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Photo-reactivation of micro-organisms suspended in the air and deposited on surfaces after exposure to UV-C radiation

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Abstract

Introduction and Objective. Many environmental microorganisms live in constant balance between UV damage and repair. The simplest repair process called photoreactivation starts immediately when microbial cells face sunlight irradiation. The aim of the study is to assess the ability of bacteria, virus, and mould suspended in the air and deposited on different surfaces to photoreactivation after their exposure to UV-C radiation produced by two disinfection devices, i.e. low-pressure mercury lamp (LPML) and light-emitting diodes (LEDs).

Materials and Method. Five microbial strains (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 260, *Aspergillus versicolor* ATCC 9577, and bacteriophage PhiX174 ATCC 13706-B1) deposited on metal, plastic, and glass surfaces, as well as dispersed in the air as bioaerosols, were irradiated with high UV-C doses (762 J/m^2 and 832 J/m^2), and subsequently exposed for 24 h to visible light with a wide (380–780 nm) spectral range to check their ability to photorecovery.

Results. UV-C radiation emitted by LPML and LEDs effectively inactivated the tested microorganisms deposited on metal, plastic and glass surfaces, as well as dispersed in the air. However, this type of inactivation is not an irreversible process and subsequent exposure of microbiologically contaminated elements of the environment with visible light may partially rebuild the population of pathogenic microorganisms in photoreactivation process.

Conclusions. Effective cleaning of both the surfaces and air cannot be limited to their exposure to UV-C radiation, but should be supplemented with other techniques for neutralizing microorganisms, which need be subsequently applied after such exposure.

Key words

ultraviolet radiation, UV-C low-pressure mercury lamp, UV-C LEDs, microorganisms, bioaerosol, metal, plastic, glass, photoreactivation, survivability

INTRODUCTION

In 1877, Downes and Blunt made one of the most groundbreaking discoveries in the history of photobiology by demonstrating the ability to prevent the growth of bacteria in test tubes after their exposure to sunlight and concluded that this process depended on the intensity, duration, and wavelength of such radiation [1]. Today, almost 150 years after this discovery, this technology is still in use to improve the microbiological quality of the environment around us. It is well known that shortwave Ultraviolet-C (UV-C) radiation is an established means of eliminating, or at least significantly reducing, both the spread of airborne pathogens and microbial contamination of various equipment and fomite surfaces [2]. Nucleic acids are the most sensitive target of UV-C radiation, although unsaturated bonds present in biological molecules, such as coenzymes, hormones, and electron carriers, may also be susceptible to this type of damage [3].

The mechanism of UV disinfection by absorption of radiation photons depends on the sensitivity of the genetic material of a given microorganism to the UV wavelength. Single-stranded viruses (e.g. parvoviruses) are more sensitive to UV radiation due to the lack of redundant genetic information in the second strand, which allows double-stranded viruses (e.g. herpesviruses, poxviruses, adenoviruses) to repair damage [4]. Non-enveloped viruses (e.g. adenoviruses, parvoviruses, calciviruses, picornaviruses) are usually more resistant to UV radiation than enveloped viruses (e.g. influenza, human cytomegalovirus, HIV, respiratory syncytial virus, vaccinia virus, human coronaviruses including SARS-CoV-2), because envelope proteins and lipids can be more easily destroyed than other parts of the virus. Usually, an increased environmental dose of UV radiation leads to an increased rate of virus mutation, and the lethal effect of nucleic acid (DNA or RNA) damage depends on the location of the changes in the virus genome [5]. UV radiation affects bacteria mainly at the cellular level, damaging their key biomolecules (mainly nucleic acids, as

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well as proteins and lipids), which consequently translates into the growth efficiency and species composition of the bacterial biome. UV radiation leads to the formation of pyrimidine dimers, in particular thymine dimers (T-T) or cyclobutane-pyrimidine dimers (CPD), as well as 6–4 pyrimidine-pyrimidine photoproducts or Dewar isomers. These mutagenic interactions change the structure of DNA, inhibit polymerases and stop replication, which usually leads to cell death [6]. A set of specific photosignalling pathways is also responsible for the developmental and metabolic changes induced by UV radiation in fungi [7]. Direct exposure to UV-C radiation can result in both the destruction of conidia [8] and the inhibition of toxin production or hyphal development [9]. Fungal death caused by UV-C radiation is associated with DNA mutations, and these with the development of pyrimidine adducts and cyclobutyl (pyrimidine) dimers [10]. UV radiation can also cause excessive accumulation of reactive oxygen species (ROS), inhibit critical cellular enzymes, and oxidize membrane lipids [11].

