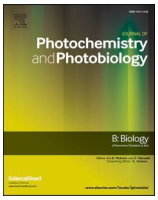
Journal of Photochemistry & Photobiology, B: Biology 217 (2021) 112155

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Selection of LED lighting systems for the reduction of the biodeterioration of speleothems induced by photosynthetic biofilms in the Nerja Cave (Malaga, Spain)

J. Muñoz-Fernández [a,*], Y. Del Rosal [b], F. Álvarez-Gómez [a], M. Hernández-Mariné [c], R. Guzmán-Sepúlveda [d], N. Korbee [a], F.L. Figueroa [a]

[a] Malaga University, Institute of Biotechnology and Blue Development (IBYDA), Department of Ecology and Geology, Campus Universitario de Teatinos s/n, 29071 Málaga, Spain
[b] Nerja Cave Foundation, Research Institute, Carretera de Maro s/n, 29787 Nerja, Málaga, Spain
[c] Barcelona University, Department of Biology, Health and Environment, 08028 Barcelona, Spain
[d] Malaga University, Department of Graphic Expression, Design and Project, Campus Universitario de Teatinos s/n, 29071 Málaga, Spain

ARTICLE INFO

Keywords:
Algae
Cyanobacteria
Biofilms
LEDs
Photonic assimilation performance
Photosynthetic efficiency

ABSTRACT

Electrical lighting favours the development of photosynthetic biofilms in caves which can induce biodeterioration in the colonized substrates. The use of specific lights as a limiting factor for biofilm growth could be effective in their control and represents an alternative to chemical methods since they can damage the substrate. However, studies about lighting and the photosynthetic activity of organisms in caves are scarce. In order to select the most effective LED light source in reducing photosynthesis and therefore, in reducing the growth rates of microalgae and cyanobacteria, four biofilms in the Nerja Cave were illuminated by several light emitted diodes (LEDs) with different spectral compositions and the photobiological responses were measured both by empirical and theoretical methodologies. The empirical approach was based on the photosynthetic efficiency, by measuring the *in vivo* chlorophyll *a* (Chl *a*) fluorescence and the theoretical approach was based on the photonic assimilation performance related to the proportion of the light quality used for photosynthesis, according to the action spectra for photosynthesis available in the literature. The photobiological responses showed differences between the empirical and theoretical approach mainly in biofilms dominated by cyanobacteria and red algae, probably because the available action spectra were not useful for monitoring these Nerja Cave biofilms. However, the expected spectral responses of photosynthesis were observed in green microalgal biofilms with maximum photosynthetic efficiency in red and blue light although the green light was also unexpectedly high. The high photosynthetic efficiency in green light could be explained by the predictable high chlorophyll content due to a very dark environment. The results were not conclusive enough for all the biofilm types to be able to recommend a specific lighting system for the photocontrol of biofilm expansion. Therefore, new action spectra for photosynthesis of the extremophile organisms of the Nerja Cave are required. This approach, based on theoretical and empirical methodologies, is a useful tool to obtain information to allow the design of the most adequate lighting systems to reduce photosynthetic activity and favour the conservation of the caves.

1. Introduction

Caves are natural underground chambers which are considered as extreme environments. They are mainly characterized by total darkness or low levels of light, stable temperatures and high humidity. Some of these subterranean environments had been used over time for human activity and may be used as a tourist attraction or as a geological, biological or archaeological laboratory. For recreational purposes the most important change, which alters the initial conditions, is the viewing light. Visitors cause the so-called “anthropic impact” that may be related to hydrogeological, geomorphological, environmental, microbiological or faunistic changes [1]. In tourist caves, electric lighting encourages the development of photosynthetic microorganisms on the surface of speleothems and walls. These microbial communities

* Corresponding author.
E-mail address: ecojuanma@gmail.com (J. Muñoz-Fernández).

https://doi.org/10.1016/j.jphotobiol.2021.112155
Received 22 July 2020; Received in revised form 16 January 2021; Accepted 12 February 2021
Available online 17 February 2021

are usually organized in biofilms, complex microbial structures enclosed in an exopolysaccharide matrix [2,3]. Regarding their biodiversity, the most frequent are made up of microalgae and cyanobacteria and many of them produce extracellular polymeric substances (EPSs) such as heteropolysaccharides, proteins and DNA [4,5] which may act as a buffer zone and contribute to desiccation tolerance which allow them to develop on practically any illuminated substrate. Uncontrolled growth of microorganisms in the biofilm can induce deterioration of the substratum, known as biodeterioration [2], which can severely damage the substratum and indeed the natural and cultural heritage of the cave. Therefore, it is vital to design preventive and corrective strategies for the control of the growth of these microbial communities.

Several physical, chemical and biological cleaning methods could be adopted [6], but most of the treatments are ineffective or even dangerous for the substrate or the subterranean environment [7,8] and they also favour fungi, which colonises any available organic matter [9,10]. Light treatments such as UV-C radiation, based on LED technology for biofilm control [11], have to be applied with a strict security approach due to the damage that could be caused to human skin and eyes [12,13]. Due to its high energy (200–280 nm) this technique is too energetic for photosynthesis and is destructive for microorganisms [14]. The wavelength of 260 nm coincides with the maximum absorption of DNA, so it is effectively altered and a biocide effect takes place. However, recently it has been found that a Kr—Cl gas mixture lamp of far-UV-C (200–222 nm) has similar anti-microbial and germicidal properties to the UV light of 254 nm but without inducing any mammalian skin damage [15]. This can be explained by the fact that far-UV-C does not penetrate the outer (non-living) layers of human skin or eyes, whereas the bacteria and virus have micrometer or even smaller dimensions and the DNA or RNA can be damaged [16,17]. Nevertheless, cave biofilms are made up of microorganisms adapted to the subterranean environment (fungi, bacteria, cyanobacteria, microalgae, etc), amongst them troglobial species, which may have effective DNA repair mechanisms when they are subjected to UV radiation due to the need to repair the damage caused by other stress factors that exist in cavities [18]. In addition, the exopolysaccharyde sheath increases the path of light and may reduce the effectivennes of UV lamps.

Therefore, more innocuous and effective methods should be investigated [19] such as those based on PAR light (400–700 nm), for the decrease of microbial growth [20–22]. PAR light is the most important factor related to the growth of photosynthetic organisms [23], and lighting is the factor that is the easiest to control in caves. The irradiance, spectral quality, and photoperiod, affecting the photosynthetic process are basic aspects in the production or reduction of growth through photosynthesis [24].

The use of LEDs for the growth control of photosynthetic organisms has increased in recent years as this lighting technology allows the creation of compositions or narrow band spectral forms, with a smaller size and volume for the light emitting source and with a longer useful life [25–27]. In addition, LED lighting has a series of advantages compared to traditional lighting: high efficiency, great thermal dissipation, increasing its durability and a chromatic reproduction index (CRI) above 80%, a wide range of colours from 2700 K to more than 6000 K, high durability and easily programming, on and off cycles, as well as spectral combinations that are interesting, depending on the photosynthetic community of a specific moment, amongst others [28]. Previous studies, aimed at designing a specific lighting system with a lower photosynthetic effectiveness, have been based on the pigment composition of the microorganisms and the overlapping of the emission spectra of the lights with the absorption spectra of a culture [29]. Amongst the results, Bruno et al. [30] confirmed that cyanobacteria are able to acclimatize to different lighting conditions by adjusting their pigment content and a very short shift in the light emission determines different growth responses of biofilms and Albertano and Bruno [31] designed wavelengths less favourable for photosynthetic growth which seem to span a narrow band around 500 nm and above 700 nm [31]. On the other hand, Olson [21] confirmed that 595 nm yellow LED lamps successfully prevented lamp flora growth and Roldán et al. [32] results suggested that a monochromatic green light could prevent the growth of photosynthetic organisms in hypogea environments.

