Iranian Journal of Fisheries Sciences
http: doi.org/ 10.22092/ijfs.2026.135503 2026, 25(3) 495-508

Research Article

Cyanophycin granule polypeptide from some cyanobacteria along Damietta branch of the River Nile, Egypt

Deyab M.A.[1], Ward F.M.[1*]

1Department of Botany and Microbiology, Faculty of Science, Damietta University, New Damietta City, Egypt
*Correspondence: Fatma2028@yahoo.com

Keywords

Biomass,
Cyanobacteria,
Cyanophycin granule polypeptide,
CGP,
Damietta branch,
Nile River

Article info

Received: February 2025
Accepted: May 2025
Published: May 2026

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract

Cyanobacteria are one of the most common and widespread microorganisms that produce cyanophycin granule polypeptide (CGP). This biopolymer could be used to produce CGP-derived dipeptides and as a potential precursor for the synthesis of polyaspartic acid. To extend the applications of this polymer, it is therefore of interest to study its isolation and purification. The present study was undertaken to follow up CGP production by some cyanobacteria isolated from the River Nile, Damietta Branch, Egypt, and to morphologically identify its algal diversity. The results of this study reported that Cyanophytes dominated at AL Zarqa, Faraskur, and Al Adliya meanwhile Chlorophytes and Bacillariophytes dominated at El Serw and Kafr Al Arab. *Anabaena anomala*, *A. cylindrica*, *A. oryzae*, *A. variabilis*, *Nostoc* sp., *Nostoc punctiforme*, *Oscillatoria obscura*, *Spirulina platensis*, *Synechococcus* sp. were identified by microscopic examination. The isolated cyanobacteria produced CGP in different amounts ranging from 0.030 ± 0.001 g L^{-1} in *Oscillatoria obscura* to 0.224 ± 0.007 g L^{-1} in *Anabaena cylindrica*. Transmission Electron Microscopy revealed that CGP produced by *Anabaena cylindrica* was dark and spherical with irregular parameters bodies. Based on this study, regular monitoring of cyanobacteria and their CGP production is necessary to extend their applications.

[Downloaded from jifro.ir on 2026-11-24]

Introduction

The Nile River is the main freshwater resource needed for nearly all drinking and irrigation water demands. Its water runs from the south to the north and ends at two branches, Damietta and Rosetta. Damietta branch ecosystem is exposed to a complex interaction of natural and anthropogenic activities that influence the floral and faunal composition.

Cyanobacteria found in the Damietta branch along 242 km of the Nile River are rich with valuable by-products that are useful for various industrial applications. These cyanobacteria consist of various organic inclusion bodies that occur in the cytoplasm. These insoluble inclusion bodies include cyanophycin granule polypeptide (CGP), a non protein, synthesized amino acid polymer that is composed of an aspartic acid backbone and arginine side groups. The water-insoluble CGP accumulates inside the cells as membrane-less granules and is degraded by the cells when growth is resumed. CGP is synthesized by cyanophycin synthetase, which adds aspartic acid and arginine to a cyanophycin primer. Meanwhile, CGP is degraded stepwise by cyanophycinase which releases β-aspartyl-arginine dipeptide, and isoaspartyl dipeptidase which cleaves the dipeptide into the free amino acids aspartate and arginine (Canizales *et al*., 2023; Sharon and Schmeing, 2023).

In the presence of sufficient nitrogen, cyanobacteria can utilize inorganic nitrogen forms, atmospheric nitrogen, and some amino acids, whereas many cyanobacterial species accumulate CGP at the heterocyst poles under limiting conditions, including low light intensity, low temperature, and sulfur, potassium, or phosphorus limitation (Aravind *et al*., 2016; Flores *et al*., 2019). CGP is extremely rich in nitrogen as it contains five nitrogen atoms in every building block and therefore CGP accumulation enables cyanobacteria to optimize nitrogen assimilation under nitrogen-poor conditions, during the fluctuation of the nitrogen supply, and day/night cycles, by allowing continuous nitrogen assimilation and storage (Watzer and Forchhammer, 2018). Frommeyer *et al*. (2016) reported that CGP is considered a transient storage for nitrogen, carbon, and energy.

CGP is typically produced in the form of opaque light-scattering cytoplasmic granules by different species of heterocystous cyanobacteria including *Toxifilum mysidocida, Anabaena variabilis*, *Aphanocapsa, Nostoc ellipsosporum*, *Scytonema,*and *Synechoncystis* species. Recently, the occurrence of genes encoding proteins homologous to cyanobacterial cyanophycins has been described for other eubacteria that do not belong to the cyanobacterial group, for example, *Acinetobacter* sp., *Bordetella bronchiseptica*, *B. pertussis*, *B. parapertussis*, *Clostridium botulinum*, *Desulfitobacterium hafniense*, and *Nitrosomonas european* (Krehenbrink *et al*., 2002; Ziegler *et al*., 2002; Füser and Steinbüchel, 2007; Zimba *et al.*, 2017; Du *et al*., 2019).

