SHORT COMMUNICATION

Annals of Agricultural and Environmental Medicine
www.aaem.pl

Effectiveness of UV-C radiation in inactivation of microorganisms on materials with different surface structures

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Górny RL, Gołofit-Szymczak M, Pawlak A, Ławniczek-Wałczyk A, Cyprowski M, Stobnicka A, Płocińska M, Kowalska J. Effectiveness of UV-C radiation in inactivation of microorganisms on materials with different surface structures. Ann Agric Environ Med. doi: 10.26444/aaem/189695

Abstract

Introduction and Objective. Ultraviolet light in the UV-C band is known as germicidal radiation and was widely used for both sterilization of the equipment and creation of a sterile environment. The aim of the study is to assess the effectiveness of inactivation of microorganisms deposited on surfaces with various textures by UV-C radiation disinfection devices.

Materials and Method. Five microorganisms (3 bacteria, virus, and fungus) deposited on metal, plastic, and glass surfaces with smooth and rough textures were irradiated with UV-C light emitted by low-pressure mercury lamp and ultraviolet emitting diodes (LEDs), from a distance of 0.5 m, 1 m, and 1.5 m to check their survivability after 20-minute exposure.

Results and Conclusions. Both tested UV-C sources were effective in inactivation of microorganisms; however, LED emitter was more efficient in this respect than the mercury lamp. The survival rate of microorganisms depended on the UV-C dose, conditioned by the distance from UV-C source being the highest at 0.5 m and the lowest at 1.5 m. For the tested microorganisms, the highest survival rate after UV-C irradiation was usually visible on glass and plastic surfaces. This observation should be considered in all environments where the type of material (from which the elements of technical equipment are manufactured and may be contaminated by specific activities) is important for maintaining the proper level of hygiene and avoiding the unwanted and uncontrolled spread of microbiological pollution.

Key words

microorganisms, plastic, glass, metal, ultraviolet radiation, inactivation, surface structure, UV-C low-pressure mercury lamp, UV-C LED

INTRODUCTION

On the electromagnetic spectrum, ultraviolet (UV) radiation falls between visible light and X-rays. UV light is divided by wavelength into UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) [1]. Ultraviolet light in the UV-C band (especially within the range of 250–280 nm with peak at 253.7 nm) is known as germicidal radiation and is widely used for both sterilization of equipment and the creation of sterile environment. UV light emits photon energy which, if absorbed by microbial nucleic acids (DNA or RNA) and proteins, leads to photochemical transformations that underlie UV disinfection. UV-C radiation absorbed by these components does not kill microorganisms but rather inactivates them, rendering them unable to reproduce or cause infections, but usually remain viable [2–7]. Although conventional UV-C emitters are quite convenient to use, they have significant limitations. They utilize tubed lamps that contain mercury, which is known to be a hazardous material. They also operate at frequencies that may cause ozone to be created, which may influence both their effectiveness and operational safety [2]. Moreover, the heat dissipation from conventional UV-C lamps results in a considerable temperature gradient in their vicinity, which may change the flavour, colour, odour and/or shelf life of numerous products and/or materials.

Against this background, ultraviolet light-emitting diodes (UV LEDs) have emerged as a superior alternative to conventional UV lamps, owing to their tailorable optical characteristics, ultra-low power consumption, durability and rapid climbing efficiencies, practically within the entire UV spectral range from 210 nm to 400 nm [3, 8–12]. Hence, the aim of the study was to assess the effectiveness of inactivation of microorganisms (3 bacteria, virus, and fungus) deposited on surfaces (metal, plastic, glass) with various textures by UV-C radiation disinfection devices – mercury lamp and light-emitting diodes.

MATERIALS AND METHOD

Representatives (in all cases reference strains from American Type Culture Collection, ATCC, were used) of 3 microbial groups were selected for UV-C inactivation tests, i.e. from among bacteria: *Bacillus subtilis* ATCC 6633 representing Gram-positive bacilli, *Staphylococcus aureus* ATCC 6538, representing Gram-positive cocci, *Pseudomonas aeruginosa* ATCC 260, representing Gram-negative rods; from among viruses: bacteriophage PhiX174 ATCC 13706-B1; and from among fungi: *Aspergillus versicolor* ATCC 9577. Inactivation