As the absorption spectra of the pigments is not enough to predict photosynthetic efficiency, both empirical and theoretical photobiological methodologies can be used to select the most effective LED light source in reducing photosynthesis and therefore, in reducing the growth rates of microalgae and cyanobacteria. Empirical methodology is based on the use of a pulse amplitude modulated (PAM) fluorometry to measure, *in situ*, the biofilm photosynthetic activity (the effective and maximum quantum yield of photosystem II, $\Delta F/F_m'$ and F_v/Fm respectively) [33]. In spite of the great advantages of *in vivo* Chl *a* fluorescence determination, the use of PAM fluorometer in cave biofilms is very recent [34], whereas, theoretical methodology is based on the photobiological knowledge of algae and cyanobacteria (action spectra of photosynthesis) published in the literature. Action spectra for photosynthesis have been conducted in higher plants, algae and cyanobacteria by using both short bandwidth interference and cut-off filters by exposure at above 10 different wavelengths and intensities and measuring oxygen evolution or carbon assimilation [35–38]. Quantum yield of photosynthesis is dependent on the incident wavelength as measured by oxygen production [38,39], carbon assimilation [40] and by *in vivo* absorption spectra and fluorescence excitation spectra [41,42].

In this study, the photosynthetic effectiveness of studied LEDs is expressed through the photonic assimilation performance (APP%) based on the different spectral qualities studied [43]. It involves measuring the relative efficiency for the generation of a response or biological effect depending on the incident wavelength such as, for example, the liberation of oxygen, photosynthetic efficiency or the rate of electronic transport during the photosynthetic process, amongst others [36,44].

The Nerja Cave is one of the most important tourist caves in Spain, with about 450,000 visitors annually. It is located on the coast of Malaga (36°45′43"N, 3°50′41"W, Andalusia Region, Spain) and it covers an area of 35,000 m^2 and a volume about 300,000 m^3, but only a third of the cave has been adapted for visits, the so-called *Tourist Galleries*. Due to its cultural and natural heritage, the cave has been declared an Asset of Cultural Interest in the category of Archaeological Sites and is an internationally recognized Heritage Site of Geological Relevance, respectively. Amongst the work carried out in 1960 for adapting the cave for tourist use, a lighting system was installed which favoured the development of photosynthetic biofilms in the tourist area of the cave.

We present the case study of the Nerja Cave where three biofilms were lighted by four LED lamps with different spectral compositions and the photosynthetic activity was analysed. The aim of this study is to use both theoretical and empirical methodologies to measure the photosynthetic activity according to the light spectral composition. The obtained information will allow the design of an adequate lighting system to aid the conservation of the heritage of the Nerja Cave.

2. Material and Methods

2.1. Study Site

For this study, four photosynthetic biofilms located in the *Tourist Galleries* of the cave were selected (Fig. 1). All the biofilms (Ne.8, Ne.16, Ne.12-above and Ne.12-below) were located just a few metres apart and, therefore, affected by similar environmental factors [46,47]. All the biofilms were previously investigated from a taxonomic point of view, which allowed us to know about their biodiversity. Biofilms Ne.8 and Ne.16, dominated by cyanobacteria *Chroococcidiopsis* sp. and red alga *Cyanidium* sp., respectively [19], were greenish in different shades and powdery in appearance and were illuminated by white low-pressure fluorescent lamps (model Roblan ES27, 18 W with a colour temperature of 4845 K and 1030 lm) (Fig. 2a). Biofilm Ne.12 was divided into two parts (above and below) according to its morphologically different

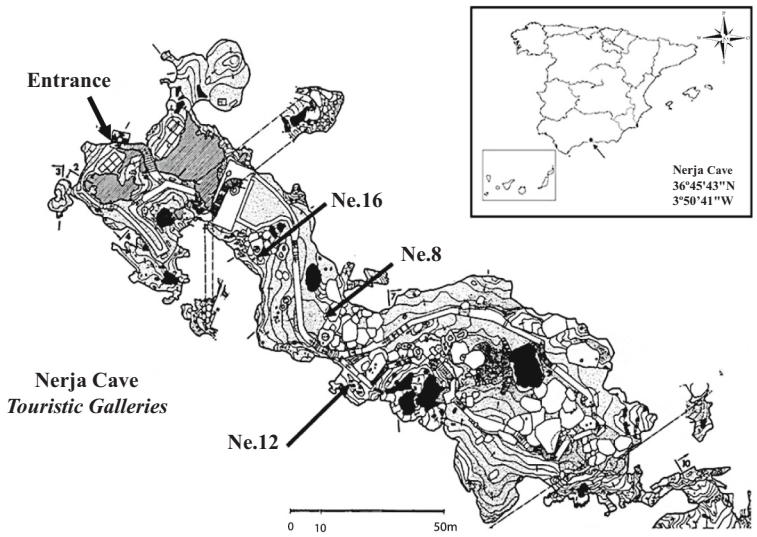


Fig. 1. Topographic map of the *Tourist Galleries* of the Nerja Cave (Spain) [from 52] and location of Ne.8, Ne.12 (above and down) and Ne.16 biofilms.

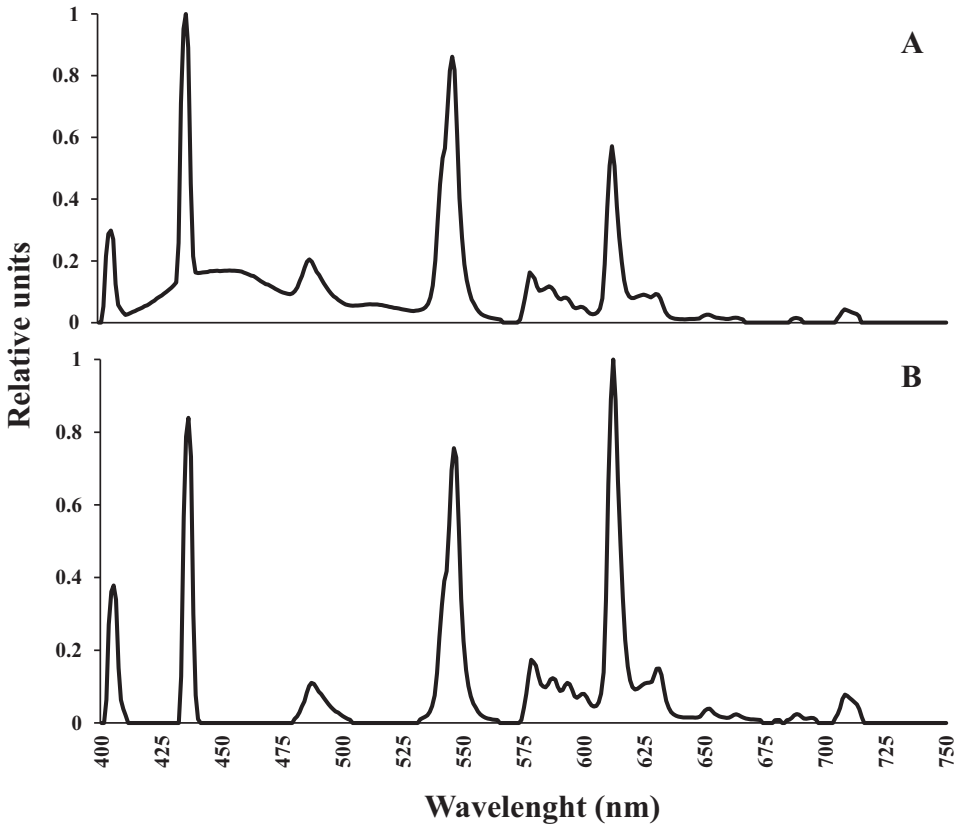
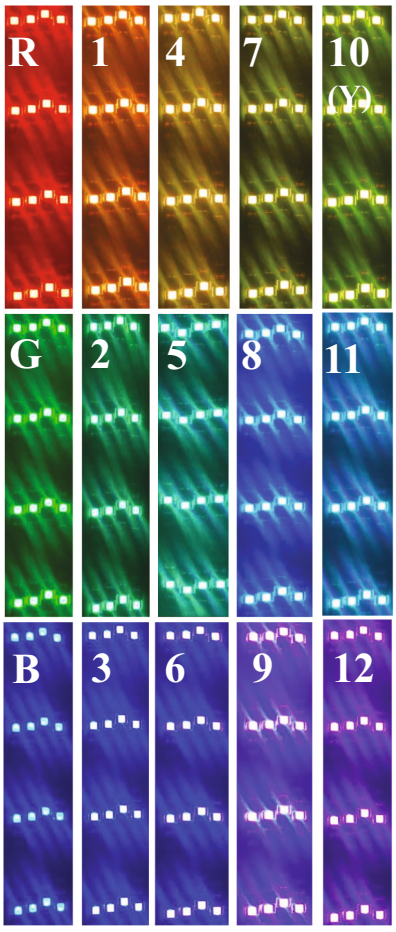


Fig. 2. Relative emission lamp spectrum in Ne.8 and Ne.16 (A).and in Ne.12 (above and down) (B).

characteristics. Ne.12-above grew on a continuous dripping water surface and was wet, gelatinous and mainly composed of green algae whilst Ne.12-below was dry, powdery and composed of cyanobacteria [19]. Both were illuminated by white low-pressure fluorescent lamps (model Philips Genie 18 W with a colour temperature of 2700 K and 1100 lm) (Fig. 2b). Finally, it was confirmed that the selected biofilms were not affected by other lamps in the cave.