Previous studies reported that several factors, including light, carbon dioxide, sulfur, and phosphorus starvation as well as the addition of arginine to culture

[Downloaded from jifro.ir on 2026-11-24]

media increased the amount of CGP formed in the cyanobacteria. Cyanophycin has unusual soluble properties as it is insoluble under neutral pH and is soluble under acidic (pH<2) or alkaline (pH>9) conditions. It is also insoluble in water-containing metal ions, EDTA, sodium deoxycholate, and organic solvents. The high viscosity, high solubility, and complete biodegradability of CGP enable it to have potential applications in the fields of biomedicine, agro-chemistry, pharmacy, food industry, and personal care. CGP can also serve as a natural source of amino acids such as arginine and aspartate, and the poly-aspartate backbone of CGP could also be used as a biodegradable substitute for polyacrylates (Aravind *et al*., 2016). CGP has unique chemical and physical properties that make it suitable for several applications such as industrial food, medicine, cosmetics, water treatment, and agriculture applications (Zou *et al*., 2022).

Damietta branch of the Nile River is often inhabited by many aquatic organisms, which affect and reflect its water characteristics. CGP produced by cyanobacteria is an important compound that has many biotechnological and industrial applications but it remains largely uninvestigated. Therefore, the present study aims to investigate the algal diversity and to focus on CGP production by cyanobacterium *Anabaena cylindrica* collected from the Damietta branch of the Nile River.

Materials and methods

Sampling site and algal collection

Surface water samples were collected at day time during November 2022 from 5 different sites along River-Nile Damietta Branch, Damietta Governorate, Egypt (latitude: 30° 10' 26" N, longitude: 31° 08' 22" E and latitude: 31° 31' 37" N, longitude: 31° 50' 41" E) (Aborahma *et al*., 2018). The samples were collected using a 90 mm mesh phytoplankton net and transported in an ice box to the laboratory until further analysis. The chosen sites are represented in the following map where Sites I, II, III, IV, and V are AL Zarqa, El Serw, Kafr Al Arab, Faraskur, and Al Adliya, respectively (Fig. 1). The filtered samples were fixed with Lugol's solution and 4% of formalin, enumerated using an inverted Olympus light microscope (Sharma, 2002), and sedimented according to Utermohle (1936). Examination of algal species was made using an EXACTA+OPTECH GmbH biological light microscope (Model B3) - Code K7161, Germany (Krammer and Lange-Bertalot, 1986; Botes, 2003).

Identification and classification of microalgae

Microalgae were isolated from water samples on BG-11 medium whose components have been purchased from Sigma–Aldrich, USA (Atıcı, 2020). Water samples were centrifuged, and the pellet was streaked on an agar plate containing sterilized BG-11 medium. It consisted per litre of the following; $NaNO_3$ (1.5 g), K_2HPO_4 (30 mg), $MgSO_4$. $7H_2O$ (75 mg), $CaCl_2$. H_2O (36 mg), Na_2CO_3 (20 mg), citric acid (6 mg), ferric ammonium citrate (6 mg), disodium magnesium EDTA (1 mg), and 1 ml of micronutrients solution that consisted of (per liter) H_3BO_3 (2.86 mg), $MnCl_2$. $4H_2O$ (1.81 mg), $ZnSO_4$. $7H_2O$ (220 mg), Na_2MoO_4. $2H_2O$ (390 mg),

[Downloaded from jifro.ir on 2026-11-24]

$CuSO_4$. $5H_2O$ (80 mg), and $CoCl_2$. $6H_2O$ (40 mg). The final pH of BG-11 medium after autoclaving was 7.1. Agar (1.0 - 1.5% w/v) was added to solidify the media. Each petri was incubated at 27°C under continuous fluorescent light illumination 45 $\mu E\ m^{-2}\ s^{-1}$ in a JSR-Growth Chamber 3-Side Illumination (model JSPC-960C2) for three weeks. The light/dark regime was adjusted to 12:12 h at a light intensity of 28 $\mu E\ m^{-2}\ s^{-1}$.

