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Effect of Ultraviolet-C Radiation on the Morphology of Cyanobacteria *Nostoc* sp. LBALBR-2 Isolated from Supply Reservoir (Belém, Pará, Brazil)

Efeito da Radiação Ultravioleta-C na Morfologia da Cianobactéria *Nostoc* sp. LBALBR-2 Isolada do Reservatório de Abastecimento (Belém, Pará, Brasil)

Efecto de la Radiación Ultravioleta-C sobre la Morfología de la Cianobacteria *Nostoc* sp. LBALBR-2 Aislado del Embalse de Abastecimiento (Belém, Pará, Brasil)

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Abstract
Cyanobacteria are photosynthetic prokaryotic organisms that in aquatic environments can compose the phytoplankton and phytobenthos, being important in the primary production of these ecosystems. The objective of this work was to identify the main morphological variations of the cells, filaments and thallus of the cyanobacteria *Nostoc* sp. LBALBR-2 submitted to ultraviolet radiation (UV-C) in microcosm systems. The cyanobacteria *Nostoc* sp. was subjected to four treatments: 1- control treatment in nutrient medium; 2- treatment in nutrient medium with exposure to UV-C radiation; 3- control treatment in medium without nutrients and 4- treatment in medium without nutrients with exposure to UV-C radiation. Optical density and *chlorophyll-a* analyses were performed to determine the growth of *Nostoc* populations and microscopic analyses to characterize the morphological development of the species. Lack of nutrients generated short trichomes with terminal heterocytes, scattered solitary akinetes and appearance of hormogonia. On nutrient medium the trichomes of *Nostoc* sp. showed predominantly the vegetative serial form. Cultures exposed to UV-C produced anomalous cells, thick mucilage, fragmented trichomes and hormogonia. It is concluded that *Nostoc* sp. LBALBR-2 grew well under eutrophication conditions and although it showed cell deformation it was resistant to UV-C radiation.
Keywords: Cyanobacteria; Bloom; Microcosm.

Resumo
As cianobactérias são organismos procarióticos fotossintetizantes que no ambiente aquático podem compor o fitoplâncton e os fitobentos apresentando importância na produção primária destes ecossistemas. O objetivo deste trabalho foi identificar as principais variações morfológicas das células, dos filamentos e talo da cianobactéria *Nostoc* sp. LBALBR-2 submetida à radiação ultravioleta (UV-C) em sistemas de microcosmos. A cianobactéria *Nostoc* sp. foi submetida a quatro tratamentos: 1- tratamento controle em meio nutritivo; 2- tratamento em meio nutritivo com exposição à radiação UV-C; 3- tratamento controle em meio sem nutrientes e 4- tratamento em meio sem nutrientes

com exposição à radiação UV-C. Foram realizadas análises de densidade óptica e clorofila- *a* para determinar o crescimento das populações de *Nostoc* e análises microscópicas para caracterizar o desenvolvimento morfológico da espécie. A falta de nutrientes gerou tricomas curtos com heterócitos terminais, acinetos solitários dispersos e aparecimento de hormogônios. Em meio nutritivo os tricomas de *Nostoc* apresentaram predominantemente a forma seriada vegetativa. Culturas expostas a UV-C produziram células anômalas, mucilagem espessa, tricomas fragmentados e hormogônios. Conclui-se que *Nostoc* sp. LBALBR-2 se desenvolveu bem em condições de eutrofização e apesar de apresentar deformações celulares mostrou-se resistente às radiações UV-C.
Palavras-chave: Cianobactéria; Floração; Microcosmo.

Resumen
Las cianobacterias son organismos procarióticos fotosintéticos que en el medio acuático pueden componer fitoplancton y fitobentos, presentando importancia en la producción primaria de estos ecosistemas. El objetivo de este trabajo fue identificar las principales variaciones morfológicas de células, filamentos y talos de la cianobacteria *Nostoc* sp. LBALBR-2 sometido a radiación ultravioleta (UV-C) en sistemas de microcosmos. La cianobacteria *Nostoc* sp. se sometió a cuatro tratamientos: 1- tratamiento testigo en medio nutritivo; 2- tratamiento en medio nutritivo con exposición a radiación UV-C; 3- tratamiento control en medio sin nutrientes y 4- tratamiento en medio sin nutrientes con exposición a radiación UV-C. Se realizaron análisis de densidad óptica y clorofila-a para determinar el crecimiento de las poblaciones de *Nostoc* y análisis microscópicos para caracterizar el desarrollo morfológico de la especie. La falta de nutrientes generó tricomas cortos con heterocitos terminales, acinetas solitarias dispersas y aparición de hormonas. En medio nutritivo, los tricomas de *Nostoc* presentaron predominantemente la forma seriada vegetativa. Los cultivos expuestos a UV-C produjeron células anómalas, mucílago espeso, tricomas fragmentados y hormonas. Se concluye que *Nostoc* sp. LBALBR-2 se desarrolló bien en condiciones de eutrofización y, a pesar de mostrar deformaciones celulares, fue resistente a la radiación UV-C.
Palabras clave: Cianobacterias; Floración; Microcosmo.

1. Introduction

Cyanobacteria are oxygenic photosynthetic Gram negative prokaryotes that in the aquatic environment can compose the phytoplankton and phytobenthos, being important in the primary production of this ecosystem (Komárek, 2013; Silva et al., 2020).