2.2. Spectral Characterization of LED Lights

RGB-type LEDs were used in this study. The optoelectronic principle for the illumination in this type of LED is known as electroluminescence and the colour of the light is determined from the semi-conductor energy band. These diodes have three semi-conductors per diode for each colour (red-R, green-G and blue-B) and by controlling these primary colours it is possible to obtain twelve different combinations of colours by regulating the electrical current that passes through the semi-conductors (Fig. 3).



Lamp	E (μmol m^{-2} s^{-1})	Lux (Lumen m^{-2})	λ_{max} (nm)	Colour Temp (K)
R	25.2	2009	626	4629
G	29.7	4498	520	5573
B	48.3	1271	465	6232
1	29.4	2734	627	4622
2	34.3	4576	521	5562
3	51.5	1484	465	6232
4	36.7	3861	626	4629
5	40.0	4723	521	5562
6	54.2	1701	465	6232
7	43.6	4938	626	4629
8	46.3	2484	465	6232
9	58.9	2121	465	6232
10 (Y)	51.5	6125	626	4629
11	64.1	5321	465	6232
12	64.6	2600	465	6232

Fig. 3. Combined light sources (1–12) from the primary colours (R, G, B) and spectral properties at 20 cm from the source.

The different spectral configurations were measured with a multi-diode spectroradiometer (Sphere Optics model SMS 500, Contoocook, NH, USA). This instrument allowed us to quantify the energy associated to each wavelength (nm) in W m^{-2} later converted to photonic units (μmol photons m^{-2} s^{-1}) from the Planck Law [48]. The colour temperature (K) was calculated from the maximum wavelength (λ_{max}) using the Wien Displacement Law [49]. The illuminance, expressed as Lux (lumen m^{-2}), was calculated from the phototopic constant [50] (Fig. 3).

2.3. Theoretical Methodology- action Spectra for Photosynthesis

In order to know the lowest possible effective LED spectral configuration to reduce the photosynthetic capacity of the biofilms, the expected effectiveness of the lightsfor photosynthesis was tested, according to the photosynthesis action spectra reported in the literature for green algae *i.e.*, *Ulva lactuca* (Ul) [37], red alga *Porphyra perforata*, both green and red portion (Pp-, Pp*) [36] and the cyanobacteria *Microcoleus* sp. (M) [38] since cyanobacteria and red and green microalgae are present in the Nerja Cave (Table 1, Fig. 4). This approach was used since there are no available action spectra for the photosynthesis of the organisms that are growing in the Nerja Cave biofilms.

Based on this, we calculated the effective photosynthetic spectrum (E_{ef}) for the photosynthetic organisms according to Álvarez et al. [43] being $E_{ef} = \Sigma (E_{LED} \times E_{action})$ where E_{LED} is the absolute spectrum of the LED source expressed in units of energy (W m^{-2}) for each action spectra (E_{action}) (Fig. 4). We also calculated the assimilation performance photons (APP) expressed as a percentage by APP (%) = (E_{ef}/E_{LED})*100. APP is the proportion of effective photosynthetic spectrum for each LED source related to the incident irradiance. So, the higher values of APP indicate a greater use of incident photons by a light source in the photosynthetic process.

It is important to emphasize that, for this theoretical study, based on taxonomical classification, photosynthetic organisms have different pigmentary compositions as well as other accessory pigments which may complement the absorption of photons [51].

Table 1
Taxonomy and references for the action spectra available in the scientific literature (*Pp-*: Red portion. *Pp**: Green portion).

Division	Specie	Abbreviation	Reference
Chlorophyta	*Ulva lactuca*	*Ul*	Lüning and Dring 1985
Rhodophyta	*Porphyra perforata*	*Pp-*	Haxo and Blinks 1950
Rhodophyta	*Porphyra perforata**	*Pp**	Haxo and Blinks 1950
Cyanobacteria	*Microcoleus* sp.	*M*	Jørgensen et al. 1987

2.4. Empirical Methodology - In Vivo Chl a Fluorescence

The empirical methodology was based on lighting the biofilms with the selected LEDs and measuring *in vivo* Chl *a* fluorescence to obtain the effective quantum yield (ΔF/Fm′) and the relative electron transport rate (rETR) of photosystem II as reported by Figueroa et al. [52]. According to the available knowledge about Nerja Cave biofilms, the spectral configurations R, G, B and 10 were selected (Fig. 5).

The biofilms were light adapted for 15 minutes for every LED spectral configuration before starting the *in situ* Chl *a* fluorescence measurements. These were carried out with a pulse amplitude modulated (PAM) fluorometer (Diving PAM, Walz GmbH, Germany) equipped with red light as the measuring light and a halogen lamp provided actinic

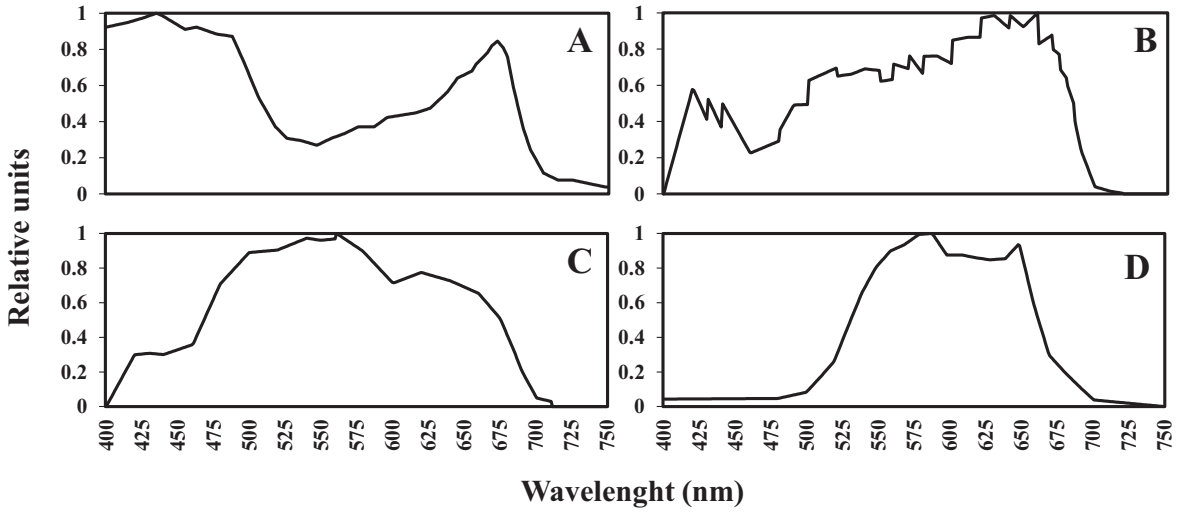


Fig. 4. Normalized action spectra for *Ulva lactuca* (A), *Porphyra perforata* (green portion) (B), *Porphyra perforata* (red portion) (C) and *Microcoleus* sp. (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

