As can be seen from the data in the Table 5, the use of bactericidal UV LED lamps for three months leads to a decrease in the total number of microbes in the auditoriums by 2.8 times ($p < 0.05$). While the combined use of UV LED lamps and air recirculators leads to a more modest result – a reduction of CFU of microbes by 1.6 times. This fact requires further research from the point of view of possible antagonistic relationships between microbial flora

Table 3

Doses of UV-C required to achieve 90 % bactericidal efficiency for certain microorganisms

Microorganism	Dose of UV-C (280 nm) for bactericidal efficiency 90 %, J/m^2
Streptococcus viridans	23
Escherichia coli	34
Corynebacterium diphtheria	38
Enterococcus	41
Staphylococcus aureus	55
Candida	136
Mold fungi	136–2052
Exposure limit value (ELV) for eye and skin, J/m^2	34

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Table 4

The quantity of CFUs per 1 dm^2 of mold fungi in selection points of the auditoriums

Mold fungi, CFU/ dm^2									
Date of study	A.482/points of research			A.478/ points of research			A.476/ points of research		
	P.1	P.2	P.3	P.1	P.2	P.3	P.1	P.2	P.3
21.09.2022	120	8	10	27	7	9	25	15	36
21.12.2022	3	4	0	7	6	6	18	44	27
Effect (p-level)	*CFU reduction by 20 times ($p < 0,01$)*			*CFU reduction by 2,3 times ($p < 0,05$)*			*Practically unchanged ($p > 0,05$)*		

and mycetes (mold fungi) [24], the number of which is significantly reduced when using air recirculators.

According to the measurements, there is a weak negative correlation between the number of CFU of mold fungi and the total microbial quantity. the Spearman correlation coefficient is «−0.314» before UV exposure and after three months the correlation increases and is $r = -0.463$ ($p < 0.05$).

The carried out microbiological studies showed a presence of small amount (1−7 CFU/dm^2) of *Staphylococcus aureus* on the tables' surfaces in the auditoriums among 33−44 % of the samples (Table 6).

The number of CFU/dm^2 of *Staphylococcus aureus* on table surfaces decreases during UV exposure, but the number of samples in which it is detected remains practically unchanged ($p > 0.05$).

Table 7 presents a total assessment of microbial contamination according to the SBM-2015 Guidlines in auditoriums when using air and surface cleaning equipment.

As can be seen from the showen above data, the use of open-type bactericidal monochrome ultraviolet LED UVC T5-5W-275NM lamps in the presence of people leads to a decrease in microbial contamination on surfaces in all examinated places by 1−2 degrees, in accordance with the criteria of the SBM 2015 Standard [15]. At the same time, the exposure limit value for humans does not exceed 30 J/m^2 and meets the requirements of biological safety according to DSTU EN 62471:2017.

The use of a combined system consisting of LED UVC T5-5W-275NM lamps and recirculators creates a smaller effect in terms of general microbial contamination, but is very effective in destroying the activity of mold fungi.

In the premises without the use of bactericidal equipment, no changes in the assessment of microbial pollution were observed.

In our opinion, the ratio and type of interaction of the microbial part of the microbiome of the prenises and the myceti should be the subject of further study in order to develop regulations for the use of modern bactericidal systems for disinfecting air and surfaces.

Table 5

The total microbial amount in certain points of the auditoriums

Total microbial quantity, CFU/dm^2									
Date of study	A.482/points of research			A.478/ points of research			A.476/ points of research		
	T.1	T.2	T.3	T.1	T.2	T.3	T.1	T.2	T.3
21.09.2022	80	194	42	66	70	114	33	23	36
21.12.2022	77	90	33	23	16	52	40	27	38
Effect	*CFU reduction by 1,6 times ($p > 0,05$)*			*CFU reduction by 2,8 ($p > 0,05$)*			*Practically unchanged ($p > 0,05$)*		

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Table 6

***Staphylococcus aureus* contamination in auditoriums**

Date of study	*Staphylococcus aureus*, CFU/dm²								
	A.482/points of research			A.478/ points of research			A.476/ points of research		
	T.1	T.2	T.3	T.1	T.2	T.3	T.1	T.2	T.3
21.09.2022	0	7	0	0	0	1	0	1	0
21.12.2022	0	0	1	0	0	1	1	1	0
Effect	*CFU reduction by 7 times (p > 0,05)*			*Practically unchanged (p > 0,05)*			*Practically unchanged (p > 0,05)*		

Thus, the introduction of modern energy-saving LED sources of bactericidal UV radiation is a fairly promising direction for improving environment quality in premises of various purposes where a person is permanently or temporarily under the conditions of compliance with the requirements of biological safety in accordance with the Order of the Ministry of Health of May 6, 2021 No. 882 and DSTU EN 62471:2017. At the same time, the existing regulatory framework regarding the use of bactericidal UV radiation for preventive purposes needs to be improved, taking into account the functional and cytological features of the microbiome that is formed in these premises.

Conclusions

1. In the auditiriums of the higher education institution where students study, microbial contamination is detected on the surfaces of the tables from «light» (up to 20 CFU/dm²) to «abnormal» (> 100 CFU/dm²) degree according to the criteria of the Guidelines for Biological Assessment of Buildings SBM 2015 (Germany). At the same time, the microflora is represented by various types of bacteria, molds, and a small amount of Staphylococcus aureus.
2. The use of bactericidal LED UVC T5-5W-275NM lamps of the open type in the presence of people leads to a decrease in microbial contamination of surfaces in all places of the researches by 2.8 times ($p < 0.05$) or by 1–2 degrees, according to the criteria of the SBM 2015 standard.
3. The combined use of UV LED lamps and air recirculators allows to reduce the number of colony-forming units of mold fungi in the room by 20 times ($p < 0.01$), despite a sufficiently high dose of direct UV radiation necessary for

Table 7

Total assessment of microbial contamination according to the SBM-2015

Date of study, criterion	Total assessment of microbial contamination, degree								
	A.482/points of research			A.478/ points of research			A.476/ points of research		
	T.1	T.2	T.3	T.1	T.2	T.3	T.1	T.2	T.3
21.09.2022, *degree of pollution*	severy anomaly	extreme anomaly	light anomaly	severy anomaly	severy anomaly	extreme anomaly	light anomaly	light anomaly	light anomaly
21.12.2022, *degree of pollution*	severy anomaly	severy anomaly	light anomaly	light anomaly	no anomaly	severy anomaly	light anomaly	light anomaly	light anomaly
The presence of the effect	–	+	–	+	++	+	–	–	–

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90% bactericidal effect (136–2052 J/m^2), which significantly exceeds the exposure limit for a human body (30 J/m^2).

4. The introduction of modern energy-saving LED sources of bactericidal UV radiation is a promising direction for indoor air improvement. At the same time, there is a need to develop appropriate hygienic regulations for their use, taking into account the requirements of biological safety in accordance with the Order of the Ministry of Health of May 6, 2021 No. 882 and DSTU EN 62471:2017 «Safety of lamps and lamp systems photobiological (EN 62471:2008, IDT; IES 62471:2006, MOD)».

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