Many environmental microorganisms live in constant balance between UV damage and repair. The simplest repair process starts immediately when microbial cells face the sunlight irradiation. In this case, UV-induced nucleic acid damage can be reverse by the photo-reactivation mechanism performed by photolyase enzymes, which use visible (or near-UV) light as the energy source. This process is initiated by the absorption of photons by the photolyase chromophores. This in turn causes the excitation and subsequent electron donation of a co-factor (i.e. the reduced form of flavin adenine dinucleotide, $FADH^+$), which splits the pyrimidine dimers, returning them to their monomeric form. Photolyases have a high affinity for pyrimidine dimers and 6–4 photoproducts, and their activity can be observed, for example, as a reduction in the level of UV-induced mutations after irradiation of microbial cells with visible light [3].

The aim of this study was to assess the ability of bacteria, virus, and mould suspended in the air and deposited on metal, glass, and plastic surfaces to photo-reactivation (by visible light as the energy source) after their exposure to UV-C radiation produced by two disinfection devices, i.e. traditional low-pressure mercury lamp and modern light-emitting diodes.

MATERIALS AND METHOD

Five reference strains from the American Type Culture Collection (ATCC) were used for the tests, representing: bacteria (average initial concentrations, $\bar{x}$: ~6×10^7 CFU/cm^3, where CFU means colony-forming unit), including *Staphylococcus aureus* ATCC 6538 Gram-positive cocci, *Bacillus subtilis* ATCC 6633 Gram-positive bacilli, and *Pseudomonas aeruginosa* ATCC 260 Gram-negative rods; viruses, including bacteriophage PhiX174 ATCC 13706-B1 ($\bar{x}$: 4.2×10^5 PFU/cm^3, where PFU means plaque-forming unit); and fungi, including *Aspergillus versicolor* ATCC 9577 ($\bar{x}$: 5.5×10^5 CFU/cm^3). For the UV-C inactivation tests, aqueous suspensions of the above-listed microorganisms were applied to three types of smooth metal surfaces: stainless steel, plastic (polypropylene), and glass (SiO_2 content 72–73%). All tested materials had the same circular token shape with an area of 4.9 cm^2, thickness 2 mm, which were autoclaved before use. The same suspensions were also used to generate monobioaerosols of these microorganisms into the aerosolization chamber (Patent No. 235437, Patent Office of the Republic of Poland, 2020) using a six-nozzle Collison nebulizer (model MRE CN25, BGI Incorporated, Waltham, USA) – see below. For the inactivation experiments, each of the tested surfaces was separately inoculated with appropriate microbial suspensions (inoculum volume 0.2 cm^3 each time), and subsequently exposed from a distance of 0.5 m for 20 min to UV-C radiation emitted by two tested devices, i.e. low-pressure mercury lamp, LPML (model G15T8, 15 W, Sankyo Denki Co., Ltd., Kanagawa, Japan) and ultraviolet emitting diodes, LEDs (model UVM002A-0401U1-RM, 9 W, Citizen Electronics Co., Ltd., Yamanashi, Japan).

During these experiments, UV-C radiation was directed perpendicularly to the exposed surfaces and the effective UV-C doses (fluences) were 762 J/m^2 (radiation peak at 253.2 nm) and 832 J/m^2 (radiation peak at 270 nm), respectively. These UV doses were selected as the highest fluences required to inactivate 90% of the most resistant microbial propagules (in this case *A. versicolor* conidia) [12]. For each of the five microbial strains and for each of the three tested surfaces, a total of 60 samples (15 strain/surface combinations × 4 replicates for each combination) were tested. Because both emitters, in addition to UV germicidal irradiation, also emit radiation with a shorter wavelength, which may create ozone from the oxygen contained in the air, 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 immanent part of the performed tests.

After UV-C exposure, all samples were divided into two equal parts. The first batch of samples (30) was immediately laboratory-processed by washing the microorganisms 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. The suspensions obtained in this way were elaborated by spread plate method on microbiological media appropriate for a given microorganism (i.e. blood trypticase soy agar for bacteria; nutrient agar and nutrient broth, both with 5% addition of NaCl, 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 the inactivation process. After 24 h of incubation, the grown colonies of bacteria and fungi were counted (as CFU) or, in the case of bacteriophage, the visible plaques on a bacterial lawn (as PFU), and the number of culturable microorganisms in the control sample, C_{cont}, or on the tested surfaces, $C_{CFU/PFU}$, was determined according to the formula 1:

$$C_{cont} \text{ or } C_{CFU/PFU} = (N/10^{-D})(V_1/V_2)$$

where: N – average number of colonies grown on the surface substrate [CFU], or average number of visible plaques on a bacterial lawn [PFU], D – dilution factor, V_1 – volume of extraction solution [ml], V_2 – volume of sample inoculated on agar [ml].