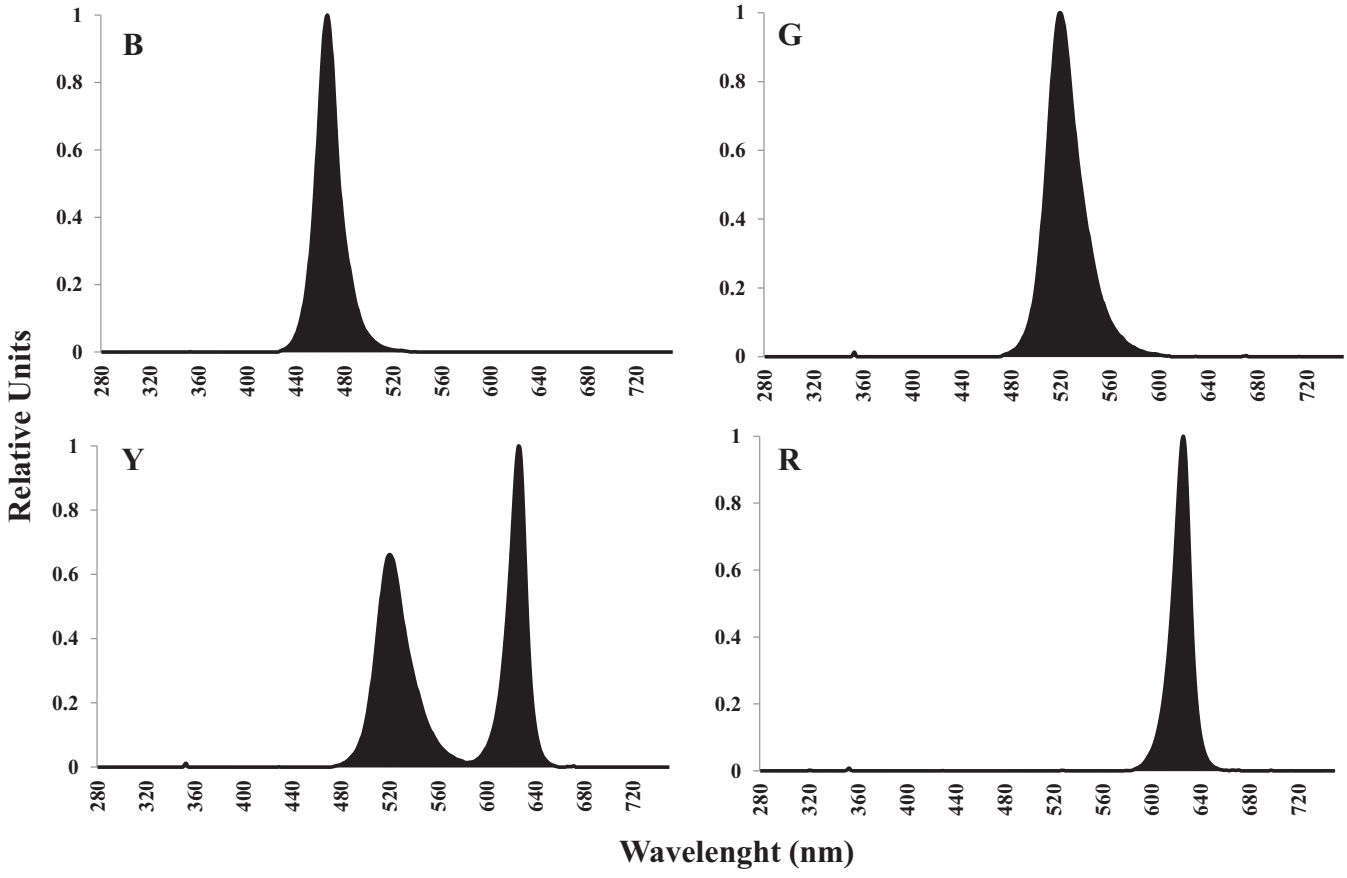


Fig. 5. Emission spectrum (280–730 nm) for selected LEDs: red (R), green (G), blue (B) and yellow colour (Y) as mixture of green and red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

light and saturation light pulses. The fiber of the PAM fluorometer was placed around 1–2 mm away from the biofilm length. The fiber has a length of 100 cm and a diameter of 5.5 mm. In each biofilm 5 areas were delimited along an irradiance gradient from the light source (0.5, 2, 5, 7 and 10 μmol photons m^{-2} s^{-1}) where *in vivo* Chl *a* fluorescence was measured. Irradiances (E_{LED}) were determined with a broad band spherical PAR sensor US-SQS (Walz GmbH) connected to a radiometer (Licor 250A). For each light quality and area irradiance, six measurements of effective quantum yield were made.

$\Delta F/Fm'$ was obtained as $\Delta F = F_m' - F_t$ where F_m' is the maximum fluorescence yield of an illuminated sample and F_t is the instantaneous fluorescence of illuminated microorganism measured briefly before the application of a saturation pulse. Values of F_m' were recorded after applying saturating pulses using the fluorometer. Then, the rETR

through PSII (expressed as μmol electrons m^{-2} s^{-1}) was determined using the incident irradiance of photosynthetic active radiation (E_{LED}, expressed in μmol photons m^{-2} s^{-1}) by rETR = $\Delta F/Fm' \cdot E_{LED}$. Finally, we calculated the photosynthetic efficiency as the slope (α) of the linear function rETR *versus* irradiance [53].

2.5. Statistical Analysis

To determine whether there were significant differences between light quality (blue, green, yellow and red) and photosynthetic efficiency in the studied biofilms, a one-way analysis of variance (one-way ANOVA) was done ($p < 0.05$). Once the ANOVA was significant, in order to identify which pairs of groups were significantly different in each biofilm, a multiple comparisons analysis, the Student Neuman-Keuls (SNK) [54], was performed as post-hoc test. Software Statistica v.10 (StatSoft) was used to verify the normality and the homoscedasticity of $\Delta F/Fm'$ and rETR by Cochram test.

3. Results

The empirical approach was based on the photosynthetic efficiency value and the theoretical on the photonic assimilation performance (APP%) value, with both parameters being index related to the proportion of the light quality use for photosynthesis. The regression coefficients obtained from the linear functions (R^2) of photosynthetic efficiency, were higher than 0.95 in all cases. So, we carried out a comparison of the theoretical (based on APP) and empirical results (based on photosynthetic efficiency) reached in each biofilm which were as follows:

For Ne.8, where the cyanobacteria *Chroococcidiopsis* sp. is dominant and according to the theoretical methodology based on the action spectra of cyanobacteria *Microcoleus* sp., red and yellow lights were the most effective for photosynthesis, whilst the blue light showed the lowest effectiveness (Table 2, Fig. 4d). However, according to the empirical methodology the blue light was related to the highest photosynthetic efficiency ($\alpha = 0.55 \pm 0.02$) and the yellow one with the lowest ($\alpha = 0.41 \pm 0.01$) (Fig. 6). Significant differences in photosynthetic efficiency were observed between the blue and yellow lights, whilst no significant differences were observed for the green and red lights.

For Ne.12-above biofilm, mostly formed by green algae, the highest photosynthetic efficiency expected according to the action spectra for photosynthesis were blue or red lights and the green-yellow light was the least effective, *i.e.* the action spectra of the green algae *U. lactuca*, (Table 2, Fig. 4a). Empirical values showed the highest photosynthetic efficiency under a red light ($\alpha = 0.64 \pm 0.01$) with the green light being high ($\alpha = 0.63 \pm 0.01$) and also the blue light ($\alpha = 0.62 \pm 0.01$) whilst the yellow light showed the lowest value ($\alpha = 0.43 \pm 0.01$) (Fig. 6). They also showed statistically significant differences between photosynthetic efficiency for all the light qualities.

For Ne.12-down, where the red algae and cyanobacteria dominated, the red and green lights showed the highest effectiveness whilst blue light was the least effective, according to the action spectra of the red alga *P. perforata* (red and green portion) and the cyanobacteria *Microcoleus* sp. (Fig. 4b, c, d). However, according to the empirical results, the highest photosynthetic efficiency was related to the blue light ($\alpha = 0.60 \pm 0.02$) and the lowest was to the yellow light ($\alpha = 0.35 \pm 0.02$), similar to the Ne.8 empirical results (Fig. 6). No statistically significant differences were observed between the red and green lights.

Finally, for Ne.16, where the mesophilic red alga *Cyanidium* sp. was the dominant microorganism, the green and red lights showed the highest effectiveness for photosynthesis (Fig. 4b, c) whilst blue light was the least effective, according to the action spectra of *P. perforata*. However, *in situ* monitoring showed the highest photosynthetic efficiency under the green light ($\alpha = 0.65 \pm 0.01$) and the lowest under the red light ($\alpha = 0.48 \pm 0.03$) (Fig. 6). No statistically significant differences were observed in between the blue and yellow lights.

Table 2
Photonic assimilation performance (APP%) for species of divisions observed Nerja Cave biofilms according to the action spectra of photosysnthesis and lighting used (see Table 1). *i.e.* Chlorophyta, Rhodophyta and Cyanobacteria.