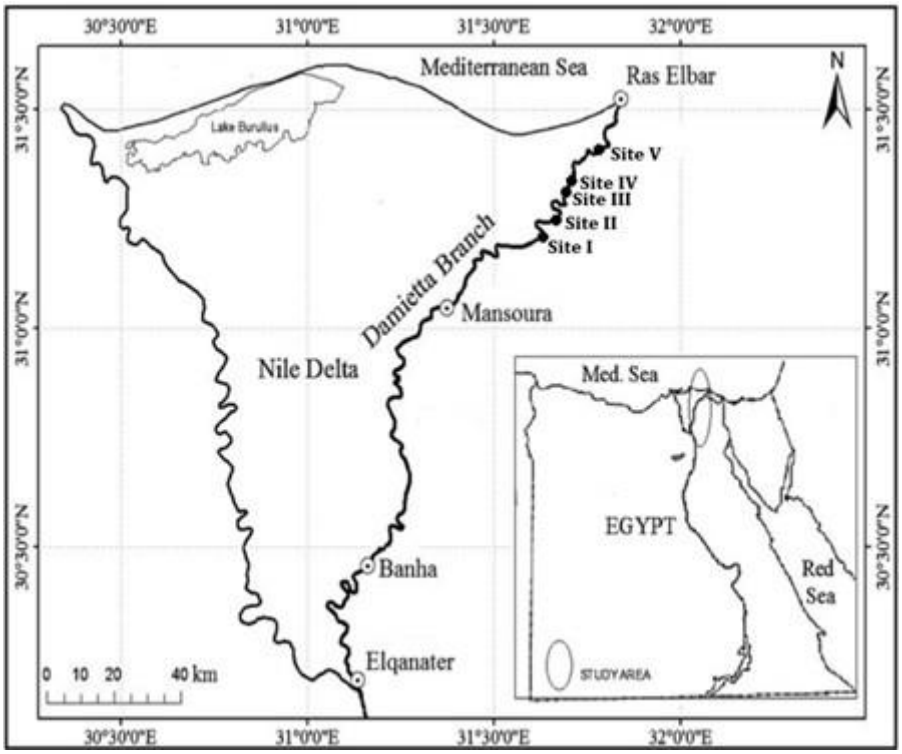

Figure 1 : A map showing the location of the study area.

Identification of the isolated cyanobacteria

Cyanobacterial colonies were repeatedly transferred to fresh plates to obtain uni-algal cultures. The growth on each plate was carefully examined using low (10X) and high-power (45X) objective lenses of the light microscope (Amer *et al*., 2013). Cyanobacterial species were identified based on their cellular morphology according to Khare *et al*. (2014) and Magana-Arachchi and Wanigatunge (2013), whereas the identification of Chlorophyta, Bacillariophyta, Dinophyta, and Euglenophyta was done according to Padisak *et al*. (2009) and Guiry (2013). Microphotographs were taken using a microscope-equipped digital camera (AMCAM camera). The cyanobacterial biomass was calculated in the total volume of algal cells according to Edler (1979) using an inverted microscope.

CGP quantification

CGP was quantified in the isolated cyanobacteria grown for 15 days under standard growth conditions according to the method described by Nausch *et al*. (2020). A known weight (30–35 mg) of freeze-dried algal samples including *Anabaena cylindrica* were homogenized with ceramic

[Downloaded from jifro.ir on 2026-11-24]

pills in a Precellys 24 homogeniser (VWR International GmbH, Erlangen, Germany) and incubated in 1 mL 50 mM Tris buffer (pH 8.0) for 30 min. After centrifugation, the pellet was resuspended in 1 mL of 0.1 M HCL and incubated for 1 hour. After another centrifugation step, 800 µL of the supernatant was used for CGP analysis. 1–10 µL of the sample was filled up with 0.1 M HCL to a final volume of 800 µL and 200 µL of 5x RotiQuant Bradford reagent (Carl Roth GmbH+Co. KG, Karlsruhe, Germany) was added. After 5 min incubation, samples were measured at 595 nm. A calibration curve was prepared with Bovine serum albumin.

Analysis of CGP from Anabaena cylindrica
Amino acid analysis

CGP was hydrolysed according to the method of Adebiyi *et al*. (2005) before determination of amino acids. HCl (6 N) was prepared by distilling a mixture of concentrated HCl and distilled water (1:1, v/v) and stored in screw-cap tubes at 4ºC. CGP was hydrolysed with 6N HCl at 110 ºC for 24 hours. Amino acid analysis was carried out using an LC 3000 Eppendorf / Biotronik amino acid analyser with column type H 125 x. The amino acid analysis was carried out in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Amino acids content was expressed as mg g^{-1} protein.