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Received: 10.04.2024; accepted: 05.06.2024; first published: 25.06.2024

tests were carried out on aqueous suspensions of the above-mentioned microorganisms. Initial concentrations in CFU/ml were as follows: *B. subtilis* – 2.2×10^6 and 4.5×10^6, *S. aureus* – 2.9×10^6 and 2.9×10^6, *P. aeruginosa* – 4.1×10^6 and 7.8×10^6, PhiX174 – 4.2×10^5 and 3.7×10^5, *A. versicolor* – 3×10^5 and 5.5×10^5 for experiments with a mercury lamp and ultraviolet emitting diodes, respectively. In each case, they were applied to 3 types of surfaces made of metal (stainless steel), plastic (polypropylene), and glass (SiO_2 content 72–73%), each with a smooth and rough texture (Fig. 1) [13]. All materials were autoclaved before use. The roughness of all tested surfaces were checked and visualized (electron accelerating voltage was 5 kV, magnification 1,000×) using scanning electron microscope, SEM (model SU-8010, Hitachi High-Technologies Corp., Tokyo, Japan). For this purpose, all specimens were placed on the carbon tape and covered with a layer of gold using sputter coater (model Q150T ES, Quorum Technologies Ltd., Lewes, UK).

For the inactivation tests, each of the native and sterilized surfaces inoculated in the above described way was exposed to UV-C radiation emitted by the tested devices. Two emitters were selected for this study, in which the source of UV-C radiation was a low-pressure mercury lamp (model G15T8, 15 W, Sankyo Denki Co., Ltd., Kanagawa, Japan),

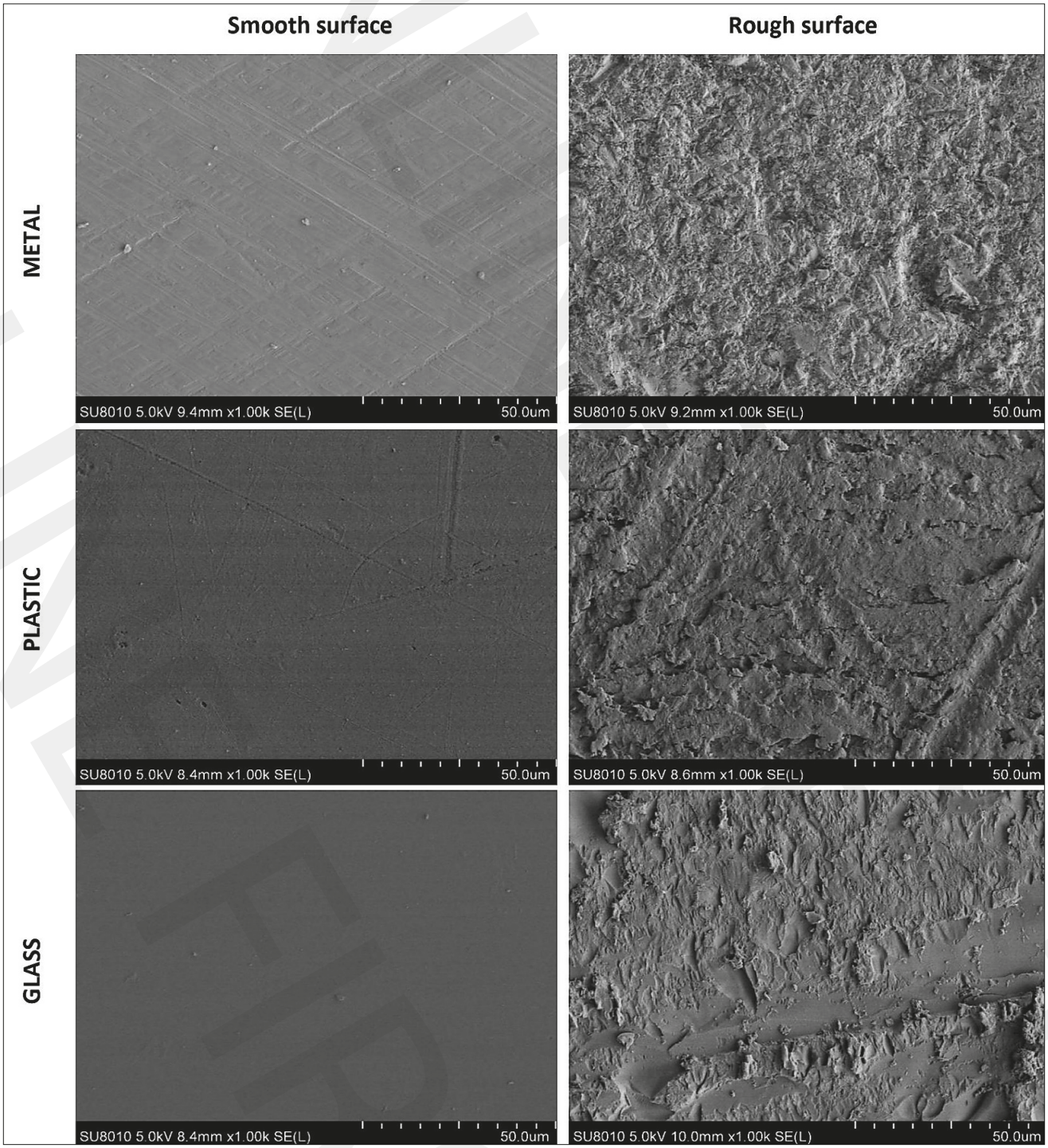
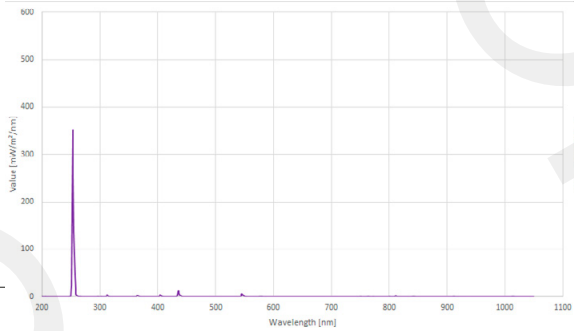
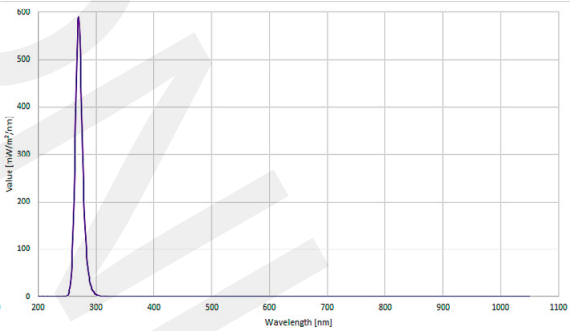