Colour	*Ulva lactuca* (Ul)	*Porphyra perforata* Red P. (Pp)	*Porphyra perforata* Green P. (Pp*)	*Microcoleus* sp. (M)
Blue (B)	89.4	50.3	29.5	5.00
Green (G)	37.9	92.1	66.1	40.9
Red (R)	48.1	75.3	92.7	85.0
Yellow (10)	42.3	84.6	78.6	61.3

4. Discussion

Previous research about photosynthetic biofilms in the Nerja Cave showed the abundance of the cyanobacteria *Chroococcidiopsis* sp. and the mesophilic red alga *Cyanidium* sp. [55,56] on the driest surfaces of the cave, whilst the wet areas were dominated by the green algae *Desmococcus endolithicus* or *Jenufa* sp. and a higher diversity of cyanobacteria with *Leptolyngbya* sp. and *Gleocapsa atrata*, amongst others [2,19]. In addition, the biofilms were highly adapted to low irradiance and this variable was only slightly influential in their growth whilst the most significant variables were the relative humidity and the CO_2 air concentration [52]. The pigmentary diversity and extracellular polymer matrix composed of heteropolysaccharides, proteins and DNA of the bio films also provides a high resistance to desiccation, thus allowing their evolution in an illuminated subterranean environment.

In this study, we estimated photosynthetic parameters by theoretical and empirical methodologies (assimilation performance photons and photosynthetic efficiency, respectively) in different biofilms lighted with different LED qualities in order to know their photosynthetic activity.

From a theoretical point of view, the evolution of assimilation performance photons and photosynthetic efficiency valuesshould be similar, as long as the Nerja Cave biofilms have similar action spectra to the species with which they were compared (see Table 2). Nevertheless, in our study, this did not happen and the empirical results did not correspond with theoretical ones for all the studied biofilms. According to the theoretical methodology and the taxonomy of biofilm mainly formed by *Chroococcidiopsis* sp. (Ne.8), the blue light was expected to reduce photosynthesis compared to the green and red light according to the action spectra for photosynthesis in cyanobacteria [38] whereas the highest photosynthesis was expected under the green and red lights due to the presence of phycocyanin and allophycocyanin pigments in the Nerja Cave *Chroococcidiopsis* sp. [19]. In the cyanobacteria, the maximum quantum yield under blue light decreases due to the interference by phycobiliproteins associated to PSII [57]. However, in cyanobacteria the highest level of Chl *a* is associated to PSI in which no phycobilisomes are attached, *i.e.* only 12% of cellular Chl *a* is associated to PSII and the fraction of absorbed quanta to PSII is 36% in *Synechococcus* sp. [42]. Wavelengths in the range of around 480 nm (blue) result in the strongest preferential excitation of PSII and therefore the strongest loss of both Φ_{CO2} or Φ_{PSII} [58]. However, Φ_{PSII} is also an unreliable measure of Φ_{CO2} for these blue wavelengths, due to the absorption by carotenoids and non-photosynthetic pigments [59]. However, at low intensities of the linear part of the light-response curves as was applied in this study, there is not limitation for the electron flow of the acceptor side of PSII and the relation between $\Phi_{CO2\ or}$ Φ_{PSII} is linear [60]. The blue light may be absorbed by the chlorophyll associated to PSI more efficiently that in PSII since in PSI blue light is not shaded by phycobiliproteins located in phycobilisomes associated to PSII and thus both blue and far-red light, have a greater role in photosynthetic activity than that absorbed by PSII [42,57].

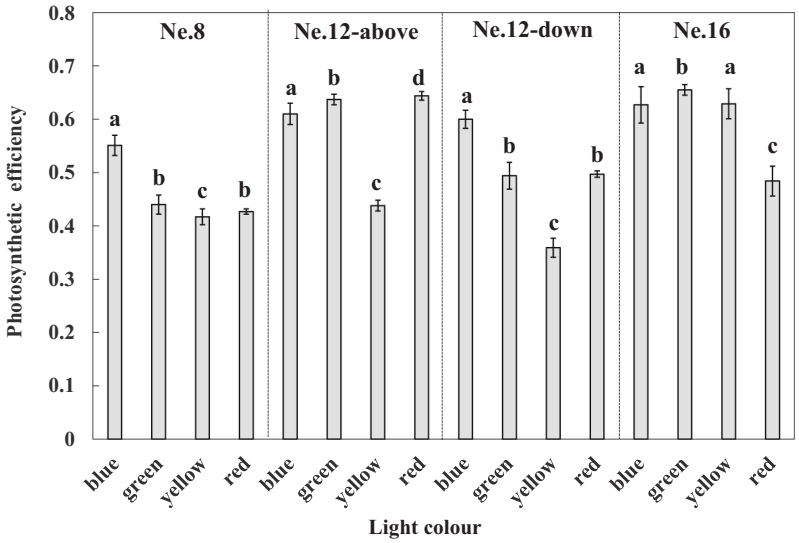


Fig. 6. Photosynthetic efficiency and standard deviation measured *in situ*, for each light quality (blue, green, yellow and red) in each biofilm (Ne.8, Ne.12-above, Ne. 12-below and Ne.16.). SNK results are showed as a, b, c, d (above the bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The *in situ* measurements showed that the highest photosynthetic activity occurred under the blue light and the lowest under the yellow light, in this case, very similar to the red light and both contrary to the theoretical model. The similarity between the red and yellow light results could be explained because the spectrum of the yellow light does not correspond to a single spectral band but consists of a combination of light in the red, green and blue bands and is more enriched in the red (Fig. 6). Therefore, it seems that the Nerja Cave *Chroococcidiopsis* sp. scarcely uses phycobiliproteins to capture light and it is well adapted to using cold light, which is the one used habitually to light the biofilm in the cave, by using Chl *a*. Other chlorophylls absorbing in red and far-red light, contrary to Chl *a,* have been found in cyanobacteria such as Chl *f* with a fluorescence emission peak at 748 nm [61] and Chl *d* [62]. Chl *f* was detected in a cyanobacteria growing in far-red light (750 nm) and the charge separation in photosystem I and II uses Chl *f* at 745 nm and Chl *f* (or *d*) at 727 nm, respectively [63]. In the Nerja Cave, *Chroococcidiopsis* sp. growing under high pressure lamps with an emission in far-red light may have adapted to the light source through other unexpected pigment compositions by using Chl *d* or *f*.

Similar results were obtained in the biofilms formed by a mixture of the red alga *Cyanidium* sp. and cyanobacteria (Ne.12-down) with the highest efficiency being in the blue light instead of the red, yellow or green lights, just as had been expected according to the theoretical model by using the action spectra for photosynthesis by Jørgensen et al. [38]. The emission spectrum of the Nerja Cave *Cyanidium* sp. indicated the presence of Chl *a* and phycobilins (phycocyanin and allophycocyanin) [19], the same as *Chroococcidiopsis* sp., therefore, the similarity response between both microorganisms is understandable. Since the cavity is an environment with dry periods and with low nutrient availability, the biofilms maybe using phycobiliproteins as a nitrogen reservoir in the driest periods of the cavity [64]. In this way, the cave microorganisms would not use these accessory pigments to capture energy; rather they could accumulate them as reserve substances in the hostile environment of the cavity, thus explaining the low photosynthetic efficiency under the red light of Ne.8 and Ne.16.

The lower efficiency related to the green light could be explained by the absence of phycoerythrin in the biofilm, which has a maximum absorption coincident with the green light maximum emission wavelength. However, this event was not observed in Ne.16, mainly composed of *Cyanidium* sp., with the highest efficiency in the green light. This fact proves that the action spectra of microorganisms has a close relationship with the absorption spectrum in terms of shape, but it is necessary to emphasize that a greater absorption by the pigment at a certain wavelength does not necessarily correspond with a greater photosynthetic action, since not only the quantity/spectral quality and the pigmentary composition have influence, but also the energy distribution in the two photosystems [45,65].

In situ, the biofilm composed mainly of green algae (Ne.12-above) showed the highest photosynthetic efficiency in the red light as expected in the action spectra for photosynthesis followed by the green and blue lights. The minimal values were reached in yellow light as was also expected (Fig. 6, Table 2). The high photosynthetic efficiency in red and blue lights was expected according to the action spectra for photosynthesis for green algae (Fig. 5, Table 2). However, the high photosynthetic efficiency in green light was an unexpected result. The action spectra used in this study was of a thin green macroalgae (*U. lactuca*), showing a decrease in photosynthetic efficiency in green and yellow light. However, thicker thalli of other green macroalgae, as *Codium fragile,* also presented high photosynthetic efficiency in green light due to the high chlorophyll content and package effect [37]. As the habitual lighting of this biofilm was a warm light (2700 K), as in Ne.8 and Ne.16, the microorganisms in Ne.12-above could have adapted and be effective in the red light. Therefore, the theoretical action spectra model used for green algae *(U. lactuca*) was representative for this biofilm because the highest photosynthetic efficiency was reached in blue and red light as expected according to the action spectra for photosynthesis in green algae with high photosynthetic efficiency in green light due to the foreseeable high chlorophyll content in green algal biofilms in the dark habitat of the Nerja Cave.

