

Research Article

# A study on the vegetative survival of some blue-green algae in the soil of Baghdad city, Iraq

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#### Abstract

Algae, photosynthetic organisms, thrive in water or wet substrates and can be found in various habitats, including arid ground, moist soil, tree trunks, and architectural structures. In the present study, the algae that live in the garden soil of the Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad was collected and tested their ability to survive in natural environments, physical and physiological pressure of water, darkness with limited light, and surviving after UV light exposure. The results obtained in this study indicated that the algae *Lyngbya major* could live on soil in a wide range of temperatures and humidity. However, during winter, the survival rate dropped to 43-64%, while in summer, the survival rate dropped more. Throughout the rainy season, the viability of species (*Leptolyngbya halophile*, and *Chroococcidiopsis cubana*) exceeded whenever the soil moisture level was increased. The chance of surviving *C. aponinum* was raised in spring but reduced in winter, with reducing the soil moisture. Nevertheless, *Myxosarcina burmensis* exhibited exclusive survival throughout the rainy and spring months. *L.major* showed comparable survival rates during both immersed and air-exposed environments over 15 days. However, their survival rates decreased when submerged for extended periods compared to exposure to air on a moist soil surface. *C.aponinum* exhibited superior and prolonged survival when immersed in a liquid media as opposed to being exposed to air on a damp earth surface. This the first study to identify the ability of algae that live in Baghdad garden soil.

Keywords: Blue-Green Algae, UV exposure, water stress, Exposed to air

# INTRODUCTION

Algae, a photosynthetic organism, is a basic vegetation that thrives in any substrate containing water or wetness. These plants serve a crucial function in the food chain since they are a significant source of nourishment for aquatic animals and fish. Additionally, they are vital oxygen producers in all habitats (Bhushan *et al.*, 2023). Algae can be found in various habitats such as arid ground, moist soil, rocky surfaces, tree trunks, architectural structures, and even in the symbiotic relationship with fungi to generate lichens. They can also be located at shallow depths, often a few centimetres below the surface, with limited illumination (Keshri, 2024). These soil areas experience extreme drought conditions and varying levels of light intensity. The oscillations in lighting and heating energy are considerably larger than the variations in physiological responses observed in aquatic environments. Consequently, the land experiences environmental stressors such as depletion and elevated temperatures (Alalaf, 2020). Specific species of algae, characterized by their greenish-blue colour, and certain eukaryotic organisms, seek out secluded microhabitats to shield themselves from harm. These organisms have a crucial impact on the augmentation of organic material with nitrogen levels in the environment. This process improves the adhesion between soil particles, rendering them more impervious to damage induced by both water and winds (Büdel, 2024). Numerous global researchers have shown interest in

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diagnosing and categorising terrestrial algae, which are crucial in sequestering carbon and enhancing soil fertility(Rai *et al.*, 2023). The majority of research conducted on the abundance and characteristics of algae in aquatic environments has focused on Phytoplankton and Seafloor Algae (Epipelic Algae and Epiphytic algae) (Keshari *et al.*, 2021). However, there have been limited studies specifically examining algae that are attached to the soil in local areas. Hence, the ongoing research focuses on diagnosing and classifying algae that adhere to the earth's surface in residential gardening.

The present study aimed to examine the ability of the most abundant types of blue-green algae, namely *Lyngbya major*, *Cyanobacterium aponinum*, *Leptolyngbya halophile*, *Chroococcidiopsis cubana* and *Myxosarcina burmensis*, to survive in different seasons of the year. Also, investigate their survival under natural conditions and when submerged or exposed to air. Additionally, the study examined their ability to withstand water stress, dry storage, darkness, dim light, and UV exposure in a controlled culture chamber. This is the first study to identify the ability of algae that live in garden soil in the Biology Department, College of Education, Ibn al-Haytham, University of Baghdad, to withstand the conditions.

# MATERIALS AND METHODS

#### Samples collection

Soil samples were taken from the gardens of the College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad. The samples were collected from October 2022 to May 2023, covering every year's season. Algae were collected. The algae L. major MENEGH ex GOMONT filaments threads formed a dark bluish-green coating on the cement-covered surfaces of buildings (Halder, 2016)., whilst C. aponinum SMITH, as an illustration, gomont filaments were observed individually on the top layer of wet soil, forming greenish-blue mats (Moro et al., 2007)). On the other hand, L. halophile (KUTZ.) RABENH and C. cubana (TURP.) KUTZ can be seen independently on the surface of damp soil, appearing as rounder and/or irregularly dark brown masses of mucilage, with diameters ranging from 1 to 15 mm (Hatem and Al-Sultan, 2023). M. burmensis SKUJA cell packets, with a diameter ranging from 23 to 30 µm, were collected from a tiny freshwater pond where they were suspended in water (KovAcIK, 1988).