Figure 1. Smooth and rough surface structures of tested materials: metal (stainless steel), plastic (polypropylene), and glass (SiO_2 content = 72–73%)

Table 1. Characteristics of tested UV-C radiation devices (low-pressure mercury lamp and ultraviolet emitting diodes, LEDs) with their spectra, as well as irradiances (fluence rates) and doses emitted during 20-minute exposure from 3 distances between UV-C emitter and surface with microbial sample

UV-C emitter	Low-pressure mercury lamp			UV-C emitting diodes (UV-C LEDs)		
Electromagnetic spectrum						
Distance between UV-C emitter and surface with microbial sample [m]	0.635	0.182	0.098	0.686	0.224	0.104
Irradiance (fluence rate) [W/m^2]	762	218	118	823	269	125
UV-C dose (fluence) [J/m^2]						

and ultraviolet emitting diodes, LEDs (model UVM002A-0401U1-RM, 9 W, Citizen Electronics Co., Ltd., Yamanashi, Japan). During the experiments, UV-C radiation emitted by the tested devices was directed perpendicular to the exposed surfaces. Inoculated surface samples were placed at 3 distances: 0.5 m, 1 m and 1.5 m from the UV-C radiation source to verify the effectiveness of a given emitter in inactivating microorganisms, depending on the distance from the contaminated surface. Each tested device was initially subjected to appropriate characteristics using a spectroradiometer with measuring head (model GL SPECTIS 5.0 Touch with measuring head GL OPTI PROBE 5.1.50, GL Optic Polska Sp. z o.o. Sp.k., Puszczykowo, Poland), showing the spectra of tested UV-C emitters and specifying irradiance (in W/m^2) measured using a light meter with actinic optical radiation hazard detector (model ILT2400 with SEL 240/ACT5/W, International Light Technologies, Peabody, USA), and subjected to a 20-minute exposure (in J/m^2) at 3 tested distances (Tab. 1).