In cyanobacteria, the most action spectra for photosynthesis have been determined as the quantum yield for oxygen production and carbon uptake [38–40]. In addition, other authors have used the combination of *in vivo* absorption spectra and fluorescence excitation spectra in both algae and cyanobacteria [41,42,66]. Relative excitation of Chl *a* fluorescence is very close to relative action spectra of photosystem II O_2 evolution in algae [66]. The action spectra were interpreted in relation to the light-harvesting pigments and their association with the two photosystems [42,66]. In this study we have used as photosynthetic indicator the effective quantum yield and rETR determined by *in vivo* Chl *a* fluorescence. Linear relation between effective quantum by fluorescence (Φ_{PSII}) and by oxygen evolution (Φ_{O2}) or carbon uptake (Φ_{CO2}) has been shown although the linear relation is lost under high light intensity

[53,60,67]. A lower fraction of incident light reaching the photosystems will directly result in a loss of effective quantum yield by CO_2 uptake (Φ_{CO2}) on an incident light basis [60]. However, at low light intensities in the linear part of the light-response curve, there are no limitations for the electron flow on the acceptor side of PSII [59]. Therefore, within a range of low-light intensities (1–50 μmol m^{-2} s^{-1} or even narrower range in the dark- as is the case for the Nerja Cave cyanobacteria and microalgae) effective quantum yield by fluorescence (Φ_{PSII}) does not change as results of a small changes in light intensity and good relations to Φ_{CO2} or Φ_{O2} are observed [53,60,67].

The review by Kalaji et al. [59], reports that Φ_{PSII} is an unsuitable parameter for dependence of Φ_{CO2} due the wavelength dependence changes in absorbed light fraction by carotenoids and non-photosynthetic pigments. However, at low intensities changes in the fraction of photons reaching the photosystems may not affect Φ_{PSII} and thus, due the incident light used to illuminate the algae and cyanobacteria biofilms in this study being very low, a good relationship between Φ_{PSII} and Φ_{CO2} was expected. Thus, the effect of broad band light qualities used in this study on effective quantum yield and ETR could be suitable related to the photosynthetic rate and eventually related to the growth rate. In this study, we applied broad-band light qualities as the first steps of wavelength dependence for photosynthesis in the biofilms of the Nerja Cave and the next pending study step is to conduct action spectra by using narrow wavelength light sources and a higher number of wavelengths than the ones used in this study.

According to the results of this study, where the expected photosynthetic response pattern did not follow the observed pattern under different light sources in cyanobacteria and red algae whereas in green algae the expected patterns were observed, further research effort is needed. Thus, the development of specific action spectra should be considered [45,65] especially for the dominant biofilms in the Nerja Cave constituted by the red alga (*Cyanidium* sp.) and cyanobacteria (*Chroococcidiopsis* sp). Nevertheless, our results showed that the spectral quality of the lighting source is a major factor in the physiological response of biofilms, since they are limited by the light quality to which they have adapted and by the low nutrient environment, which can induce the biofilms to use phycobiliproteins as a reservoir substance or heterotrophic nutrition.

5. Conclusions

Because the development of photosynthetic biofilms causes biodeterioration processes in the substrate where they grow, we suggested controlling them by the design of a specific lighting system (LED) with less photosynthetic effectiveness according to the spectral light emission of lamps. In order to obtain the required information, theoretical and empirical approaches were developed and the results were different for each methodology. Therefore, our results were not conclusive enough to be able to recommend a specific lighting system for the Nerja Cave for the all the types of biofilms there may be in the cave because the action spectra available in the scientific literature were not useful for predicting the photosynthetic activity of the Nerja Cave biofilm. Thus, further research is needed to obtain the specific action spectra for the Nerja Cave biofilms, in order to rigorously predict the bio-optical responses and thus allow the design of the most adequate lighting system according to the conservation of the cave without compromising the quality and visual safety of the visitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is part of the Interdisciplinary Research Project of the Nerja Cave, applied to the conservation of the cave. The project is financed by Cueva de Nerja Public Services Foundation and authorized by the Ministry of Culture of the Andalusian Government. This work is also a contribution to the Research Group FYBOA (RNM-295) of the Junta de Andalucía.

References

[1] D. Gillieson, P. Van Beynen (Eds.), Karst Management, Springer, New York, 2011, pp. 141–158, https://doi.org/10.1007/978-94-007-1207-2_6.
[2] Y. Del Rosal, M. Hernández-Mariné, M. Roldán, Phototrophic microorganisms in the tourist cave of Nerja, Sci. Technol. Cult. Herit. (2014) 229–234, https://doi.org/10.1201/b17802.
[3] P. Albertano, in: Whitton B.A. (Eds.), Ecology of Cyanobacteria II, Springer, Dordrecht, 2012, pp. 317–343, https://doi.org/10.1007/978-94-007-3855-3_11.
[4] T.R. Neu, K.C. Marshall, Bacterial polymers: physicochemical aspects of their interactions at interfaces, J. Biomater. Appl. 5 (2) (1990) 107–133, https://doi.org/10.1177/088532829000500203.
[5] S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. De Philippis, P. Tamagnini, Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly, FEMS Microbiol. Rev. 33 (5) (2009) 917–941, https://doi.org/10.1111/j.1574-6976.2009.00183.x.
[6] J. Mulec, G. Kosi, Lampenflora algae and methods of growth control, J. Cave Karst Stud. 71 (2) (2009) 109–115.
[7] P. Boston, in: J. Gunn (Ed.), Encyclopedia of Caves and Karst Science, New York, 2004, pp. 1568–1570.
[8] C.B. Estévez, L.M. Merino, A. Román, J.J.D. Valsero, The lampenflora in show caves and its treatment: An emerging ecological problem, Int. J. Speleol. 48 (3) (2019) 249–277, https://doi.org/10.5038/1827-806X.48.3.2263.
[9] Lefèvre, La 'maladie verte' de lascaux, Stud. Conserv. 19 (3) (1974) 126–156, https://doi.org/10.1179/sic.1974.013.
[10] V. Jurado Lobo, C. Saiz Jiménez, Vida microbiana en las cavernas: el fascinante mundo de la biodiversidad subterránea y su papel en los procesos de deterioro, Enseñanza Las Ciencias La Tierra Rev. La Asoc. Española Para La Enseñanza Las Ciencias La Tierra. 24 (1) (2016) 51–60.
[11] F. Borderie, B. Alaoui-Sossé, L. Aleya, Heritage materials and biofouling mitigation through UV-C irradiation in show caves: state-of-the-art practices and future challenges, Environ. Sci. Pollut. Res. 22 (6) (2015) 4144–4172, https://doi.org/10.1007/s11356-014-4001-6.
[12] G.P. Pfeifer, A. Besaratinia, UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer, Photochem. Photobiol. Sci. 11 (1) (2012) 90–97, https://doi.org/10.1039/c1pp05144j.
[13] J.G. Jose, D.G. Pitts, Wavelength dependency of cataracts in albino mice following chronic exposure, Exp. Eye Res. 41 (4) (1985) 545–563, https://doi.org/10.1016/S0014-4835(85)80011-7.
[14] A. Norman, The nuclear role in the ultraviolet inactivation of Neurospora conidia, J. Cell. Comp. Physiol. 44 (1) (1954) 1–10, https://doi.org/10.1002/jcp.1030440102.
[15] M. Buonanno, B. Ponnaiya, D. Welch, M. Stanislauskas, G. Randers-Pehrson, L. Smilenov, F.D. Lowy, D.M. Owens, D.J. Brenner, Germicidal efficacy and mammalian skin safety of 222-nm UV light, Radiat. Res. 187 (4) (2017) 493–501, https://doi.org/10.1667/rr0010cc.1.
[16] D. Welch, M. Buonanno, V. Grilj, I. Shuryak, C. Crickmore, A.W. Bigelow, G. Randers-Pehrson, G.W. Johnson, D.J. Brenner, Far-UVC light: A new tool to control the spread of airborne-mediated microbial diseases, Sci. Rep. 8 (1) (2018) 1–7, https://doi.org/10.1038/s41598-018-21058-w.
[17] N. Yamano, M. Kunisada, S. Kaidzu, K. Sugihara, A. Nishiaki-Sawada, H. Ohashi, A. Yoshioka, T. Igarashi, A. Ohira, M. Tanito, C. Nishigori, Long-term effects of 222-nm ultraviolet radiation C sterilizing lamps on mice susceptible to ultraviolet radiation, Photochem. Photobiol. 96 (4) (2020) 853–862, https://doi.org/10.1111/php.13269.
[18] J.R. Snider, C. Goin, R.V. Miller, P.J. Boston, D.E. Northup, Ultraviolet radiation sensitivity in cave bacteria: evidence of adaptation to the subsurface? Int. J. Speleol. 38 (1) (2009) 11–22, https://doi.org/10.5038/1827-806X.38.1.2.
[19] Y. del Rosal Padial, M.C. Hernández-Mariné, M. Roldán, Biodiversidad, estructura y fisiología de los biofilms fotosintéticos en la Cueva de Nerja, in: Fundación Pública de Servicios Cueva de Nerja (Eds.), Análisis, impacto y evolución de biofilms fotosintéticos en espeleotemas: el caso de la Cueva de Nerja (Málaga, España). Patronato de la Cueva de Nerja, 2018, pp. 145–228.
[20] J. Gurnee, Management of some unusual features in the show caves of the United States, Int. J. Speleol. 23 (1) (1994) 13–17, https://doi.org/10.5038/1827-806x.23.1.2.
[21] R. Olson, Control of lamp flora in developed caves, in: Val Hildreth-Werker, Jim C. Werker (Eds.), Cave Conserv. Restor, National Speleological Society publisher, Huntsville, 2006, pp. 343–348.
[22] R. Olson, Control of lamp flora in Mammoth cave national park, in: T. Hazslinszky (Ed.), Int. Conf. Cave Light, Hungarian Speleol. Soc. Publisher, Budapest, 2002, pp. 131–136.