Nostocaceae C. A. Agardh (ex Kirchner) constitute a family of filamentous, heterocystic, isopolar cyanobacteria with unbranched uniseriate trichomes, or false branches, which inhabit diverse environments (Komárek, 2013; Komárek et al., 2014). These cyanobacteria present importance to the global scientific Community because they have a significant number of strains producing amino acids, fatty acids, polysaccharides, flavonoids, vitamins, and many types of minerals that have antitumor, antiviral, antibacterial, and anti-inflammatory effects (Gheda & Ismail, 2020; Khalifa et al., 2021; Deyab et al., 2020; Mazur-Marzec et al., 2021).

In addition, Nostocaceae fix atmospheric nitrogen and contribute to the fertility of agricultural soils worldwide, and some species behave as harmful microorganisms in aquatic ecosystems due to toxic bloom events (Huisman et al. 2018).

Nostoc Vaucher ex Bornet et Flahault is a genus of Nostocaceae well distributed worldwide and has more than 300 recognized species, however, the genus is heterogeneous and certainly divided into several generic clusters and most species need revision at the morphological and molecular level (Komárek, 2013). In Brazil, 12 species are recognized (Werner et al., 2015) and only one species is cited for the state of Pará, Brazilian Amazon (Costa et al., 2014).

In recent decades, increasing research on cyanobacteria in simulated environments (mesocosm and microcosm) is observed in an attempt to evaluate their behavior in relation to different environmental factors, such as exposure to heavy metals (Mota et al., 2015), nitrogen and phosphorus concentrations (Hughes & Marion, 2021), stimulation to different conditions of temperatures, light, photoperiod (Huisman et al. 2018) and ultraviolet- UV light (Rastogi et al., 2014).

Regarding UV, studies address the behavior of cyanobacteria in the face of light as a stressor of biochemical processes (Jantaro et al., 2011). Cyanobacterial cells react and produce some photoprotective and antioxidant compounds such as

mycosporine-like amino acids- MAAs that have been researched for application in sunscreens and anti- aging products (Soule et al., 2016; Latifi et al., 2009; Geraldes, 2019).

On the other hand, there are studies on the behavior, morphogenesis and toxin production by cyanobacteria when exposed to UV projecting a future climate change scenario due to the sharp increase in solar ultraviolet radiation (UVR) caused by the continuous depletion of the ozone layer, fueling serious concerns about the ecological consequences for all living organisms, including cyanobacteria (Rastogi et al., 2014). UV-C used for germicidal purposes is artificial and is an alternative to prevent cyanobacterial blooms in lakes and reservoirs (Tao et al., 2010; Skai et al., 2009; Usepa, 2006; Jiang et al., 2020), as shortwave UV-C radiation has shown effect on cyanobacterial cells by inhibiting their growth and reducing cyanotoxin concentration through repression of photosynthetic activity and DNA damage (Ou et al., 2012), but no effect on green algae (Tao et al., 2010).

Few studies show the changes that occur in the morphology of cyanobacteria under the effects of UV-C radiation, especially cyanobacteria from Amazonian environments. The objective of this study was to identify the main morphological variations of the cells, filaments and thallus of the cyanobacteria *Nostoc* sp. LBALBR-2 submitted to ultraviolet radiation (UV-C) in a simulated environmental system.

2. Methodology

2.1 Culture

The cyanobacteria *Nostoc* sp. LBALBR- 2 was isolated from roots of the decomposed aquatic macrophyte *Eichhornia crassipes* Mart. (Solms) on the banks of Bolonha reservoir (1°25'14.6"S and -48°25'58.4"W), which belongs to the water supply system of Belém Metropolitan Region (Pará, Brazil). The species was isolated in May/2018 and is cultured in BG-11 medium (Rippka et al., 1979) in triplicate in non- continuous culture with repotting every 15 days. The incubation temperature is 30°C ± 5°, luminosity of approximately 20-60 µmol.photon.m^{-2}.s^{-1} and photoperiod of 12 h light.

2.2 Experimental Design

Four experimental treatments were used in microcosm to verify their effects on the cyanobacteria *Nostoc* sp. LBALBR- 2 (Table 1). Experiments 1 and 2 contain nutrients (BG-11 medium) and experiments 3 and 4 contain ultrapure water type I.

Table 1. Experimental design submitted to *Nostoc* sp. LBALBR-2, cyanobacteria isolated from the roots of macrophytes from Bolonha reservoir (Belém, Pará, Brazil).

Experiment	Temperature (°C)	Humidity (%)	Light (µmol.photon.m^{-2}.s^{-1})	Photoperiod (h)	UV-C (h. day^{-1})	Medium
Experiment 1 (nutrient control)	20 ± 5	60 ± 2	40 - 60	12	Absent	BG- 11
Experiment 2 (nutrient UV-C)	20 ± 5	60 ± 2	40 - 60	12	1 a 3	BG- 11
Experiment 3 (water control)	20 ± 5	60 ± 2	40 - 60	12	Absent	Absent
Experiment 4 (water UV-C)	20 ± 5	60 ± 2	40 - 60	12	1 a 3	Absent

Source: Authors.

For each experiment, samples were taken daily for microscopic analysis, aliquots for *chlorophyll-a* and optical density analysis until the fourth day, then the withdrawals occurred at 3-day intervals until day 22nd. All treatments took place in a

climatic chamber for plant growth (Humidity, Panasonic). The radiation source was a 30W UV-C germicidal tubular lamp, Redy.