Because the emitters, in addition to the inactivating UV-C radiation, also emit radiation with a shorter wavelength which may create ozone from the oxygen contained in the air (increased concentration of ozone in the air may lead to inflammatory reactions in the eyes or respiratory diseases, including intensification of asthma symptoms and cardiovascular diseases), the control of its concentration using a single-gas detector (model Micro 5 G222E, Gesellschaft für Gerätebau mbH, Dortmund, Germany) during sample exposure was an inherent part of UV-C emitters' testing. After exposure, the microorganisms were washed from the tested surfaces using a programmable rotator-mixer (model Multi RS-60, Biosan, Riga, Latvia) at 800 rpm for 5 min at room temperature, and the suspensions thus obtained were microbiologically processed by the spread plate method on microbiological media appropriate for a given microorganism (i.e. blood trypticase soy agar for bacteria, nutrient agar and nutrient broth for bacteriophage, malt extract agar for fungi – all media: Becton Dickinson & Co., Sparks, USA).

The survival of microorganisms under the influence of UV-C radiation was quantitatively determined to assess the effectiveness of inactivation process. All tests were conducted in a Class 2 biological safety cabinet (model SafeFAST Classic 218, Faster, Ferrara, Italy). Each experiment was performed in triplicate and repeated 3 times for each material. After checking the normality of data distributions with the Shapiro-Wilk test, the collected data were statistically elaborated by analysis of variance (ANOVA) and *t*-test using Statistica (data analysis software system) version 10. (StatSoft, Inc., Tulsa, USA). Probability values were treated as statistically significant at $P<0.05$.

RESULTS

The study showed that both the tested UV-C emitters were effective in inactivating microorganisms; however, the efficiency of this process, determined by microorganisms' survivability, in the case of UV-C LEDs was higher than those in which the UV-C emitter was the mercury lamp (*t*-tests – mercury lamp vs. LEDs: in the case of *B. subtilis*, *S. aureus*, *P. aeruginosa*, bacteriophage PhiX174 – $P<0.05$; in the case of *A. versi*color – $P<0.00001$) (Fig. 2). Effectiveness of inactivation of the microorganisms measured by the percentage of their survival after exposure to UV-C radiation, depended primarily on the distance of the tested sample from the UV-C emitter (i.e. 0.5 m, 1 m and 1.5 m), and consequently, on the UV-C dose acting on the microorganisms deposited on a given surface (average exposure values for mercury lamp – 762 J/m^2, 218 J/m^2 and 118 J/m^2, respectively; for LED emitter – 823 J/m^2, 269 J/m^2 and 125 J/m^2, respectively). To a lesser extent, the survivability of the tested microbial strains depended on the type of material from which a given surface was made or its texture, and varied depending on the type of tested microorganism exposed to UV-C radiation. Analyzing these relationships in detail, the performed tests revealed the following:

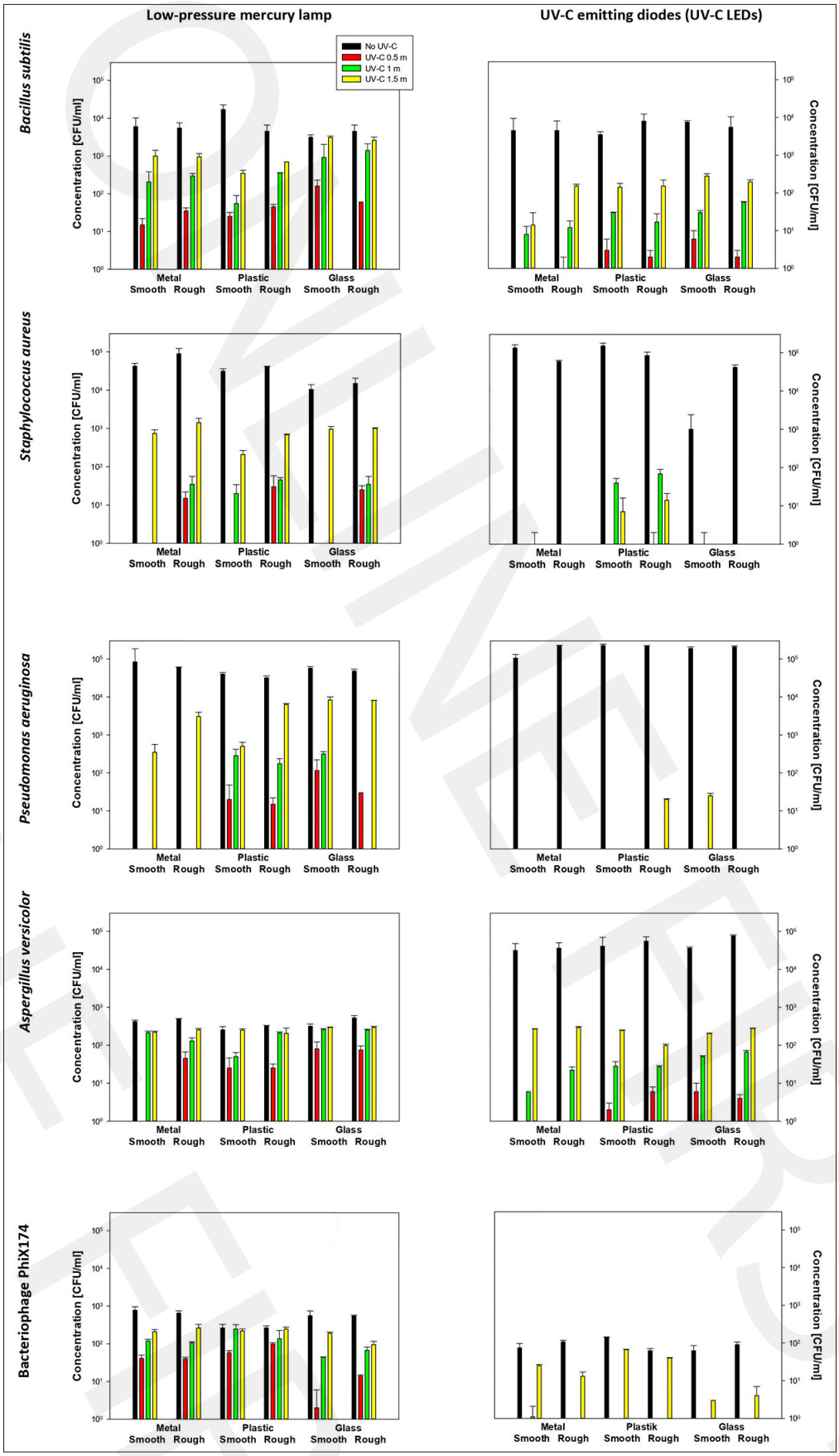


Figure 2. Survivability of microorganisms deposited as water suspensions on smooth and rough metal, plastic, and glass surfaces after exposure to UV-C radiation at the distances of 0.5 m, 1 m and 1.5 from tested aurfaces, which translate into UV-C doses (fluences) of 762 J/m^2, 218 J/m^2, and 118 J/m^2 as well as 823 J/m^2, 269 J/m^2, and 125 J/m^2, for mercury lamp as well as light-emitting diodes (LEDs), respectively

1) for all tested microorganisms, regardless of the type of surface and its texture, the highest reduction in the number of viable microorganisms was observed in the case of the mercury lamp placed at a distance of 0.5 m from the tested samples (0.5 m vs. 1.5 m – Scheffé test: $P < 0.01$), and in the case of LEDs at a distances of 0.5 m and 1m (0.5 m vs. 1.5 m and 1 m vs. 1.5 m – in both cases Scheffé tests – $P<0.05$). In relation to the individual tested microorganisms, these relationships were identical, although their statistical significance was confirmed in the case of the mercury lamp for *S. aureus* and *P. aeruginosa* (0.5 m vs. 1.5 m and 1 m vs. 1.5 m – in all 4 cases Scheffé tests – $P < 0.05$), as well as for *A. versicolor* (0.5 m vs. 1 m – Scheffé test – $P<0.05$ and 0.5 m vs. 1.5 m, Scheffé test – $P<0.001$); in the case of LEDs: for *B. subtilis* (0.5 m vs. 1.5 m and 1 m vs. 1.5 m, in both cases Scheffé tests – $P<0.001$), for *A. versicolor* (0.5 m vs. 1.5 m, Scheffé test – $P<0.0001$ and 1 m vs. 1.5 m – Scheffé test – $P<0.001$), and for bacteriophage PhiX174 (0.5 m vs. 1.5 m and 1 m vs. 1.5 m, in both cases Scheffé tests – $P<0.05$). Since the radiation dose received by a microorganism deposited on the irradiated surface is the product of the radiation intensity and the exposure time, it can be assumed that extending the exposure time (over 20 min) to UV-C radiation for the tested microorganisms would further reduce their survivability.