The second batch of samples (30) was re-exposed for 24 h to visible light emitted by two compact 'natural sunshine' fluorescent lamps with the following light parameters: 380–780 nm spectral range with three radiation peaks at 404 nm, 435 nm, and 545 nm; correlated colour temperature – 6500

K; colour rendering index – 96 (model MASTER TL-D 90 Graphica 18W/965 SLV/10, Philips Lighting B.V. – Signify Holding, Piła, Poland). As before, during these radiation experiments, visible light was directed perpendicul to the tested surfaces. After this re-exposure, all samples were laboratory elaborated in the same manner as the first batch samples.

In the case of aerosolized microorganisms, the UV-C emitters were placed in an aerosolization chamber opposite the nebulizer outlet nozzle located at a distance of 0.5 m from the UV-C source (to simulate the same effective UV-C doses of 762 J/m^2 and 832 J/m^2 for LPML and LEDs, respectively). In the aerosolization chamber, the generated particles were homogenized by mixing them using a mixer with a variable propeller speed. In the tests, the forced air flow velocity within the aerosolization chamber was 0.3 m/s, which is typical for the indoor environment. The aerosol mixing velocity was controlled by an anemometer (model Testo 435–4 with IAQ probe, Testo Sp. z o.o., Pruszków, Poland). Before starting the measurement with each of the tested microorganisms, the aerosolization chamber was cleaned mechanically and chemically using an alcohol-based surface disinfectant with antimicrobial properties (Desprej, BOCHEMIE, s.r.o., Bohumín, Czech Republic). Each actual measurement of bioaerosol concentration was preceded by a measurement checking the cleanliness of the aerosolization chamber. For this purpose, at the beginning of each measurement session, the test set was operated in the absence of microbiological material in the nebulizer (i.e. only water aerosol was generated), and the air samples were collected using a Button Aerosol Sampler (SKC Inc., Eighty Four, USA) with a 1.2 µm polycarbonate filter (Merck Millipore, Tullagreen, Ireland) at a flow rate of 5 l/min for five minutes in two repetitions. Each time, laboratory processing of the air sample collected in this way showed no microbial growth. The actual measurements of microbial concentration consisted of 20-minute nebulization of the tested bioaerosol of a given microorganism into the aerosolization chamber, in which the tested UV-C radiator (LPML or LEDs) was placed, which was not switched on at this stage of the study. In the chamber, the bioaerosol was subjected to continuous mixing (by an air stream with a speed of 0.3 m/s), and after this time, a control sample of the bioaerosol was taken using a Button Aerosol Sampler with a polycarbonate filter (as described above). After the control measurement, the tested UV-C source (LPML or LEDs) was switched on and irradiated the aerosol of a given microorganism mixed in the chamber for the next 20 minutes. After this period of time, the tested radiator was turned off and the appropriate bioaerosol samples were aspirated in the manner described above.

All of the above bioaerosol measurements were performed in duplicate. Immediately after bioaerosol sampling, each filter was placed in a Falcon tube (Sarstedt AG & Co. KG, Nümbrecht, Germany) with 10 ml of 0.9% physiological saline extraction solution (Baxter Manufacturing Sp. z o.o., Lublin, Poland) with 0.05% Tween 80 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and shaken for one hour at room temperature on a laboratory shaker (model Promax 1020, Heidolph Instruments GmbH & Co., Schwabach, Germany). From the obtained suspension of microorganisms, a series of serial dilutions (from 10^{-1} to 10^{-6}) were made, and then 0.1 ml of the tested sample was inoculated (in three replicates) onto Petri plates with the following microbiological media: TSA (bacteria), MEA (fungus), and nutrient agar with *E. coli* lawn (bacteriophage). After inoculation, all Petri plates were divided into two parts. The first batch was immediately incubated at room temperature, the second batch (as in the case of tested surface samples) was re-exposed for 24 h to visible light emitted by a 'natural sunshine' fluorescent lamp. After this re-exposure, all of these samples were laboratory processed in the same manner as the first batch of air samples. In both cases, after the incubation period, the concentration of culturable bacteria and fungi (C_{CFU}) and bacteriophage (C_{PFU}) in one liter of air of the tested sample (CFU/l or PFU/l) was calculated according to formula 1.