#### Algae's ability to survive in natural environments

Algal were collected through the environment and transported to the laboratory regularly throughout the year, according to their availability. These substances demonstrated their capacity to survive and grow under different season conditions, including changes in air temperature (AT), relative humidity (RH), soil moisture content (SMC), and plant water availability (PWT).

The vegetative survival rate in *L. major, C. aponinum, L. halophile ,and C. cubana* was assessed by calculating the proportion of living vegetation cells compared to dead vegetative cells that displayed features such as becoming hyaline, empty, twisted, or malformed. For *M. burmensis*, the probability of survival was determined by quantifying the total number of viable cell packets and comparing it to the total number of non-viable cell packets, which displayed traits such as being transparent, unclear, reduced, or broken down. The counts were conducted on around 6000-7000 vegetative cells, vegetative filaments, and/or cell packets obtained from five distinct places.

# Surviving in conditions of both physical and physiological pressure of water

Seven days before, materials obtained from liquid BG11 medium were individually introduced into a solid medium consisting of 2 to 8 % agar and then into BG11 with a concentration of 0.2-0.5 mol/L Sodium chloride (NaCl). These samples were then placed in a culture chamber, and the survival percentage was calculated (Li *et al.*, 2023).

# Assessing the survivability of drying algae stored in the open air and desiccators

The five-day-before materials of BG11 were thoroughly dried using blotting and then placed individually on filter papers in watching glasses. They were then stored in the culture chambers under two different conditions: one group was exposed to air at a relative humidity of 47-55%, while the other group was housed within desiccators with fused calcium chloride (CaCl<sub>2</sub>), maintaining a relative humidity of 30-33%. The survival of dried algae, regardless of the storage method, was assessed by quantifying the surviving vegetative cells, filaments of matter, or cell packets after a 3-day incubation of the dehydrated materials in BG11 medium within the culture container (Le Loeuff *et al.*, 2023).

### Adaptation to survive in conditions of darkness with limited levels of light

The alage, which were seven days before and taken from BG11, were subjected to light intensities of 40  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> in the culture chambers. The exposure was done independently for two intensities: 2 and 10  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. This was achieved by altering the distance between the infected culture tubes with a light source. The light level was quantified using a lux meter manufactured by Lutron Electronics, based in the United States. A portion of the cultured tubes were enveloped in opaque black paper and stored in a lightless environment. The percentage of vegetative survival was calculated using standard methods.

# Surviving after UV light exposure

Algaes seven days before were individually placed within sterile BG11 solution, with each material spread out in open Petri dish containers of 90 mm in diameter. The materials were then subjected to UV radiation emitted by Phillips germicidal lamps. The amount of energy flounce, determined by increasing the duration of the exposure period from 5 to 20 minutes, varied between 0.96 and 3.84 KJ/m<sup>2</sup>. Following irradiation, the materials were immediately moved to the culture container and regularly evaluated for vegetative life. Controls were used, and these consisted of materials that had not been irradiated (Hussein, 2016).

#### **RESULTS AND DISCUSSION**

# Algae's ability to survive in natural environments

The wall algae *L. major* survived throughout the year, with a maximum survival rate of over 75%. The high survival rate was consistently observed in both the springtime (March and April) and rainfall (January and February) seasons, while the air temperature varied from 15 to 30 and 28 to 40 °C, and the relative humidity (RH) was between 65-95% and %, 70-98 respectively. However, during the colder months (December, January, and February), the survival rate was reduced by around 55% as the air temperature dropped to 5°C, while the relative humidity (RH) stayed between 70-98%. In the summer, the survival rate significantly decreased to around 30% or less when the air temperature increased to 55 °C and the relative humidity decreased to 20% or lower.

Nevertheless, the soil algae *C. aponinum*, disappeared during the hot summer after the soil moisture content decreased 15 to 35% (measured by the weight for dried soil). They resurfaced during precipitation and flourished when the soil moisture content achieved their maximum capacity of 100%. Their survival rate de-

creased approximately 1/3 to 1/6 during the winter when the SMC was less than half of what it was during the rainy season. In the spring, their survival rate dropped to over 3/4 when the soil moisture content ranged from 50% to 90%. The soil algae *L. halophile* and *C. cubana* were able to survive.