Ultrastructural analysis of CGP produced by Anabaena cylindrica

CGP produced by *Anabaena cylindrica* was analyzed by Transmission Electron Microscopy (TEM) according to the modified method of Parveen *et al.* (2013). Cells of *A. cylindrica* were centrifuged and mixed with a mixture of glutaraldehyde (2%), formaldehyde (2%), sucrose (0.18 M), picric acid (0.1% in 0.1 M), pH 7.4 Sorensen phosphate buffer for 1 hour. The cyanobacteria sample was washed with the previous buffer and fixed with an osmium tetroxide (1% osmium tetroxide in 0.1 M cacodylate buffer) for 1 hour. The sample was embedded in LX-112 Resin and sectioned at 0.5 µm using an ultra-microtome (Reichert-Jung Ultracut) with a diamond knife and transferred onto 150-mesh copper grids. The prepared sections were stained with 2% uranyl acetate / 50% ethanol for 15 min and washed 3 times in 50% distilled water / 50% ethanol and finally twice in distilled water. The copper grids were then dried and examined using a Jeol JSM-5300 transmission electron microscope (Faculty of Science, Alexandria University, El Shatby, Alexandria, Egypt).

Statistical analyses

Data were analysed using two-way ANOVA. Mean separation was performed using Duncan's multiple range test at $p<0.05$.

Results

In this study, different algal groups (Cyanophyta, Chlorophyta, Bacillariophyta, Dinophyta, and Euglenophyta) were identified at different sites of the River Nile, Damietta Branch. There are significant differences in algal classes and species numbers among sites. The present study reported a total of 26 algal species representing five algal groups as 9 Cyanophyta species, 5 Chlorophyta

[Downloaded from jifro.ir on 2026-11-24]

species, 7 Bacillariophyta species, 3 Dinophyta species, and 2 Euglenophyta species. Cyanobacteria was represented by *Anabaena anomala*, *A. cylindrical*, *A. oryzae*, *A. variabilis*, *Nostoc* sp., *Nostoc punctiforme*, *Oscillatoria obscura*, *Spirulina platensis*, and *Synechococcus* sp. Chlorophyta was represented by *Cosmarium* sp., *Chlorella vulgaris, Closterium accrosum*, *Mougeotia* sp., and *Pediastrum simplex*, while Bacillariophyta was represented by *Amphora ovalis*, *Fragilla* sp., *Synedra acus*, *S. ulna*, *Nitzschia filiformis*, *Pleurosigma elongatum*, and *Cyclotella meneghiniana*. Dinophyta species were *Exuviaella apora*, *Phormidium* sp., and *Peridinium penardiforme*. Euglenophyta was represented by *Euglena* sp., and *Phacus triqueter*.

As shown in Figure 2, Cyanophytes dominated at sites I, IV, and V meanwhile Chlorophytes and Bacillariophytes dominated at sites II and III, respectively.