All tests were performed in a class 2 biosafety cabinet (model SafeFAST Classic 218, Faster, Ferrara, Italy) at a room temperature (SD) of 22.6 (1.4)°C and relative air humidity (SD) 29.4(2.2)%.

All experimental data were statistically processed. After checking the normality of data distributions with the Shapiro-Wilk test, the collected data were statistically elaborated by Kruskal-Wallis and Mann-Whitney tests, using Statistica (data analysis software system) version 10 (StatSoft, Inc., Tulsa, USA). Probability values were treated as statistically significant at $P<0.05$.

RESULTS

Survivability of microorganisms deposited as water suspensions on metal, plastic and glass surfaces, as well as suspended in air (due to their aerosolization from water suspensions) after 20 min exposure to UV-C radiation, generated by low-pressure mercury lamp (LPML) and light-emitting diodes (LEDs), and subsequent 24 h exposure to visible light is presented in Figure 1.

The doses of UV-C radiation generated by both tested lamps showed high efficiency in inactivating all tested microorganisms. Inactivation was effective both for microorganisms present on surfaces made of metal, plastic and glass, as well as those suspended in the air in the form of bioaerosol; however, statistical analysis revealed that LEDs were more effective in this respect than LPML regarding all irradiated surfaces (Mann-Whitney test: $P<0.0001$). In terms of bioaerosol samples, the differences between the tested UV-C sources were not statistically significant (Mann-Whitney test: $P > 0.05$). In relation to the individual tested microorganisms, irradiation of the tested samples showed that:

a) The applied dose of UV-C radiation emitted by LPML caused a complete (100%, i.e. greater than 6-log) reduction in the number of *S. aureus* cocci suspended in the air, and significantly reduced the number of these bacteria deposited on the tested surfaces (by 97.4–99.5%, i.e. 1.6–2.3-log, on average). In the case of the LED emitter, the applied dose of UV-C radiation practically eliminated the contamination of the tested surfaces with *S. aureus* cocci (average reduction in the concentration of bacteria after irradiation – 99.98–100%, i.e. 3.7-log–greater than 6-log), and in the case of air pollution it significantly (by 98.6%, i.e. 1.8-log, on average) reduced the number of these bacteria.
b) In the case of *B. subtilis* bacilli, UV-C radiations emitted by LPML and LEDs reduced the survivability of microorganisms deposited on the tested surfaces by 96.4–99.5% (1.4–2.3-log) and 95.7–99.7% (1.4–2.5-log),