The mortality rate was about 100% during the wet season and the organisms were missing during the seasons (winter, summer, and spring) (Table I). Therefore, *L. major* exhibited a higher tolerance to environmental challenges than other soil algae.

The presence of algae on the surface of the soil throughout rainy months can either attributed to the revival of hardy and dormant cells that previously existed in the soil's structure or to the dispersal of viable growing cells by rainwater runoff in nearby favorable microenvironments. Nevertheless, when the desiccated soils from the summer months were transported into a controlled environment and subjected to prolonged exposure to tap or filtered water, none of the existing soil algae were observed on the soil surface. In a study conducted by (Gupta and Agrawal, 2006) noted that Rhizoclonium crassipellitum, a species alga associated with soil, exhibited considerable survival only during spring time. However, it perished without generating any structures for reproduction upon the arrival of summer. This alga was completely absent during the summer, rainy, and winter seasons. On the other hand, Scytonema millei, Phormidium bohneri, and Lyngbya mesotricha, which are all types of soil algae, survived at a rate of 100% in the season of rain, cold months, and spring time. Nevertheless, their chance of surviving declined to 15-25% over the summer season.

*Myxosarcina burmensis* exhibited a survival rate of over 75 % during the rain period, while the water temperature ranged from 16 to 24 °C. However, its survival rate dropped to 30-60% during spring when water temperatures ranged from 17 to 28 °C. This indicates that

**Table 1.** Influence of seasonal physical conditions on the survival (%) of vegetative cells and\ or cell packets in *L. major*, *C. aponinum*, *L. halophile*, *C. cubana*, and *M. burmensis* 

Seasonal	Springer Summer Rainy (March and )May, June,July, (January, and April) and Augest) February)		Winter (December, Janu- ary, and February		
Variables	Survival Rate (%)				
Atmospheric temperature (°C)	15-30	30-55	28-40	5-25	
Relative Humidity(%)	70-98	23-66	70-95	65-95	
Soil moisture content % <sup>b</sup>	50-90	15-35	89-100	39-44	
Water temperature (°C)	17-28	20-38	16-24	5-18	
Lyngbya major	78-96	15-23	81-94	42-64	
Cyanobacterium aponinum	70-95	0	90-96	12-31	
Leptolyngbya halophile,and Chroococcidiopsis cubana	0	0	96-100	0	
Myxosarcina burmensis	30-60	0	75-85	0	

*M. burmensis* constitutes a temperature-sensitive alga. It vanished throughout both the winter and summer months, when the water temperature ranged from 20 to 38 °C and 5 to 18 °C, as shown in Table 1. During unfavourable periods, plankton diatoms survive by retaining a small, gradually decreasing population of vegetative cells neither floating in the water nor resting on sediments, as registered in a drinking water reservoir in Central China (Huang et al., 2021). The production and degeneration of Aphanizomenon flos-aquae blooms in a small pond were influenced by the seasonal variations in temperatures of the water and day length, as observed earlier in Lake Chaohu, Lake Zhiyin (China), Reservoir Orlík (Czechia) and Reservoir Stefanski (Poland) and five different potash tailings pile sites in Lower Saxony and Saxony-Anhalt, Germany (Chen et al., 2020; Sommer et al., 2020). In their study, Xu et al. (2021) observed a swift initiation of photosynthesis after the dried Microcoleus crust was rehydrated in Inner Mongolia, China.

# Survival in both submerged and exposure to air situations

The soil algae C. aponinum, and C. cubana, exhibited enhanced survival rates and longer lifespans when submerged in BG11 compared to being exposed to air on a wet surface of the soil in cultivation chambers (Table 2). Therefore, if they were immersed in rainwater accumulated on the ground, they would remain unharmed. The wall alga L. major and soil alga L. halophile exhibited similar levels of survival under both submerged and air-exposed settings for up to 15 days. However, their survival rates were slightly lower when submerged for longer periods compared to being exposed to air. M. burmensis exhibited lower survival rates when cultivated on a damp soil surface exposed to air compared to when it was submerged in BG11, which replicates its native planktonic environment. Trentepohlia aurea, a resilient aerial alga, demonstrated enhanced survival and sporangia formation exclusively under settings with exposure to air. In a study conducted by Ceccarelli et al. observed that elevated precipitation levels resulted in nearly total eradication of the macroalgal colony linked to the substrates (Ceccarelli et al., 2020). Other study conducted by (Gupta and Agrawal, 2006) found the soil alga P. bohneri exhibited higher survival rates and longer survival times when submerged in BG11 compared to being exposed to air on a moist surface of the soil. On the other hand, the structure of the wall or otherwise bark alga S. millei and the soil algae M. chthonoplastes, L. mesotricha, and R. crassipellitum showed similar or little better rates of survival when exposed to air on a moist surface of soil compared to being suspended in BG11.