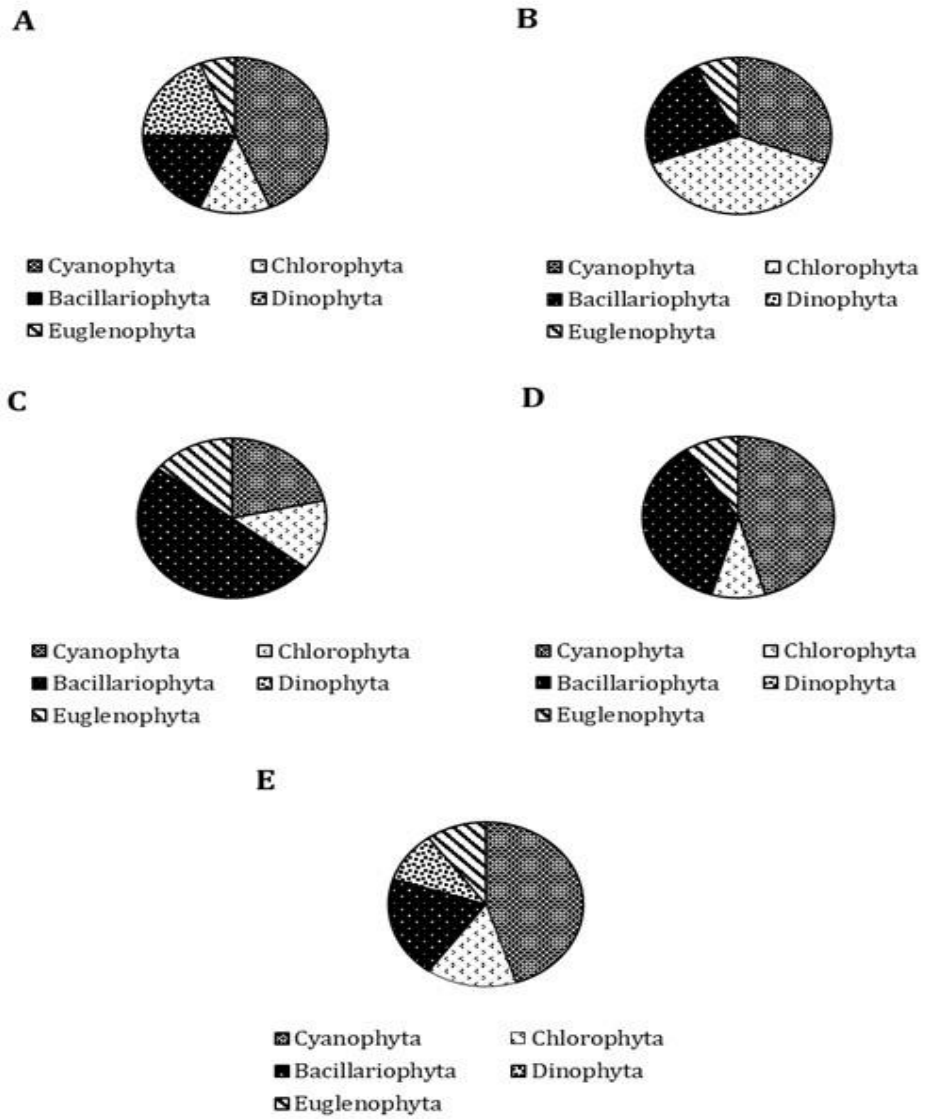

Figure 2: Variations in algal group percentages at different sites of the River-Nile Damietta Branch, Egypt (where A: site I, B: site II, C: site III, D: site IV, E: site V).

[Downloaded from jifro.ir on 2026-11-24]

Dinophyta was absent at sites II, III, and IV. Euglenophyta recorded the lowest number of species at different collection sites. A total of 16, 13, 14, 11, and 20 algal species were reported at sites I, II, III, IV, and V, respectively. A high number of Cyanophyta species (9) was recorded at site V, followed 7 species at site I. Whereas, the minimum number of Cyanophyta species (3) was recorded at site III.

Isolation of cyanobacteria

In the present study, water samples were collected from the River Nile Damietta Branch - Egypt during the time when the cyanobacteria are expected to be available on the photosynthetic area of the water body. Then, water samples were screened on standard algal BG-11 media. A total of nine cyanobacteria (*Anabaena anomala*, *A. cylindrical*, *A. oryzae*, *A. variabilis*, *Nostoc* sp., *Nostoc punctiforme*, *Oscillatoria obscura*, *Spirulina platensis*, and *Synechococcus* sp.) were isolated in pure form. Their morphological characteristics and photomicrographs are shown in Table 1 and Figure 3, respectively.

Table 1: Morphological characteristics of the identified cyanobacteria collected from five sites along River Nile Damietta Branch – Egypt.

Serial no.	Cyanobacterial species	Morphological characteristics
1	*Anabaena anomala*	Bent filaments, apical cell rounded, ellipsoidal akinete, barrel heterocyst.
2	*A. cylindrica*	Bluish-green filaments, Solitary straight trichome, No mucous sheath, Barrel-shaped cells (2.56-5.28 µm in wide, 4.59-10.42 µm in length), Barrel-shaped to cylindrical heterocyst, Cylindrical to barrel-shaped akinete
3	*A. oryzae*	Bluish green short straight filaments; colorless sheath, cylindrical vegetative cell (1.5-3.5 µm in wide, 3.0-5.0 µm in length), Trichomes with terminal oval heterocyst (2.5-3.5 µm in wide, 3.0-4.0 µm in length), ellipsoidal akinete.
4	*A. variabilis*	Greyish-green filaments, Bundle straight trichome, No mucous sheath, Cylindrical to barrel-shaped cells (2.29-5.23 µm in wide, 2.61-8.48 µm in length) with rounded apical cell, Ellipsoidal heterocyst (bigger than vegetative cells), Ellisoidal to long ellipsoidal akinete.
5	*Nostoc* sp.	Bluish-green heterocystous filaments, straight to slightly curved long trichome, gelatinous and oblong cells (3.73-6.59 µm wide, 4.34-11.38 µm in length), ellipsoidal heterocyst, spherical akinete.
6	*Nostoc punctiforme*	Dark blue-green heterocystous filaments, Bundle straight to amorphous trichome, Mucilaginous sheath present, Spherical to sub-spherical cells (2.6 – 5.8 µm in wide, 2.8-5.8 µm in length) with rounded apical cell, Spherical to sub-spherical heterocyst, No akinete
7	*Oscillatoria obscura*	Green to blue-green filaments, broad vegetative cell (3.5 - 4.0 µm in wide, 1.5-2.5µm in length), cross wall granulated, trichome ends rounded.
8	*Spirulina platensis*	Bluish-green multicellular unbranched long filaments, spiral-shaped trichomes, Septa present, spiral cell (7.0 – 8.0 µm in width, 4.0 - 5.5 µm in length).
9	*Synechococcus* sp.	Small single solitary cells, ellipsoidal cells (diameter 0.6 and 2.1 µm), no flagella or other organelles of motility.