[23] K.J. McCree, in: O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler (Eds.), Encycl. Plant Physiol. New Ser vol. 12A, Springer, Berlin, 1981, pp. 41–55, https://doi.org/10.1007/978-3-642-68090-8_3.
[24] N. Korbee, F.L. Figueroa, J. Aguilera, Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga *Porphyra leucosticta* (Bangiales, Rhodophyta), J. Photochem. Photobiol. B Biol. 80 (2) (2005) 71–78, https://doi.org/10.1016/j.jphotobiol.2005.03.002.
[25] C.S. Brown, A.C. Schuerger, J.C. Sager, Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting, J. Am. Soc. Hortic. Sci. 120 (5) (1995) 808–813, https://doi.org/10.21273/JASHS.120.5.808.
[26] R.J. Bula, R.C. Morrow, T.W. Tibbitts, D.J. Barta, R.W. Ignatius, T.S. Martin, Light-emitting diodes as a radiation source for plants, HortScience 26 (2) (1991) 203–205, https://doi.org/10.21273/HORTSCI.26.2.203.
[27] M.M. Hasan, T. Bashir, R. Ghosh, S.K. Lee, H. Bae, An overview of LEDs' effects on the production of bioactive compounds and crop quality, Molecules. 22 (9) (2017) 1420, https://doi.org/10.3390/molecules22091420.
[28] R. Lenk, C. Lenk, Practical Lighting Design with LEDs, John Wiley & Sons, Hoboken, 2017.
[29] J. Sanmartín, D. Vázquez-Nion, B. Silva, B. Prieto, J. Arines, in: Mosquera, Gil (Eds.), Conserving Cultural Heritage, Cádiz, 2018, pp. 313–318, https://doi.org/10.1201/9781315158648.
[30] L. Bruno, V. Valle, Effect of white and monochromatic lights on cyanobacteria and biofilms from Roman catacombs, Int. Biodeterior. Biodegrad. 123 (2017) 286–295, https://doi.org/10.1016/j.ibiod.2017.07.013.
[31] P. Albertano, L. Bruno, in: C. Saiz-Jimenez (Ed.), Molecular Biology and Cultural Heritage, E-Publishing, London, 2017, pp. 171–178, https://doi.org/10.1201/9780203746578.
[32] M. Roldán, F. Oliva, M.A. Gónzalez Del Valle, C. Saiz-Jimenez, M. Hernández-Mariné, Does green light influence the fluorescence properties and structure of phototrophic biofilms? Appl. Environ. Microbiol. 72 (4) (2006) 3026–3031, https://doi.org/10.1128/AEM.72.4.3026-3031.2006.
[33] J. Serôdio, S. Vieira, S. Cruz, Photosynthetic activity, photoprotection and photoinhibition in intertidal microphytobenthos as studied in situ using variable chlorophyll fluorescence, Cont. Shelf Res. 28 (10–11) (2008) 1363–1375, https://doi.org/10.1016/j.csr.2008.03.019.
[34] E. Piano, F. Bona, E. Falasco, V. La Morgia, G. Badino, M. Isaia, Environmental drivers of phototrophic biofilms in an Alpine show cave (SW-Italian Alps), Sci. Total Environ. 536 (2015) 1007–1018, https://doi.org/10.1016/j.scitotenv.2015.05.089.
[35] K.J. McCree, The action spectrum, absorptance and quantum yield of photosynthesis in crop plants, Agric. Meteorol. 9 (1971) 191–216, https://doi.org/10.1016/0002-1571(71)90022-7.
[36] F.T. Haxo, L.R. Blinks, Photosynthetic action spectra of marine algae, J. Gen. Physiol. 33 (4) (1950) 389–422, https://doi.org/10.1085/jgp.33.4.389.
[37] K. Lüning, M.J. Dring, Action spectra and spectral quantum yield of photosynthesis in marine macroalgae with thin and thick thalli, Mar. Biol. 87 (2) (1985) 119–129, https://doi.org/10.1007/BF00539419.
[38] B.B. Jørgensen, Y. Cohen, D.J. Des Marais, Photosynthetic action spectra and adaptation to spectral light distribution in a benthic cyanobacterial mat, Appl. Environ. Microbiol. 53 (4) (1987) 879–886, https://doi.org/10.1128/AEM.53.4.879-886.1987.
[39] R.T. Wang, C.L.R. Stevens, J. Myers, Action spectra for photoreactions I and II of photosynthesis in the blue-green alga *Anacystis nidulans*, J. Photochem. Photobiol. 25 (1) (1977) 103–108, https://doi.org/10.1111/j.1751-1097.1977.tb07429.x.
[40] M.R. Lewis, O. Ulloa, T. Platt, Photosynthetic action, absorption, and quantum yield spectra for a natural population of *Oscillatoria* in the North Atlantic, Limnol. Oceanogr. 33 (1) (1988) 92–98, https://doi.org/10.4319/lo.1988.33.1.0092.
[41] A. Subramaniam, E.J. Carpenter, P.G. Falkowski, Bio-optical properties of the marine diazotrophic cyanobacteria *Trichodesmium* spp. II. A reflectance model for remote-sensing, Limnol. Oceanogr. 44 (3) (1999) 618–627, https://doi.org/10.4319/lo.1999.44.3.0618.
[42] G. Johnsen, E. Sakshaug, Biooptical characteristics of PSII and PSI in 33 species (13 pigment groups) of marine phytoplankton, and the relevance for pulseamplitude-modulated and fast-repetition-rate fluorometry, J. Phycol. 43 (6) (2007) 1236–1251, https://doi.org/10.1111/j.1529-8817.2007.00422.x.
[43] F. Álvarez-Gómez, Y. del Rosal, R. Guzmán, S. Mohamed, S. Merino, M. Hernández-Mariné, N. Korbee, F.L. Figueroa, Selection of LED lighting system in caves based on action spectra of photosyntesis: reducing biodeterioration of speleothems by biofilms of algae and cyanobacteria, in: B. Andreo, J.J. Durán (Eds.), VI Congr. Español Sobre Cuevas Turísticas. El Karst y El Hombre Las Cuevas Como Patimonio La Humanidad, Asociación Española de Cuevas Turísticas (ACTE) Publisher, Málaga, 2016, pp. 71–79.
[44] K.J. McCree, Test of current definitions of photosynthetically active radiation against leaf photosynthesis data, Agric. Meteorol. 10 (1972) 443–453, https://doi.org/10.1016/0002-1571(72)90045-3.
[45] F.L. Figueroa, R. Guzmán-Sepulveda, F. Alvarez-Gomez, G. González-González, S. Mohamed-Mohamed, P. Celis-Plá, Y. del Rosal, M. Hernandez-Mariné, S. Merino-Córdoba, Procedimiento bio-óptico basado en los espectros de acción de la fotosíntesis y lámparas LEDs para el control del biodeterioro por biofilms de algas y cianobacterias, in: M. Moreno Oliva, M.A. Rogerio Candelera, J. Teodomiro López Navarrete, V. Hernández Jolín (Eds.), Estudio y Conservación del patrimonio cultural, Red de Ciencia y Tecnología para la Conservación del Patrimonio Cultural y Universidad de Málaga Publishers, Sevilla, 2015, pp. 174–178.