# Surviving in conditions of both physical and physiological water stress

The survival rates of *L. major*, *C. aponinum*, *L. halophile*, *C. cubana*, and *M. burmensis* reduced when the media included 2-9% agar and when the media was supplemented with 0.3-0.6 mol/L Sodium Chloride (Table 2). The filamentous parts of *C. cubana* and *L. halophile*, which lack a sheath, appeared more susceptible to desiccation than the other cells, and *C. aponinum* and *L. major*, which have a hard sheath covering their filaments. The filamentous structures of *L. major*, which had a somewhat thicker sheath than those of *C. aponinum*, exhibited a higher tolerance to desiccations than the latter (Wiśniewska *et al.*, 2021).

L. halophile and C. cubana vegetative cells did not undergo cell division when grown on agarized media with an agar concentration of 7% or higher and in a salinized solution with a concentration greater than 0.5 mol/L. However, they were able to separate to a maximum percentage of approximately between (10 to 14%) when grown on agarized media with a concentration of 3% and around 5-10% on media with a concentration of 5%, in cultures that were 8-14 days ago. In the case of L. halophile, the maximum division rate was approximately 9% in a salinized solution with a concentration of 0.5 mol/L, while C, cubana had a maximum division rate of 10-14% under the same conditions in cultures that were 10-15 days old. Furthermore, L. halophile had a maximum division rate of approximately 9 to12% on a moist-soil surface seven days after incubation after inoculation, while C. cubana had a maximum division rate of 5% under the same conditions. High salt levels inhibit photosynthesis and oxygen consumption in blue-green algal cells, as demonstrated by studies conducted by (Dąbrowski et al., 2021; Singh et al., 2022).

Every blue-green algal cell perished and no cells remained dormant when cultivated on any solid media containing agar or in BG11 treated with salt (Table 2). Nevertheless, many algae can withstand electrolytes by creating cysts or impermeable protective coverings (Agrawal and Singh, 1999). In their study, (Scherer and Potts, 1989), discovered that a *Nostoc commune*, obtained from the field, produced a distinctive protein after undergoing multiple drying cycles. This protein could enhance the alga's ability to withstand desiccation. Chroococcidiopsis cells in dried-out cultures protect against dehydration by increasing the thickness of their outer layer (Napoli *et al.*, 2021).

# Survival of dried algal kept in the open air and in desiccators

Under controlled conditions in a culture container with a relative humidity of 46-57%, *C. aponinum* and *L. halophile* filaments missed their ability to survive and

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Algo	Controlb	UV exposure		
Alga		0.96	2.88	3.84
Lyngbya major	54	33	14	10
Cyanobacterium aponinum	34	15	11	9
Leptolyngbya halophile	46	18	15	5
Chroococcidiopsis cubana	45	25	14	7
Myxosarcina burmensis	50	36	28	15

Table 3. Death day of algae following UV exposure (0.96, 2.88, 3.84 kJ/m2)

aMaintained in culture chamber following UV exposure.;bNo UV treatment.

then died after a period of 13-14 days, respectively. Similarly, *C. cubana* cells, *M. burmensis* cell packets, and *L. major* filaments had a longer survival time, with cells dying after 23 - 25 days, respectively. The death of cells occurred more rapidly inside desiccators with a relative humidity (RH) of 30-33%. Therefore, water evaporation from an alga exposed to air is somewhat stopped when the cells are coated with a protective layer or slimy substance, especially at higher relative humidity.

#### Survival in darkness and at low light intensity

L. major, C. aponinum, L. halophile, C. cubana and M. burmensis survived darkness for ≥2 weeks and died without any remaining dormant cells. All algae's survival rate and duration were enhanced by low-intensity light levels of between 3 and 10  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, as shown in Table 2. Under darkness, the vegetative cells of L. halophile and C. cubana did not undergo division. However, when exposed to light intensities of 3, 10, and 40 µmol m-2 s-1, most vegetative cells split to the greatest extent of approximately 2-5%, 3-6%, and 7-10%, correspondingly 10 days after inoculation. Chlorella cells were found to be completely alive without light even after 40 -45 days, as reported by (Garg et al., 2022). C. minor, G. aeruginosa, A. nidulans, P. foveolarum, S. hofmanni, S. millei, P. bohneri, M. chthonoplastes, and R. crassipellitum were able to stay alive in darkness for different durations ranging from  $\frac{1}{2}$  to 1  $\frac{1}{2}$ or more. Additionally, these organisms could persist for even longer periods under dim light conditions(Agrawal and Pal, 2003). Lower light intensity does not appear to be a crucial determinant of their survival. According to Adir et al. (2003), exposing blue-green algal crust to a high light intensity level (1000 µmol□m<sup>-2</sup>□s<sup>-1</sup>) through the dry periods leads to significant damage (Adir et al., 2003). Conversely, a greater intensity of light increased the formation of carotenoids and phycobiliproteins in Anabaena azollae (Venugopal et al., 2006).