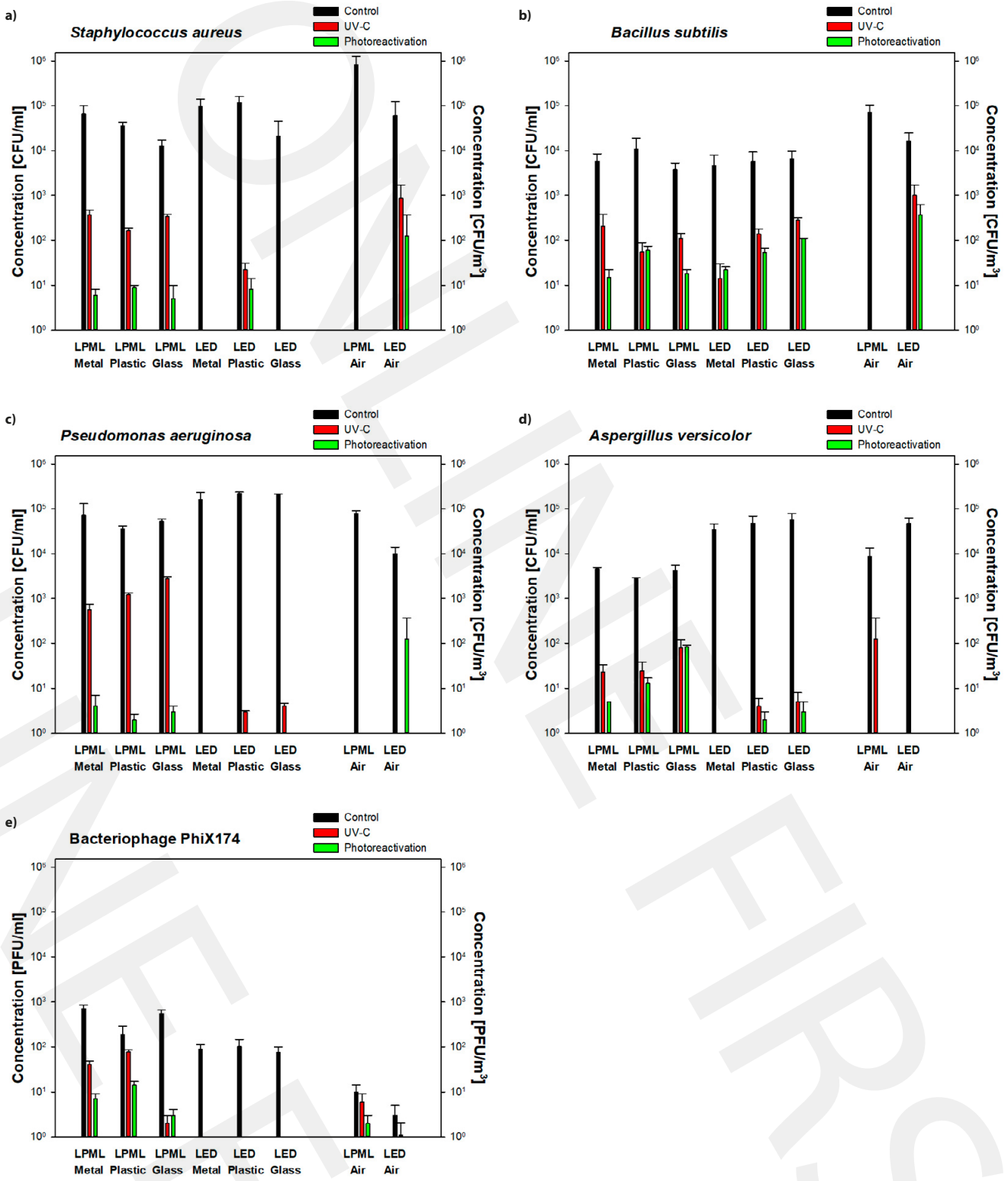

Figure 1. Survivability of micro-organisms deposited as water suspensions on metal, plastic and glass surfaces, as well as suspended in the air (due to their aerosolization from water suspensions) after 20 min exposure to UV-C radiation, generated by low-pressure mercury lamp (LPML) and light-emitting diodes (LEDs), and subsequent 24 h exposure to visible light. UV-C doses (fluences) were 762 J/m2 and 823 J/m2 for LPML and LEDs, respectively.

respectively. In the case of the bioaerosol of this bacterium, its exposure to the tested dose of UV-C radiation emitted by LPML caused the complete elimination of culturable microorganisms in the air (100%, i.e. greater than 6-log reduction), and in the case of the dose of radiation emitted by LEDs it reduced this number by 94% (1.2-log).

c) The population of *P. aeruginosa* rods deposited on the tested surfaces under the influence of UV-C radiation emitted by LPML decreased its number by 94.7% to 99.2% (1.3-log to 2.1-log), compared to its initial number, and under the influence of UV-C radiation emitted by LEDs it was practically reduced to zero (i.e. from 4.7-log to greater

than 6-log reduction). A similar 100% level of reduction in the number of these bacteria occurred in a situation in which the bioaerosol of this bacterium was exposed to UV-C radiation emitted by both LPML and LEDs (in both cases 100%, i.e. greater than 6-log reduction).

d) Reduced survival under the influence of doses of UV-C radiation emitted by LPML and LEDs was also observed in the case of *A. versicolor* conidia. Exposure to UV-C radiation emitted by LPML translated into a reduction in the number of conidia deposited on the tested surfaces by 81–94.9% (0.7–1.3-log). In the case of this radiation emitted by LEDs, the number of culturable conidia of this mould practically dropped to zero (i.e. from 4-log to greater than six-log reduction). In the case of exposure to the aerosol of *A. versicolor* conidia, UV-C radiation emitted by LPML inactivated 98.6% (1.8-log) of the cells of this fungus suspended in the air, and this radiation emitted by LEDs completely eliminated (100%, i.e. greater than 6-log reduction) the contamination of the air with this type of asexual spores.

e) In the case of bacteriophage PhiX174, exposure of this virus to UV-C radiation emitted by LEDs resulted in complete elimination from the environment (on all three surfaces tested, virus survivability was equal to 0, i.e. greater than 6-log reduction). On the other hand, exposure to UV-C radiation emitted by LPML was highly effective in the case of the virus deposited on metal and glass surfaces – a decrease in the number of culturable viruses by 94.2% (1.2-log) and 99.6% (2.4-log), respectively. Viruses deposited on the plastic surface and exposed to UV-C radiation from LPML retained their culturability, on average by 41.1% (0.4-log reduction only). UV-C radiation emitted by LPML and LEDs showed similar effectiveness in the case of aerosol of this virus. In these cases, the irradiation of bacteriophages suspended in the air caused a decrease in their numbers by 60% (0.2-log reduction) and 33.3% (0.5-log reduction), respectively.