[Downloaded from jifro.ir on 2026-11-24]

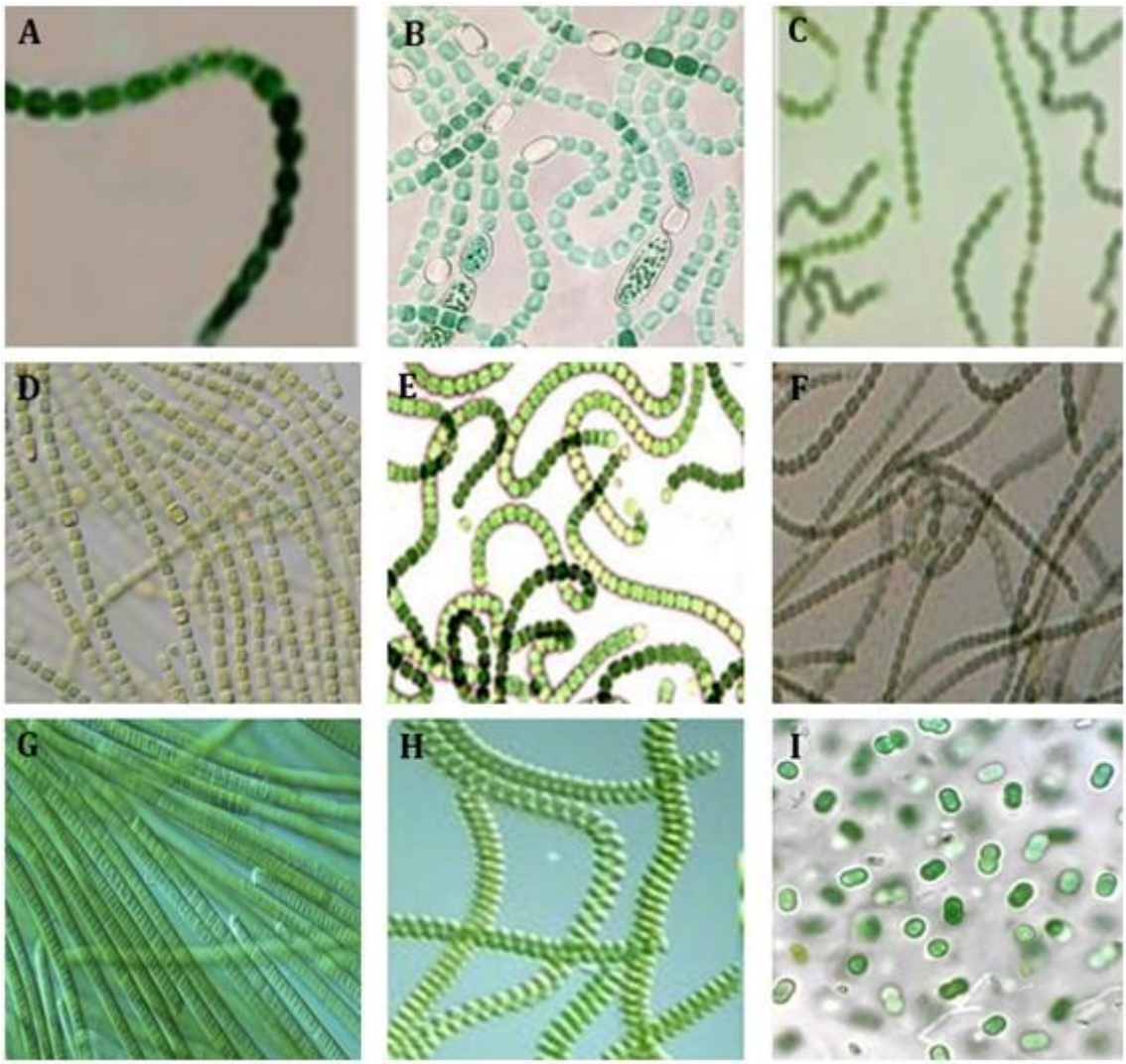

Figure 3: Photomicrographs of the identified cyanobacterial strains (20 µm) where A: *Anabaena anomala* ; B: *A. cylindrica*; C: *A. oryzae*; D: *A. variabilis*; E: *Nostoc* sp.; F: *Nostoc punctiforme*; G: *Oscillatoria obscura*; H: *Spirulina platensis*; I: *Synechococcus* sp. (Magnification of ×100).

Biomass and CGP production of different isolated cyanobacteria.

In the present study, the isolated cyanobacteria (nine species) were examined for their biomass and CGP production under similar incubation conditions. Variations in dry weight biomass of different cyanobacteria were illustrated. It is clear that algal freeze-dry weight biomass ranged from 0.25±0.01 g L^{-1} in *Oscillatoria obscura* to 0.80±0.02 g L^{-1} in *A. cylindrical*. The capability to produce CGP in cyanobacteria is widespread, whereas its amount varies between species. The results showed that CGP amounts ranged from 0.030 ±0.001 g L^{-1} in *Oscillatoria obscura* to 0.224±0.007 g L^{-1} in *A. cylindrica*. CGP in *A. cylindrical* reached 28% of cell dry weight (Table 2).