#### Survival after exposure to UV

Survival following ultraviolet (UV) radiation. Every type of algae demonstrated varying degrees of sensitivity to UV shock, with values ranging between 0.95 to 3.85% kJ/m<sup>2</sup>. Additionally, they experienced death ear-

lier than the control group, as shown in Table 3. All of the cells exhibited susceptibility after the trial. The existence of a sheath, as seen in *L. major* and *C. aponinum*, or a mucilage cover, as observed in *L. halophile* and *C. cubana*, did not offer any extra defence against UV damage compared to the absence of these characteristics, as found in *M. burmensis*.

The results of this study are consistent with the findings of (Agrawal and Singh, 1999) and (Agrawal and Pal, 2003). The presence of mucilage covering the cell walls of *C. minor, G. aeruginosa* and *A. nidulans*, in addition to the presence of hyaline or pigmentation on the cells of the hyphae of *L. martensiana* and *S. hofmanni* does not provide effective protection against damage caused by ultraviolet rays. In the study conducted by He and Häder (2002) found that increased exposure of *Anabaena* sp. algae to ultraviolet radiation led to excessive production of reactive oxygen, which led to decreased survival rates.

Amidst the detrimental consequences of ultraviolet radiation in aquatic ecosystems, Ultraviolet light suppresses the growth of algae, particularly cyanobacteria. Stress triggers a mechanism known as a reactive oxygen species (ROS) chain, which partly generates DNAmediated species, leading to a range of damage, mutations, cell senescence, carcinogenesis, and apoptosis (Pessoa, 2012). The present study recorded the death of most exposed algae to UV radiation compared with control. UV-A generally causes indirect DNA damage by forming chemical intermediates such as oxygen and hydroxyl radicals that interact with DNA to form strand breaks, DNA-protein crosslinks and alkali labile sites. On the other hand, UV-B causes direct DNA damage by inducing the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (Dahms and Lee, 2010). The results of the current study are consistent with a study conducted by Korbee et al. 2005, which indicated the harmful effect of ultraviolet radiation in the UV-A range (320-400 nm): It causes indirect damage to DNA through the production of intermediate compounds such as oxygen and free hydroxyl, which lead to the breakage of DNA chains and the formation of abnormal bonds between DNA and proteins.

A recent study was conducted by (Schneider et al.,

2022) to study the effect of ultraviolet radiation on the green and red alga cultivated under different radiation treatments. The results indicated that UV light affected development rates and the accumulation of internal substances, including carotenoids (violaxanthin, antheraxanthin, and zeaxanthin), MAA palythine, and phenolic compounds. The UV radiation treatments also exhibited a peak in blue light, indicating the presence of photoreceptors responsive to these radiation characteristics (UV-A, UV-B, and blue light). Generally, UV-A induces certain chemicals without impacting development, whereas UV-B appears to promote the accumulation of compounds at the cost of growth. The impact of light quality on growth and metabolism in G. cornea is compared to that in other species using a comparable experimental approach. Studies confirm that excessive exposure to ultraviolet radiation limits the ability of algae to photosynthesize and fix nitrogen, which negatively affects their abundance in aquatic environments and disturbs the ecological balance.

# Conclusion

The present study indicated that *L. major* algae can survive in Baghdad soil, in a wide range of temperatures and humidity, during summer and spring, while the survival rate decreased during winter. As for the rest of the algae species (*L. ptolyngbya halophile* and *Chroococcidiopsis cubana*), the chance of survival of *C. aponinum* in the spring increased. However. *Myxosarcina burmensis* showed exclusive survival throughout the rainy spring months. These results indicate that the dominant cyanobacteria have evolved distinct defense mechanisms that enable them to thrive in tropical regions' harsh environmental conditions and laboratory settings.

# **Conflict of interest**

The authors declare that they have no conflict of interest.

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