2.3 Morphological and Morphometric Characterization

The species was observed and analyzed using an inverted microscope (Axiovert A1, Zeiss Germany) at each time point of the experiments. Morphometric measurements were recorded with a camera (Axio Cam MRc, software Axio Vision, Zeiss Germany) with measurements in micrometers (µm), measuring cell diameters and lengths (width and height), length of filaments and the presence/absence of specialized cells (akinetes and heterocytes), morphological changes, presence of mucilage, among others. Staining wasestablished according to the Faber Castell palette (1761).

2.4 Chlorophyll - a and Optical Density

Chlorophyll-a analysis was performed according to the 10200 H method of SWWM (APHA, 2017) using 90 % acetone in a HANNA 2000 spectrophotometer. Optical density were analyzed at wavelengths 680, 650 and 750 nm (Śliwińska-Wilczewka et al., 2016; Ortiz-Moreno et al., 2020).

2.5 Data Analysis

The cellular values, *chlorophyll-a* and optical density were submitted to parametric ANOVA one way (F) and non-parametric Kruskal- Wallis (H) analysis of variance to verify the differences between the times and types of experiments, considering as significant variation $p< 0.05$ and the post hoc tests of Tukey and Dunn, parametric and non-parametric, respectively, both performed in Past 4.03 software (Hammer et al., 2001).

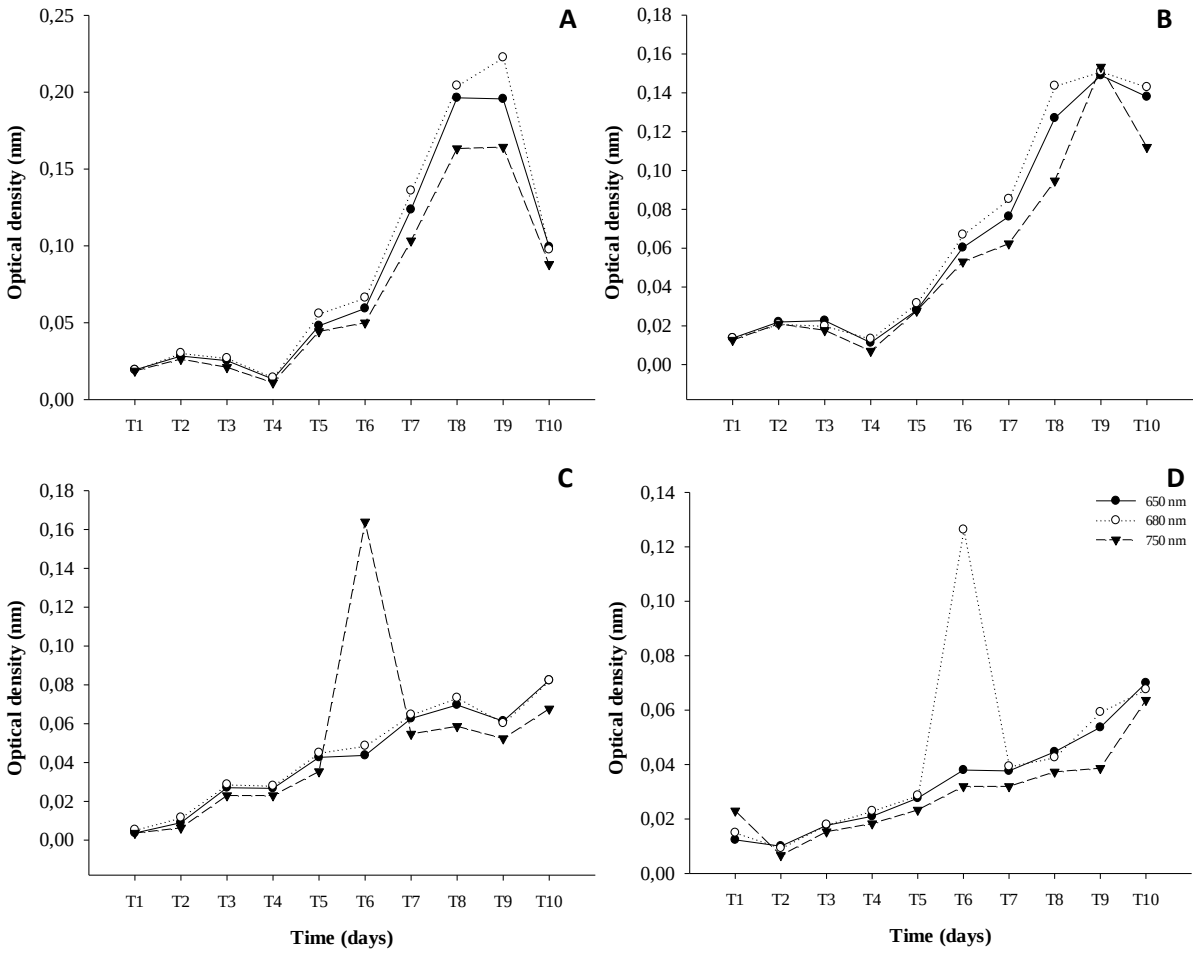
3. Results

3.1 Growth

We suggest that all the wavelengths analyzed express the growth of the species *Nostoc* sp. There was a significant difference between the times ($F= 168.2$; $df= 9$; $p= 0.0005$), where the curves start to differ from day 4 (T4) (Figure 1).

For the experiments in nutrient medium (experiments 1 and 2) the exponential phase started on day 10th (T6), suggesting a decline from day 19th (T9) (Figures 1A and 1B). The species showed slower growth in the absence of nutrients (experiments 3 and 4), in which only the exponential phase was evident (T5 to T10), and in T6 outliers were observed in both experiments (Figures 1C and 1D).