As mentioned earlier, not only the efficiency of inactivation by UV-C radiation emitted by LPML and LEDs was examined, but also the ability of the tested microorganisms to regenerate under the influence of visible light irradiation, i.e. to photo-reactivate. Daily irradiation of samples with visible light after their exposure to UV-C radiation at doses of 762 J/m^2 (for LPML) and 823 J/m^2 (for LEDs), in only five cases resulted in the regeneration of the irradiated microorganisms. In the case of surfaces irradiated with UV-C radiation, the source of which was LPML, an increase in the number of culturable microorganisms occurred in the case of: *B. subtilis* bacilli deposited on the plastic surface (an increase of 9.1% in relation to the number of culturable microorganisms after their exposure to the tested dose of UV-C radiation), conidia of *A. versicolor* mould and bacteriophages PhiX174 deposited on the glass surface (an increase of 6.3% and 50%, respectively).

In the case of exposure to UV-C radiation from LEDs, the regeneration of microorganisms irradiated with visible light was recorded only in the case of *B. subtilis* bacilli deposited on the metal surface (an increase of 57.1% in relation to the number of culturable microorganisms after their exposure to the tested dose of UV-C radiation) and *P. aeruginosa* rods dispersed in the air (in this case, the increase was the highest among all tested microorganisms, as much as 125%).

During the experiments with both LPML and LEDs, the control of the ozone emission did not reveal the presence of this gas in the air near the UV-C emitters and samples (all measured concentrations were below the limit of quantification, i.e. below 0.01 ppm – 0.02 mg/m^3).

DISCUSSION

Today, ultraviolet-C germicidal irradiation is a widely used method for the environmental control of microbial contamination, including air and surface disinfection [2, 13]. In such applications, electric radiators are usually used, including most often low-pressure (fluorescent) and medium-pressure mercury lamps and light-emitting diodes. 'Conventional' UV-C lamps contain mercury, and this as a hazardous substance, is undesirable in the environment. In addition, heat dissipation from this type of UV-C sources causes a significant temperature gradient near the emitter, which is also an unfavourable phenomenon affecting, among others, the durability of exposed products or objects. Against this background, ultraviolet C light-emitting diodes (UV-C LEDs) have become a reliable alternative to conventional UV-C lamps due to the lack of toxic substances, their tunable optical properties, low energy consumption, long life span and efficiency, as well as the possibility of shaping the emission spectrum in a wide ultraviolet range from 210 nm-400 nm [14–16]. In this study, both tested UV-C radiation sources were effective in inactivating microorganisms deposited on metal, plastic, and glass surfaces, as well as those dispersed in the air in the form of bioaerosol particles. This high inactivation efficiency of both UV-C sources in the conducted experiments was mainly the resultant of the radiation doses emitted by them. The effectiveness of this type of devices is usually determined by UV doses (fluences) necessary to obtain specific log reduction, where log reduction described as 1, 2, 3, 4, 5, and 6 corresponds to 90%, 99%, 99.9%, 99.99%, 99.999%, and 99.9999%, respectively (log reduction is defined as (N_0/N), where N_0 is the initial and N is the final, i.e. after UV exposure, viable microorganism counts). The data available in the literature on the subject show that for the microorganisms tested in these experiments, the highest fluences (in J/m^2) to obtain significant log reduction were as follows [12, 17]: for *S. aureus* – 340 for 5-log reduction, for *B. subtilis* ATCC 6633 – 750±110 for 5-log reduction, for *P. aeruginosa* – 100 for 5-log reduction; for bacteriophage PhiX174 ATCC 13706-B1 – 360 for 5-log reduction, and for *A. versicolor* – 768 for 1-log reduction. Against this background, the applied in this study UV-C doses emitted by LPML were sufficient to achieve 1.2-log to 2.3-log, 0.4-log to 2.3-log, and 0.7-log to 2.4-log, as well as 0.2-log to greater than 6-log inactivation of microorganisms deposited on metal, plastic, and glass surfaces, as well as suspended in the air, respectively. On the other hand, the fluences produced by LEDs resulted in maximal inactivation efficiencies of 2.5-log to greater than 6-log, 1.6-log to greater than 6-log, and 1.4-log to greater than 6-log, as well as 0.5-log to greater than 6-log for microorganisms deposited on metal, plastic, and glass surfaces, as well as suspended in the air, respectively. Despite these nominal values, the differences in log reductions between different surfaces and bioaerosols irradiated with LPML and LEDs were not statistically significant (for both sets of experiments – Kruskal-Wallis tests: $P > 0.05$). A certain

role in microbial inactivation also played reflectivity of exposed materials, influencing the number of UV-C photons reaching the exposed microorganisms [13]. Despite the fact that the reflectance of solar (covering UV band) light of the tested materials were different from each other (i.e. 20–28% for stainless steel [18], 10% for polypropylene [19], and 4–6.9% for SiO_2 glass [20, 21]), its influence on survivability of tested microorganisms may have been somewhat limited (for both LPML and LEDs experiments – Kruskal-Wallis tests: $P > 0.05$).