Amino acid analysis

In the present study, amino acid analysis of CGP produced by *A. cylindrica* showed that aspartic acid and arginine were the two dominating amino acids in the ratio of 1:1.3 (mass basis). Moreover, other amino acids are present in different amounts as shown in Table 3.

[Downloaded from jifro.ir on 2026-11-24]

Table 2: Variations in algal freeze-dry weight biomass (g L^{-1}) and CGP (g L^{-1}) produced by different cyanobacteria grown for 15 days under standard growth conditions. Each value was presented as Mean ± standard error, n = 3.

Cyanobacterial species	Algal freeze-dry weight biomass (g L^{-1})	CGP (g L^{-1})
Anabaena anomala	0.75 ± 0.05	0.165 ± 0.001
A. cylindrica	0.80 ± 0.02	0.224 ± 0.007
A. oryzae	0.75 ± 0.02	0.180 ± 0.005
A. variabilis	0.40 ± 0.01	0.072 ± 0.003
Nostoc sp.	0.30 ± 0.01	0.057 ± 0.001
Nostoc punctiforme	0.45 ± 0.02	0.054 ± 0.003
Oscillatoria obscura	0.25 ± 0.01	0.030± 0.001
Spirulina platensis	0.70 ± 0.05	0.091 ± 0.006
Synechococcus sp.	0.65 ± 0.04	0.130 ± 0.008

Table 3: Amino acids content of crude CGP from *A. cylindrica* grown for 15 days under standard growth conditions.

Amino acid	Concentration (mg g^{-1} protein)
L- Aspartic acid	49.9 ± 1.253
L- Glutamic acid	0.22 ± 0.003
Histidine	0.92 ± 0.016
Serine	0.10 ± 0.002
Arginine	57.6 ± 1.211
Glycine	0.01 ± 0.001
Threonine	0.81 ± 0.016
Tyrosine	0.20 ± 0.004
Alanine	0.11 ± 0.002
Valine	0.47 ± 0.008
Methionine	0.46 ± 0.008
Isoleucine	0.07 ± 0.001
Phenyl alanine	0.04 ± 0.001
Leucine	0.03 ± 0.001
Lysine	0.58 ± 0.013

Ultrastructural analysis of CGP produced by Anabaena cylindrica

SEM was used to examine CGP produced by *A. cylindrica* grown in standard BG-11 medium. SEM revealed that cells of *A. cylindrica* include intracellular inclusions that have all of the characteristics of CGP. As shown in Figure 4, CGP appears as dark and spherical with irregular parameters bodies. Moreover, CGP is not bounded by a membrane and electron-dense with a granular appearance.

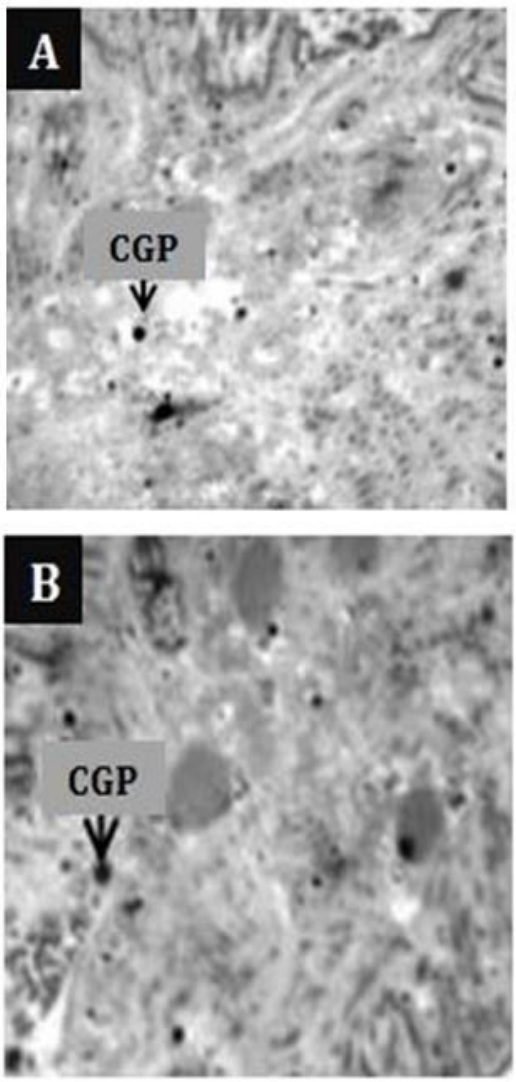

Figure 4: TEM micrographs of *A. cylindrica* collected from the River-Nile Damietta Branch showing CGP, Scale bars: a = 500 nm, b = 700 nm.