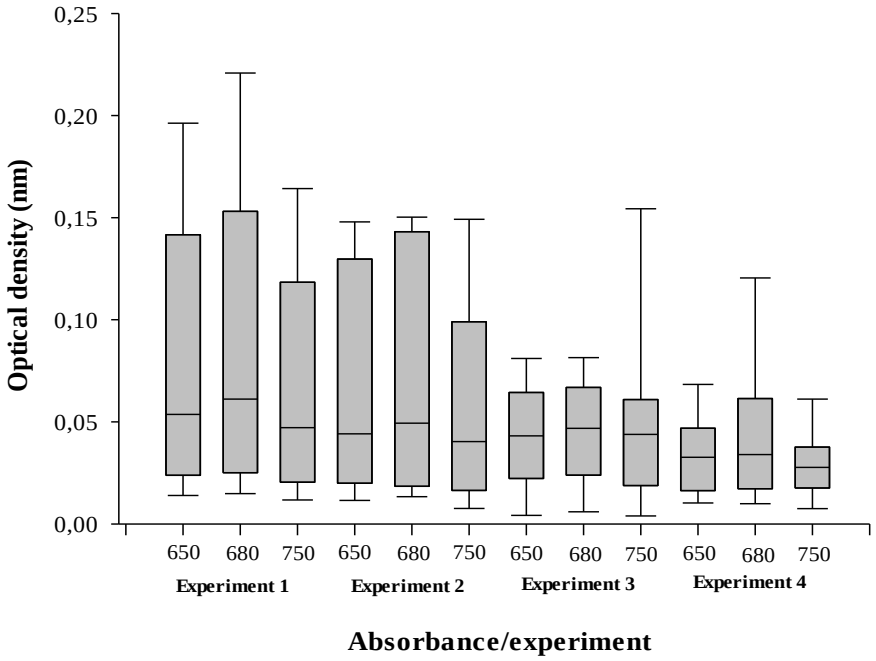
Figure 1. Growth curve of *Nostoc* sp. LBALBR- 2 at different optical densities (650 nm, 680 nm and 750 nm) and in the different treatments: A- experiment 1, nutrient control; B- experiment 2, nutrient UV-C; C- experiment 3, water control; D- experiment 4, water UV-C.



Source: Authors (2022).

The experiments were different (F= 6.0; df= 3; p= 0.0004), with the lack of nutrients being a limiting factor for the growth of the species, as experiments 3 and 4 had the lowest growth than experiments grown on nutrient medium (experiments 1 and 2), regardless of exposure to UV-C radiation (Figure 2).

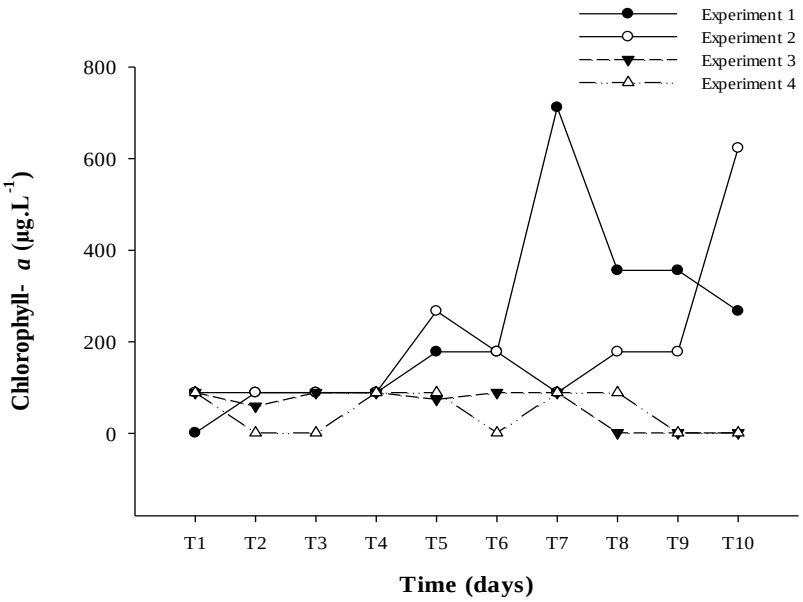
Figure 2. Growth variation of *Nostoc* LBALBR-2 at different optical densities (650 nm, 680 nm, and 750 nm) and experimental treatments (box plot showing: maximum, third quartile, median, first quartile and minimum).



Source: Authors (2022).

There was no difference in *chlorophyll-a* between times (H= 3.7; p= 0.89). However, experiments 1 and 2 showed a slight increase in chlorophyll-a concentrations from day 13th (T7) and day 7th (T5), respectively (Figure 3).