As it was already mentioned, the aim of this study was to assess the ability of bacteria, virus, and mould suspended in the air and deposited on metal, glass, and plastic surfaces to photo-reactivation after their exposure to UV-C radiation produced by LPML and LEDs. There are several processes that enable organisms to repair nucleic acid damage caused by UV radiation, including excision, error-prone, recombinational repair and photo-reactivation. In short: excision repair is a multi-step enzymatic process in which the region of DNA containing a dimer or other damage is first physically cut out, and the gap replaced by a newly-synthesized DNA fragment. In error-prone repair, the DNA polymerase shifts from template directed synthesis to catalyzing the incorporation of random nucleotides; however, such repair DNA synthesis may lead to mistakes (translated to mutations). In recombinational repair, the block of DNA replication by pyrimidine dimers results in a gap in one strand of DNA, where the dimer blocks part of it from being copied. The most common way that cells fill such a gap is through genetic recombination with another DNA molecule or chromosome containing the same or similar information.

Against this background, the photo-reactivation process described briefly in the Introduction of this article, seems to be the least complicated DNA repair mechanism after its damage by UV radiation [7]. As this is an enzymatic process, it may be, among others, dependent upon temperature, light intensity, relative humidity, pH, and ionic strength of the medium; however, most researchers agree that photo-reactivation relies primarily on the time of light exposure and relative humidity (influencing sorption capacity of exposed microbial propagules) [22]. In the current study, as relative humidity was below 50%, no rapid decrease in the rate of UV-induced inactivation was expected to be observed due to minor changes to this parameter. With respect to the irradiation time of the samples, 24 h of exposure to visible light was long enough to observe its regenerative effect among the tested microorganisms [3, 15]. As the photo-reactivation process took place at room temperature, such conditions were probably sufficient for the enzymatic activity of photolyases [23].

In the literature on the subject, to date the photo-reactivation has usually been studied in microorganisms suspended in liquids – drinking water, wastewater, aquaculture, etc. [e.g. 24–29], and much less frequently in microorganisms suspended in air [e.g. 1, 22] or deposited on surfaces [e.g. 13, 30]. Against this background, the study conducted above contributes new information to the knowledge in this area.

As shown, photo-reactivation was demonstrated by *B. subtilis* bacilli deposited on the plastic surface, conidia of *A. versicolor* mould and bacteriophages PhiX174 deposited on the glass surface after LPML irradiation, as well as *B. subtilis* bacilli deposited on the metal surface, and *P. aeruginosa* rods dispersed in the air after exposure to UV-C LEDs. In the case of *B. subtilis* bacteria, the key role in their photo-reactivation was probably played by the humid conditions of the environment. Low relative humidity did not favour the sorption of water from the atmosphere by these bacilli, and hence a sharp concurrent drop in UV-induced inactivation rate was not observed [22]. These bacteria also have the ability to form spores and, as such, in harsh conditions are more resistant to UV-C disinfection. Moreover, a specific role in its resistance to UV radiation may also be played by a dedicated DNA repair mechanism which enables direct reversal of spore photoproduct due to lyase, which splits thymine dimers to thymine *in situ* during spore germination. A certain role may also be played here by an ability of these bacterial cells to adhere to a substrate surface during the initial phase of biofilm formation. Such attachment is much easier on surfaces with increased porosity (in the case of the tested surfaces, glass showed the smallest roughness, compared to metal and plastic surfaces) [31]. In this case, natural surface irregularities may promote the 'hiding' of a certain number of these bacteria and thus isolate their cells from the adverse effects of UV-C irradiation, which increases the reactivation possibilities of the colonies after the cessation of radiation.