2) In relation to the type of material from which a given surface was made, UV-C radiation emitted by LEDs inactivated bacterial and fungal microorganisms deposited on metal, plastic or glass with the same high effectiveness (ANOVA: $P>0.05$), and their survivability did not exceed 0.85% of the initial number of microbes. It should be noted, however, that in the case of bacteriophage PhiX174, its survival rate on both smooth and rough plastic surfaces was higher than other microorganisms, reaching at the lowest tested 20-minute exposure (125 J/m^2 at 1.5 m) 46% and 64%, respectively. In the case of UV-C radiation emitted by the mercury lamp, differences in the survival of *S. aureus*, *P. aeruginosa*, and *A. versicolor* deposited on metal, plastic, and glass surfaces did not differ from one other (ANOVA: $P>0.05$). Statistically significant differences were meanwhile recorded for *B. subtilis* bacterium and bacteriophage PhiX174. Inactivation under the influence of UV-C radiation emitted by the mercury lamp of *B. subtilis* colonies deposited on smooth and rough glass surfaces was significantly lower than that on smooth and rough plastic surfaces (Tukey test: $P < 0.05$). In turn, an inactivation of bacteriophage PhiX174 by UV-C mercury radiation was a highly efficient process for this virus deposited on metal and glass surfaces only. On a plastic surface, the percentage of viable virus particles exposed to UV-C radiation reached 92.5% (smooth surface) and even 100% (rough surface) of its initial number (plastic vs. metal and plastic vs. glass, in both cases Scheffé tests – $P<0.01$).

3) With respect to the texture of the metal, plastic, and glass surfaces, when exposed to UV-C radiation emitted by the mercury lamp, for both smooth and rough specimens, the effectiveness of inactivation of the tested microorganisms deposited on them did not differ significantly (*t*-test: $P>0.05$). The same relationship was observed for smooth and rough surfaces of the tested materials under the influence of UV-C radiation emitted by LEDs (*t*-test: $P>0.05$). However, when the survival of microorganisms on smooth surfaces after exposure to UV-C radiation emitted by mercury lamp or LEDs was tested, regardless of the material from which the surface was made (i.e. metal, plastic or glass), LEDs were more effective in inactivating the tested microorganisms (*t*-test: $P < 0.001$). The same relationship, but with greater statistical significance, was noted in the case of microorganisms deposited on rough surfaces (*t*-test: $P < 0.0001$). Taking into account the group of tested microorganisms, on smooth surfaces after exposure to UV-C radiation emitted by mercury lamp, the lowest survival rate was observed for *S. aureus* and *P. aeruginosa* bacteria, and the highest survival rate for *A. versicolor* filamentous fungus (*S. aureus* vs. *A. versicolor* and *P. aeruginosa* vs. *A. versicolor,* in both cases Scheffé tests – $P < 0.05$). In the case of rough surfaces, after exposure to UV-C radiation emitted by mercury lamp, *S. aureus* bacteria had the lowest, whereas bacteriophage PhiX174 had the highest survival rate (*S. aureus* vs. PhiX174, Scheffé test – $P < 0.05$). In the case of smooth and rough surfaces after exposure to UV-C radiation emitted by LEDs, inactivation of the tested microorganisms did not differ significantly from one another (ANOVA: $P > 0.05$).

Continuous control of the ozone concentration during microbial inactivation experiments using mercury lamps and LED diodes did not show the presence of this gas in the air near the UV-C emitters and samples (concentrations below the limit of quantification, i.e. below 0.01 ppm – 0.02 mg/m^3).