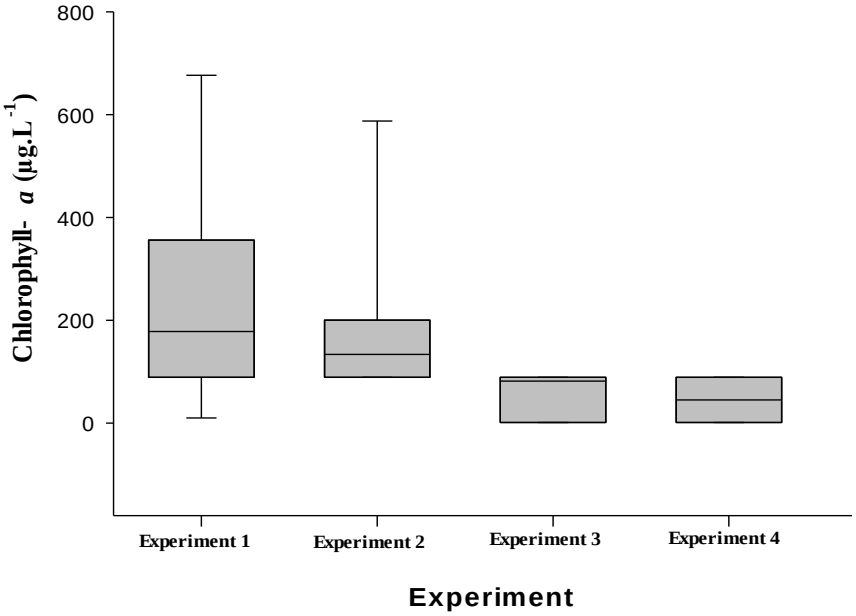
Figure 3. Growth curve of *Nostoc* sp. LBALBR-2 from *chlorophyll-a* concentrations at different times and experimental treatments.



Source: Authors (2022).

The *chlorophyll-a* concentrations also showed significant differences among experiments (F= 4.7; df= 3; p= 0.004), being experiments 3 (58.0 ± 40.0 µg.L^{-1}) and 4 (45.0 ± 46.0 µg.L^{-1}) with *chlorophyll-a* lower than the nutrient medium, which had mean concentrations of 198.5 µg.L^{-1} and 187.0 µg.L^{-1}, in experiments 1 and 2, respectively (Figure 4).

Figure 4. Variation of *chlorophyll-a* concentration of *Nostoc* sp. LBALBR-2 in the different experimental treatments (box plot showing: maximum, third quartile, median, first quartile and minimum).



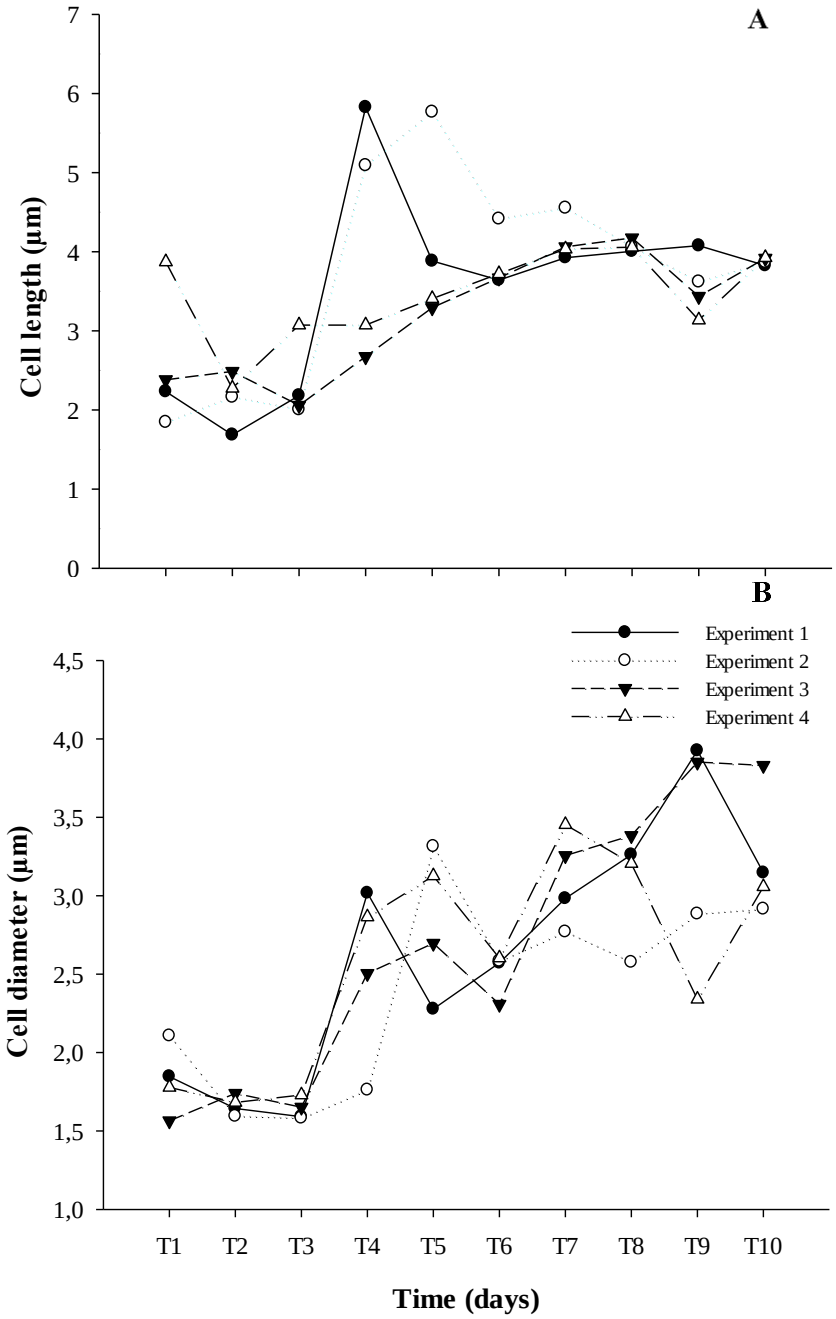
Source: Authors (2022).