Fungi have several UV protective mechanisms, which enable them to regenerate after inactivation. Among these is pigment production (including carotenoids, melanin, and mycosporins), which serves as a kind of 'sunscreen' for fungal cells [7, 32]. Some studies confirm that different pigmentation of conidia confers different resilience to UV irradiance [33]. After UV-C radiation, mould conidia usually reveal a greater survival rate when they are subsequently exposed to light repair conditions and, in the majority of fungal species, photo-reactivation is presumed to be the primary lesion repair pathway [7]. In the current study, *A. versicolor* conidia, when deposited on the tested surfaces and irradiated with the same UV-C doses, however, due to different surface reflectance (higher in the case of metal and plastic compared to glass), their whole exposure might be lightly different. Such a slight photo-reactivation was also observed by Oliveira et al. for *A. fumigatus* and *A. terreus* [34]. It is also worth mentioning that the inactivation efficiency of LPML was slightly lower than that of LED emitters, and the photo-reactivation phenomenon in the case of this mould was noted only for conidia exposed to UV-C radiation emitted by LPML.

In turn, the photo-reactivation of bacteriophage PhiX174, which represents circular single stranded DNA (ssDNA) phage and a potential surrogate for SARS-CoV-2 in terms of radiation inactivation, may be attributed to the location of its repair enzymes and be supported by the host bacteria. Some repair enzymes seem to be preferentially concentrated near the cellular DNA and virus penetrating inside the host cell, can complete the viral cycle and, due to its assistance, can repair their own genetic material [35]. Bacteriophage PhiX174 is one of the most sensitive phages to UV irradiation, and when deposited on metal or plastic surfaces, their reflectance might enhance the inactivation effect of UV-C radiation to a greater extent than a glass surface. For such a sensitive microorganism, this could be important for its reactivation [36].

In the current study, *P. aeruginosa* rods represent a group of Gram-negative bacteria, whereas *S. aureus* serves as an example of Gram-positive pathogenic bacteria. Both types of bacteria responded differently to UV-C irradiation in the

tests. *P. aeruginosa* rods showed a high capacity for photo-reactivation after being irradiated with UV-C emitted by LEDs, but only when the particles of this microorganism were dispersed in the air. In the case of *S. aureus* cocci, however, each tested variant of UV-C irradiation, i.e. emitted by LPML or LEDs, did not show a significant increase in the number of regenerated culturable cells of this bacterium, regardless of the surface on which they were deposited, or whether they were dispersed in the air as a bioaerosol.

Regarding both these bacterial strains, UV-C-induced cell damage was probably dependant on the structure of their cells. Gram-negative bacteria have a thin peptidoglycan layer covered by an outer lipid membrane, whereas Gram-positive bacteria contain a thick peptidoglycan layer devoid of a lipid shield. UV-C radiation easily penetrates the peptidoglycan layer, but is inhibited in Gram-negative bacterial cells by a lipid membrane containing polysaccharides and proteins [37]. Thus, in the case of *P. aeruginosa* rods, if these bacteria were deposited on flat surfaces, they probably had much less opportunity to avoid the adverse effect of UV-C radiation, while being suspended in the air, their environmental dispersion probably allowed them to avert the lethal effect of the UV-C radiation dose and retain their repair abilities thanks to the impermeability of the outer polysaccharide-protein coat. In the case of *S. aureus* cocci, the ease of penetration through the peptidoglycan layer caused the applied doses of UV-C radiation to destroy all possibilities of enzymatic photo-reactivation of these bacteria [3, 37]. Hence, both on the tested surfaces and in the air, the decrease in their survival was very significant.

From the proper hygienic status of the air and surfaces point of view, a quantitative determination of photo-reactivation is essential to calculate the UV-C doses necessary to eliminate, or if not, at least effectively limit the number of pathogenic microbial pollutants. More importantly, the photo-reactivation phenomenon may significantly affect the survival of microorganisms in the environment, and thus reduce the effectiveness of the disinfection process carried out using devices emitting UV-C radiation for the purposes of risk reduction caused by microbiological contamination of fomite surfaces and the air. Hence, both adequate UV-C irradiation of target environment and subsequent avoidance of visible light after UV-C inactivation, can effectively inhibit reactivation of microorganisms, which is of enormous practical importance from the point of view of public health.

CONCLUSIONS

UV-C radiation emitted by LPML and LEDs in doses of 762 J/m^2 and 832 J/m^2 effectively inactivated the tested microorganisms deposited on metal, plastic and glass surfaces, as well as dispersed in the air. However, this type of inactivation is not an irreversible process and subsequent exposure of microbiologically contaminated elements of the environment with visible light of the wide (380–780 nm) spectral range may partially rebuild the population of pathogenic microorganisms in the photo-reactivation process. Therefore, effective cleaning of both the surfaces and air cannot be limited to their exposure to UV-C radiation, but should be supplemented with other techniques for neutralizing microorganisms, which need be subsequently used after exposure to ultraviolet light.

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