DISCUSSION

For several decades, disinfection and sanitization with UV-C radiation has been a proven technology for removing viral, bacterial, and fungal pathogens from surfaces, air, and water [2, 7]. The widespread use of UV-C emitters concerns primarily [e.g. 2, 8, 9, 14–17]: hospitals, both in operating theatres and in patient wards, for the disinfection of surfaces, surgical tools and the air (which has become particularly important since the outbreak of the SARS-CoV-2 coronavirus pandemic); food processing plants (pasteurization of food products, e.g. juices, disinfection of surfaces and air within production lines, in warehouses and production halls, aseptic packaging of products, light traps for insects); pharmaceutical plants (disinfection of surfaces and air within production lines and production halls, disinfection of liquids, aseptic packaging of products); electronics industry plants (disinfection of material surfaces and production lines); water and sewage treatment plants; ventilation and air conditioning systems in buildings and means of transport, and laboratories where microbiological hazards occur. In all of these environments, products made of materials such as metal, plastic or glass, are practically ubiquitous and virtually everywhere may have a direct contact with microbiological contaminants. UV effects on materials vary significantly. Irradiance, being the sum of reflectivity, transmissivity, and absorptivity, makes materials with low reflectivity and law transmissivity likely to absorb UV at high rates. Some materials with high UV reflectivity (e.g. metals) or high transmissivity (e.g. quartz glass) may absorb very little UV. Plastics, especially polymers having large molecules, are relatively resistant to UV radiation, but impurities and residual solvents are responsible for their photodegradation. UV energy absorbed by plastics can also excite the creation of free radicals, which then cause secondary reactions and cross-linking [2]. In this study, the highest survival rate of the tested microorganisms after UV-C irradiation (i.e. above 60% of the initial number of exposed colonies/plaques) was usually visible on the glass

and plastic surfaces. The results obtained confirm the observations of Gidari et al. [13] regarding the survivability of SARS-CoV-2 virus, Pedrós-Garrido et al. [18] with respect to *Pseudomonas fluorescens*, and Różańska et al. [19], regarding *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Enterococcus faecalis*, *P. aeruginosa*, and *Candida auris* deposited on plastic surfaces. The last-mentioned researchers studying the efficacy of UV-C radiation in eliminating the microorganisms from frequently touched plastic, glass, and steel surfaces, revealed that the surface material plays a crucial role in the effectiveness of UV-C disinfection, indicating the need for surface-specific disinfection strategies in a given environment. Also, Bartolomeu et al. evaluating UV-C radiation efficiency in decontamination of inanimate surfaces with phage ϕ6, observed that the inactivation of this bacteriophage deposited on glass and plastic was lower than on stainless steel, which is consistent with the results of the current study [20]. In turn, Lorenzo-Leal et al. studying survivability of Gram-positive (*Clostridium difficile*, methicillin-resistant *S. aureus*, *Listeria monocytogenes*) and Gram-negative (*A. baumannii*, *Escherichia coli*, *P. aeruginosa*) bacteria, as well as yeasts (*Cryptococcus neoformans* var. *grubii*, *Candida albicans*) deposited on a plastic (polystyrene) surface, and exposed to far-UV-C light (222 nm) at a distance of 0.5 m, observed successful growth inhibition at even lower UV-C doses (from 93 J/m^2 to 464 J/m^2) than those in the current study [21]. Likewise, Sharma et al. confirm a significant decrease in survivability of *E. coli*, *L. monocytogenes*, and *Salmonella* Typhimurium deposited on stainless steel specimen at even lower UV-C doses (from 19 J/m^2 to 108 J/m^2) [11].

The hitherto obtained results have shown that for surface disinfection purposes, UV-C irradiation is more efficient when applied to a smooth surface without shadow areas [5, 7, 20, 22]. The current study, however, did not confirm such an observation, but showed that the effectiveness of UV-C inactivation of the tested microorganisms deposited on smooth and rough metal, plastic and glass surfaces, did not differ significantly due to the surface structure.

CONCLUSIONS

Both tested UV-C sources were effective in damaging microorganisms; however, the LED emitter was more efficient in this respect than the low-pressure mercury lamp. The survival rate of microorganisms depended on the UV-C dose, conditioned by the distance from UV-C source being the highest at 0.5 m and the lowest at 1.5 m. For the tested microorganisms, the highest survival rate after UV-C irradiation was usually visible on glass and plastic surfaces. This observation should be considered in all environments where the type of material (from which elements of technical equipment are manufactured and may be subject to both uncontrolled and/or intentional contaminations forced by specific activities) is important for maintaining the proper level of hygiene and avoiding the unwanted and uncontrolled spread of microbiological contamination.

Acknowledgement
This paper was created on the basis of results of a research task carried out within the scope of the 6th stage of the "Governmental Programme for Improvement of Safety and Working Conditions" funded by the resources of the National Centre for Research and Development (task no. I.PN.13 entitled "Test methods and criteria for assessing devices used for disinfection with UV-C radiation in the work environment and non-industrial indoor environment in terms of their safety of use and effectiveness of microbial inactivation"). The Central Institute for Labour Protection – National Research Institute is the Programme's main co-ordinator.

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