3.2 Morphology: Filament, Trichomes and Cells

There was no difference in average diameter cell *Nostoc* (F= 0.24; df= 3.0; p= 0.8) and length (F= 0.5; df= 3.0; p=0.7) between experiments, but diameter (F= 10.7; df= 9; p=0.002) and cell length (F= 5.0; df= 9; p=0.005) increased significantly over time.

The average cell diameter ranged from 1.6 µm to 3.3 µm and the average cell length ranged from 3.3 to 3.8 µm between day 1 and day 22, respectively, for all experiments (Figure 5).

Figure 5. Temporal variation of cell morphometry of *Nostoc* sp. LBALBR- 2: A- cell length (height) and B- cell diameter (width).



Source: Authors (2022).

3.3 Thallus Morphology

The thallus of *Nostoc* LBALBR- 2 presented as a thvick, foliaceus, dark green mass (colorpalette 059) in the nutrient cultures (experiments 1 and 2) (Figure 6A). The fouling started with a thin film on the microcosm wall and a thicker layer on the surface with the appearance of gas, usually after one week of colonization (Figures 6B and 6C).

On the other hand, cultures without nutrients (experiments 3 and 4) presented a thin, lemon yellow mass (color palette 004) (Figures 6D and 6E). It was found that after 30 days of incubation the stalk acquired a gelatinous consistency, especially in samples without nutrients (Figure 6F).

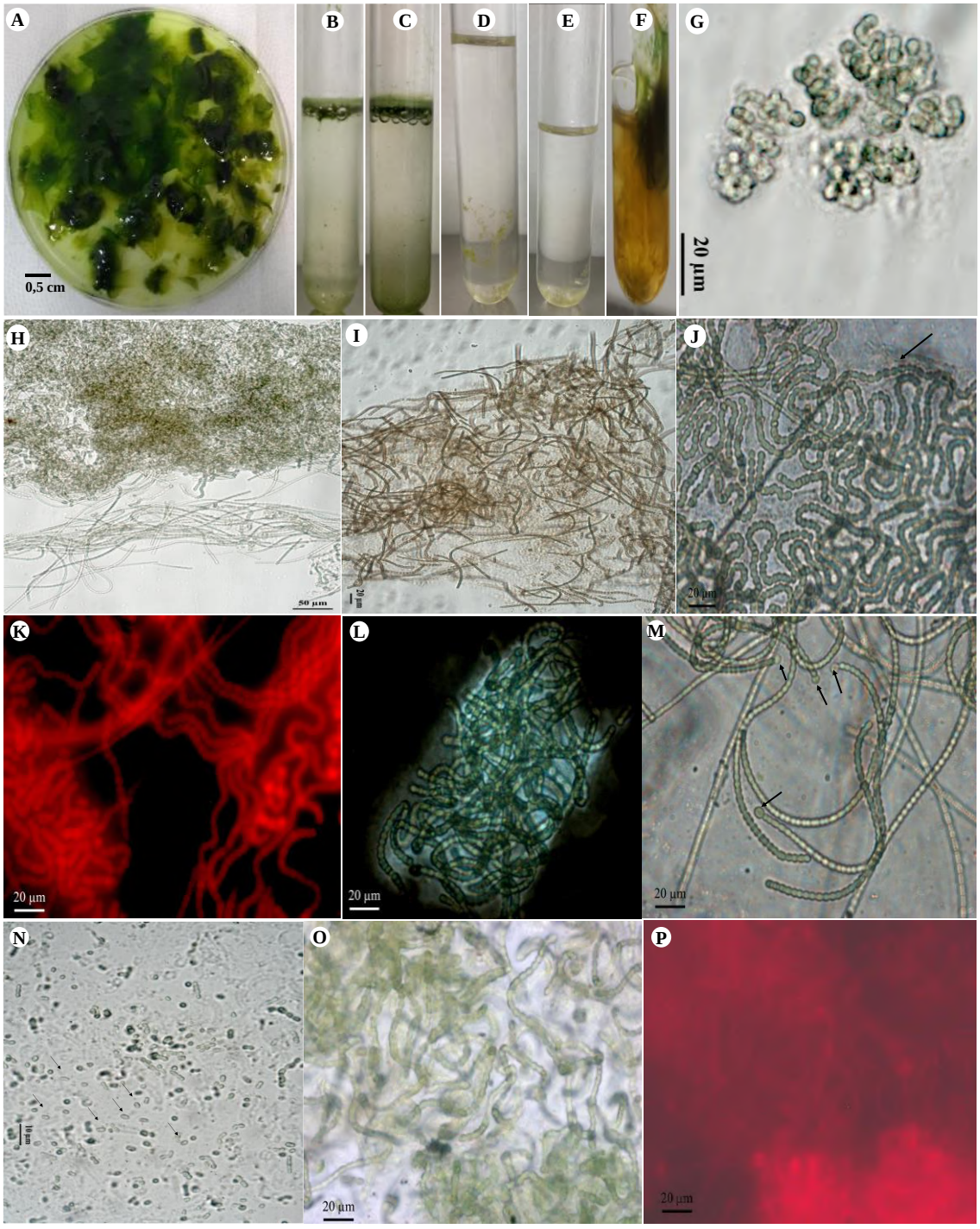
For all experiments, starting on day 4th of cultivation the thallus changed from amorphous colonies, with vegetative cells overlapping in irregular layers, to multiple trichomes with cells arranged in uniseriate sequences (Figures 6G to 6I).