[46] C. Liñán, Y. del Rosal, Procesos de ventilación natural Cueva de Nerja - Cueva Pintada (Nerja, Málaga), in: B. Andreo, J.J. Durán (Eds.), El Karst y El Hombre Las Cuevas Como Patrim. Mundial, Asociación de Cuevas Turísticas Españolas, Málaga, 2016, pp. 335–346.
[47] C. Liñán, Y. del Rosal, F. Carrasco, I. Vadillo, J. Benavente, L. Ojeda, Highlighting the importance of transitional ventilation regimes in the management of Mediterranean show caves (Nerja-Pintada system, southern Spain), Sci. Total Environ. 631 (2018) 1268–1278, https://doi.org/10.1016/j.scitotenv.2018.02.304.
[48] A. De Vos, Thermodynamics of Solar Energy Conversion, John Wiley & Sons, Weinheim, 2008.
[49] K.R. Lang, Radiation, in: N. Ashby, W. Brantley, M. Fowler, M. Inglis, E. Sassi, H. S. Sherif, H. Klose (Eds.), Essent. Astrophys, Springer, Heidelberg, 2013, pp. 33–67, https://doi.org/10.1007/978-3-642-35963-7.
[50] A. Stockman, H. Jägle, M. Pirzer, L.T. Sharpe, The dependence of luminous efficiency on chromatic adaptation, J. Vis. 8 (16) (2008) 1–26, https://doi.org/10.1167/8.16.1.
[51] K.S. Rowan, Photosynthetic Pigments of Algae, Cambridge University Press, New York, 1989.
[52] F.L. Figueroa, F. Álvarez-Gómez, Y. del Rosal, P.S.M. Celis-Plá, G. González, M. Hernández, N. Korbee, In situ photosynthetic yields of cave photoautotrophic biofilms using two different pulse amplitude modulated fluorometers, Algal Res. 22 (2017) 104–115, https://doi.org/10.1016/j.algal.2016.12.012.
[53] F.L. Figueroa, R. Conde-Álvarez, I. Gómez, Relations between electron transport rates determined by pulse amplitude modulated chlorophyll fluorescence and oxygen evolution in macroalgae under different light conditions, Photosynth. Res. 75 (3) (2003) 259–275, https://doi.org/10.1023/A:1023936313544.
[54] A.J. Underwood, Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance, Cambridge University Press, New York, 1997.
[55] C. Ciniglia, H.S. Yoon, A. Pollio, G. Pinto, D. Bhattacharya, Hidden biodiversity of the extremophilic Cyanidiales red algae, Mol. Ecol. 13 (7) (2004) 1827–1838, https://doi.org/10.1111/j.1365-294X.2004.02180.x.
[56] V. Jurado, Y. Del Rosal, M. Hernández Mariné, I. Galocha Zapata, I. Domínguez-Moñino, M.A. Rogerio Candelera, C. Sáiz-Jiménez, Biología molecular de los biofilms fotosintéticos de la Cueva de Nerja, in: Fundación Pública de Servicios Cueva de Nerja (Eds.), Análisis, impacto y evolución de los biofilms fotosintéticos en espeleotemas. El caso de la cueva de Nerja. Málaga, 2018, pp. 229–260.
[57] R.M. Schuurmans, P. Van Alphen, J.M. Schuurmans, H.C.P. Matthijs, K. J. Hellingwerf, Comparison of the photosynthetic yield of cyanobacteria and green algae: different methods give different answers, PLoS One 10 (9) (2015), e0139061, https://doi.org/10.1371/journal.pone.0139061.
[58] S.W. Hogewoning, E. Wientjes, P. Douwstra, G. Trouwborst, W. van Ieperen, R. Croce, J. Harbinson, Photosynthetic quantum yield dynamics: from photosystems to leaves, Plant Cell 24 (5) (2012) 1921–1935, https://doi.org/10.1105/tpc.112.097972.
[59] H.M. Kalaji, G. Schansker, R.J. Ladle, V. Goltsev, K. Bosa, S.I. Allakhverdiev, M. Brestic, F. Bussotti, A. Calatayud, P. Dąbrowski, N.I. Elsheery, L. Ferroni, L. Guidi, S.W. Hogewoning, A. Jajoo, A.N. Misra, S.G. Nebauer, S. Pancaldi, C. Penella, D. Poli, M. Pollastrini, Z.B. Romanowska-Duda, B. Rutkowska, J. Serôdio, K. Suresh, W. Szulc, E. Tambussi, M. Yanniccari, M. Zivcak, Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues, Photosynth. Res. 122 (2) (2014) 121–158, https://doi.org/10.1007/s11120-014-0024-6.
[60] B. Genty, J.M. Briantais, N.R. Baker, The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, Biochim. Biophys. Acta - Gen. Subj. 990 (1) (1989) 87–92, https://doi.org/10.1016/S0304-4165(89)80016-9.
[61] M. Chen, Y. Li, D. Birch, R.D. Willows, A cyanobacterium that contains chlorophyll *f* - A red-absorbing photopigment, FEBS Lett. 586 (19) (2012) 3249–3254, https://doi.org/10.1016/j.febslet.2012.06.045.
[62] H. Miyashita, S. Ohkubo, H. Komatsu, Y. Sorimachi, D. Fukayama, D. Fujinuma, S. Akutsu, M. Kobayashi, Discovery of Chlorophyll *d* in *Acaryochloris marina* and Chlorophyll *f* in a Unicellular Cyanobacterium, Strain KC1, Isolated from Lake Biwa, J. Phys. Chem. Biophys. 4 (4) (2014) 149, https://doi.org/10.4172/2161-0398.1000149.
[63] D.J. Nürnberg, J. Morton, S. Santabarbara, A. Telfer, P. Joliot, L.A. Antonaru, A. V. Ruban, T. Cardona, E. Krausz, A. Boussac, A. Fantuzzi, A. William Rutherford, Photochemistry beyond the red limit in chlorophyll *f*–containing photosystems, Science 360 (6394) (2018) 1210–1213, https://doi.org/10.1126/science.aar8313.
[64] N. Tandeau de Marsac, J. Houmard, Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms, FEMS Microbiol. Reviews 10 (1–2) (1993) 119–189, https://doi.org/10.1016/0378-1097(93)90506-W.
[65] F. Álvarez-Gómez, G. González, R. Guzmán, S. Mohamed, S. Merino, F.L. Figueroa, Utilización de distintas combinaciones espectrales e intensidades lumínicas de lámparas LEDs para el estudio de la actividad fotosintética en algas que pueden producir biodeterioro del patrimonio cultural, in: M. Moreno Oliva, M.A. Rogerio Candelera, J.T. López Navarrete, V. Hernández (Eds.), Estud. y Conserv. Del Patrim. Cult. Actas., Red de Ciencia y Tecnología para la Conservación del Patrimonio Cultural y Universidad de Málaga Publishers, Málaga, 2015, pp. 179–183.
[66] A. Neori, M. Vernet, O. Holm-Hansen, F.T. Haxo, Relationship between action spectra for chlorophyll *a* fluorescence and photosynthetic O_2 evolution in algae, J. Plankton Res. 8 (5) (1986) 1009–1010, https://doi.org/10.1093/plankt/8.5.1009.
[67] J.C. Kromkamp, J. Beardall, A. Sukenik, J. Kopecký, J. Masojidek, S. Van Bergeijk, S. Gabai, E. Shaham, A. Yamshon, Short-term variations in photosynthetic parameters of *Nannochloropsis* cultures grown in two types of outdoor mass

cultivation systems, Aquat. Microb. Ecol. 56 (2–3) (2009) 309–322, https://doi.org/10.3354/ame01318.