In cultures with nutrients (experiments 1 and 2) the trichomes were longer, on average 300 µm long (Figures 6J to 6L). In cultures without nutrients (experiments 3 and 4), the final cells had terminal heterocytes and the trichomes were short, on average 105 µm (Figure 6M), and in the final times of the experiment, akinetes appeared, measuring 2.5-3.0 µm in height by 1.6- 2.0 µm in diameter (Figure 6N).

In cultures exposed to UV radiation (experiments 2 and 4), the filaments were surrounded by a dense mucilaginous sheath that was dark green in nutrient-depleted cultures and yellowish in nutrient-free cultures (Figures 6O and 6P).

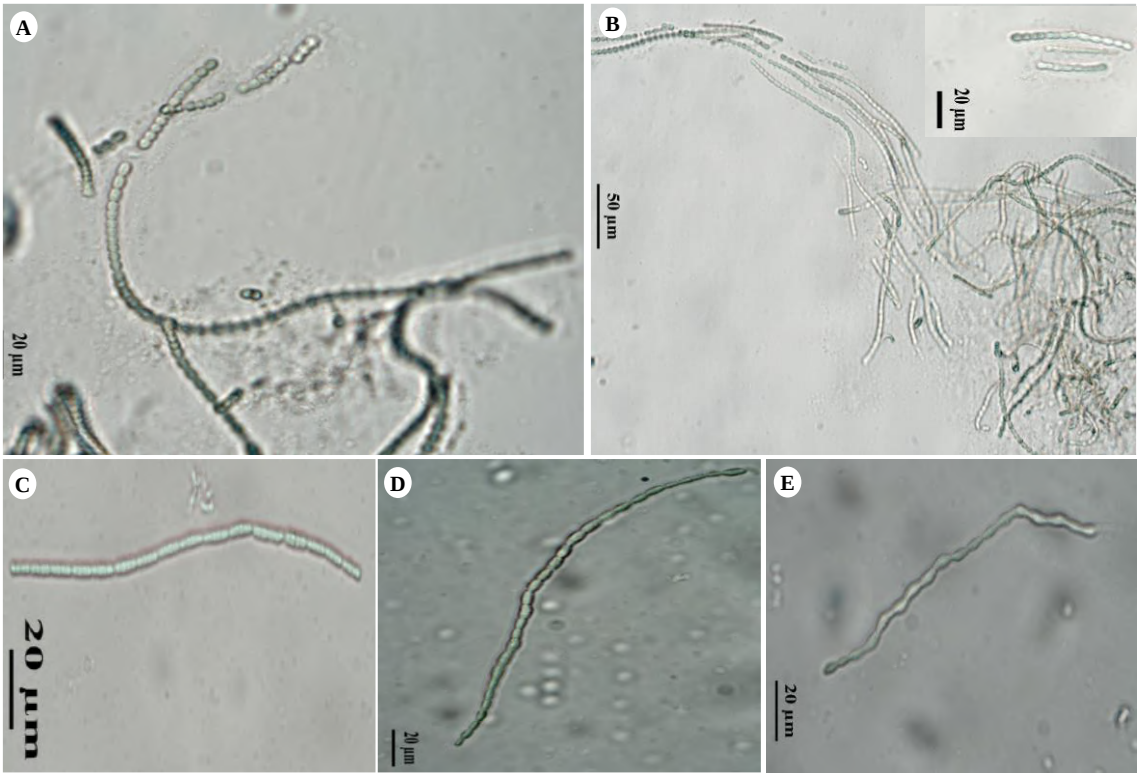
Also, after 15 days of UV exposure, fragments and hormogonia appeared and increased over time. At the final times, the trichomes showed anomalous cells as a resultof UV exposure (Figures 7A to 7E).

Figure 6 - Photomicrograph of *Nostoc* sp. LBALBR- 2: A- Macroscopic appearance of the thallus; B, C, D and E- growth and colonization of the thallus evidencing the fouling after seven days of inoculation, respectively, for the experiments nutrient control, nutrient UV-C, water control, water UV-C; F- macroscopic appearance of the water UV-C strain after 30 days of colonization; G and H- young filaments with overlapping vegetative cells; I- young filaments in cultures exposed to UV-C; J- mucilaginous mass involving the filament (arrow); K- detail of filament visualized in epifluorescence; L- mucilaginous sheath evidenced by Nanquim ink pigmentation; M- detail of trichomes showing terminal heterocytes (arrows); N- scattered akinetes (arrows) in culture without nutrients; O- production of mucilaginous material in colonies under UV-C radiation exposure conditions; P- mucilage interferes in epifluorescence.



Source: Authors (2022).

Figure 7 - Photomicrograph of *Nostoc* sp. LBALBR-2: A and B- hormogonia in colonies grown without nutrients and exposed to UV-C; C to E- anomalous cells in trichomes exposed to UV-C.



Source: Authors (2022).

4. Discussion

The absence of nutrients and UV exposure were stressors to the *Nostoc* sp. LBALBR-2 population. According to Meeks et al. (2002), *Nostoc* cyanobacterial species mature into four forms, which are determined by environmental cues.

Multilayered vegetative cells, trichomes with vegetative cells, trichomes with akinetes and heterocytes and the hormogonia are the main differentiations observed in *Nostoc* (Silva et al., 2020). Thus, the population of *Nostoc* sp. in the present study developed better in nutrient crops generating longer trichomes. For the vegetative cycle is maintained when the amount of nutrients is sufficient for the growth of the species (Silva et al., 1989).

On the other hand, lack of nutrients generated short trichomes with terminal heterocytes and scattered solitary akinetes. On this, Dextro et al. (2018) observed that the absence of nutrients, especially nitrogen, induces heterocyte formation and low light intensity induces akinetes formation in *Nostoc* strains.

Akinetes are generally recognized as resistance cells, with thick walls, that accumulate protein reserves in the form of cyanophycin granules. Akinetes are produced when environmental conditions are unfavorable (Calijuri et al., 2006; Mehnert et al., 2014; Wood et al., 2021), with temperature being one of the main factors responsible for the formation of akinetes, as some cyanobacteria initiate the production of akinetes at temperatures between 20°C and 25°C, such as *Aphanizomenon ovalisporum* (Cirés et al, 2013), and the absence of this cell occurs at temperatures below 21°C, as in the cyanobacterium *Cylindrospermopsis raciborskii* (Bittencourt-Oliveira et al., 2012). However, other environmental factors should influence the formation of these cells including carbon depletion, phosphorus limitation and symbiotic interactions (Singh et al.; 2011; Allaf &Peerhossaini, 2022).

According to Legrand et al. (2016) akinetes are formed under various abiotic stress conditions, depending on the species and also the sampling season. Thus, the resistance of akinetes and their ability to germinate seem to follow a species-specific process and are so important for the maintenance of the species that they also carry the genes for toxin production.

Perez et al. (2016) found that the trigger for differentiation of akinetes of the planktonic species *Anabaena variabilis* is low luminosity and of the terrestrial or symbiotic species *Nostoc punctiforme* is lack of phosphate. These authors suggest that atthe time of akinetes differentiations there is a decrease in photosynthesis and respiration in both species, and this remains low in the mature akinetes, this may indicate a reduction in growth of *Nostoc* sp. LBALBR-2, from the present study, in the absence of nutrients, as many dispersed acini were formed.

The occurrence and quantity of heterocytes in cyanobacterial populations dependon different environmental factors, such as aerobic fixation of atmospheric nitrogen (N_2), concentration and lack of nitrate, organic components, temperatures, X-rays, irradiation, etc. Nitrogen metabolism and environmental nitrogen concentration are considered the crucial factors in their development (Rippka et al., 1979; Komarek,2013), thus their highest abundance was in the trichomes grown in nutrient-free medium.

We suggest that hormogonia arose in response to lack of nutrients and UV exposure, although there are few studies confirming UV stress on hormogonia formation, UV is known to cause trichome fragmentation in *Nostoc* sp. (Singh et al., 2011). Hormogonia is a process of dispersal, phototaxis, reproduction and establishment of nitrogen-fixing symbioses and constitutes part of the life cycle of filamentous cyanobacteria, especially in *Nostoc*.

According to Zuniga et al. (2020) light quality and quantity, nutrient concentrations, and autogenic repressors are factors that induce hormogonia formation, which according to Gonzales et al. (2019) occurs through a regulatory network of sigmas genes that works in a cascade, coupled with specific genes that induce changes in cell architecture.

Regarding the stresses promoted by UV-C, the most significant were the production of a thick mucilaginous sheath around the *Nostoc* filaments and anomalous cells, which appeared at the end of UV exposure.

Studies have shown that *Nostoc* sp. creates enzymatic and non-enzymatic defense mechanisms to counteract the damaging effects of UV radiation, such as the production of antioxidant enzymes and mycosporin-like amino acids capable of performing UV screening, yet it dies if continuously exposed to this radiation (Richa & Sinha, 2015) or recovers when the radiation source is ceased (Phukan & Syiem, 2019). Thus, we suggest that the mucilaginous sheath that appeared on *Nostoc* in the present study is the production of these compounds in response to UV-C exposure.

Phukan & Syiem (2019) evaluated the oxidant and antioxidant homeostasis responses of the cyanobacteria *Nostoc muscorum* exposed to UV radiation and evidenced a complex defense organization developed by this species, which after being subjected to doses of UV-C radiation exhibited ultrastructural cell damage, produced reactive oxygen species (ROS) and consequently significantly induced enzymatic and non-enzymatic antioxidant production.

In the present study, we show the morphological alterations of UV-C exposure that we suppose are consequences of the biochemical and ultrastructural alterations suffered by the exposed cells. It is worth noting that not all cells showed malformations, due to the organization of the *Nostoc* tallus that takes place by the superposition of cells, allowing some cell layers to be more exposed than others which was also observed by Phukan & Syiem (2019).

The Amazon microbiota is poorly known when compared to its estimated high diversity. The behavior and development of the species can be studied from models made in *Nostoc* cultures, and their potential in biopharmaceutical production is important in contemporary scientific research. Thus, we suggest that future studies should better highlight the sequences of events identified in the present study, especially at the intracellular and biochemical level to potentiate their use as a model of cyanobacteria development.

Furthermore, the study contributes to strengthening the knowledge about the defense mechanisms of cyanobacteria and also to improving the processes of reducing blooms using UV-C radiation.

5. Conclusion

The effect of the radiation on the species occurred in the initial development of the thallus, the color of the colony, the shape of the cells and the length of the filaments.The nutrient cultures allowed the cyanobacteria population to develop further and the cells did not change in diameter and length, but UV-C exposure caused anomalous cells, the appearance of a thick mucilage and numerous fragmented trichomes and hormogonia,which we suggest are adaptive strategies to UV-C light stress and especially the lack of nutrients.

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