[Downloaded from jifro.ir on 2026-11-24]

Discussion

Microalgae are considered bioindicators of eutrophication and are effectively used to assess the quality of water (Ahmed, 2024). Some algae are known to release toxins that contaminate water while others are used to remove heavy metals from aquatic ecosystems (Kumar and Amit, 2012; Cheraghpour *et al*., 2020; Sarma *et al*., 2024).

Five algal groups; Cyanophyta, Chlorophyta, Bacillariophyta, Dinophyta, and Euglenophyta were recorded at different sites along the River Nile, Damietta Branch. It gives a brief overview of the quality of water from which *Anabaena cylindrica* was collected. Moreover, relationships between *A. cylindrica* and its associated species may be a factor affecting its CGP production.

The present study reveals the dominance of Cyanophyta at sites I, IV, and V. The dominance of Cyanophytes in most sites may be due to the availability of nutrients and other favorable environmental conditions such as light and temperature (Gao and Song, 2005). A number of Euglenophyta species was the lowest at all sites of the studied area. This result was in agreement with Elrefaey *et al.* (2017) who reported that Euglenophyta were scarcely recorded in the study area compared with other phytoplankton divisions, and were completely absent at many sites.

CGP is not considered as a nitrogen-rich reserve polymer only, it be easily degraded and utilized as the only carbon source for growth by some organisms. CGP is not the only compound whose amount changes with culture age. Canizales *et al*. (2023) reported that cyanophycin production is associated with the shift from an exponential growth phase to a stationary phase. In our results, the highest contents of CGP in *A. cylindrica* mean that it was already in the stationary phase. Compared to other cyanobacteria, *A. cylindrica* recorded high biomass (0.80±0.02 g L^{-1}) and CGP content (0.224±0.007 g L^{-1}) as presented in Table 2. CGP in *A. cylindrical* was reached to 28% of cell dry weight. This result in agreement with Elbahloul *et al.* (2005) who reported that cyanophycin can reach up to 40% of cell dry weight. Moreover, Hakkoum *et al.* (2025) reported that *A. cylindrica* contains other bioactive compounds, such as polysaccharides, proteins, indole acetic acid phytohormone, nitrogen, phosphorus, and potassium. Therefore, amongst the tested isolates, it has been decided to follow up CGP produced by *A. cylindrica* and to analyze it. Heterocysts of *A. cylindrica* express cyanophycinase to degrade cyanophycin into dipeptides, which are shuttled to vegetative cells that convert the dipeptides into free amino acids. These amino acids can be used as a feedstock in the production of a wide range of chemicals such as acrylonitrile, polyacrylic acid, 1,4-butanediamine, and urea (Burnat *et al*., 2014; Zou *et al*., 2022; Nawaz *et al*., 2024). Watzer *et al*. (2015) indicated that arginine availability is the main bottleneck of CGP structure. In the present study, amino acid analysis of CGP produced by *A. cylindrica* showed that aspartic acid and arginine were the two dominating amino acids in the ratio of 1:1.3 (mass basis). Simon (1973) also revealed the presence of arginine in purified CGP from the cyanobacterium *Anabaena cylindrica*. CGP produced by

[Downloaded from jifro.ir on 2026-11-24]

Synechococcus sp. and *E.coli* contained aspartic acid and arginine in the ratio 0.9:1 and 1.05:1, respectively (Hai *et al*., 1999). Moreover, Wingard *et al.* (2002) reported that CGP produced by *Synechococcus* collected from the Arabian Sea was composed of approximately equal molar quantities of aspartic acid and arginine with small amounts of other amino acids. This difference may be attributed to the different environmental conditions such as temperature (Chen *et al*., 2022).

Conclusion

Cyanophycin granule polypeptide (CGP) is a storage material for nitrogen, carbon, and energy that could be used in various industrial and biotechnological applications. This study is one of the first studies that focuses on CGP production by some cyanobacteria collected from the Nile River Delta, Damietta branch. Our results revealed their capability to produce CGP, whereas its amount and amino acids ratio vary between species. By following up CGP production by *Anabaena cylindrica,* SEM showed that CGP is dark and spherical with irregularly parameters bodies. Further studies are needed to study factors affecting CGP production and to optimise it for the widespread utility of this natural biopolymer.

Conflicts of interest

The authors report there are no competing interests to declare

References

Aborahma, R.A.B, Mahmod, W.E. and Fath, H.E.B., 2018. Morphometric Temporal Change Analysis for the River Nile Forced Bends using RS/GIS Techniques: Case Study of Damietta Branch of the Nile River, Egypt. *Applied Environmental Research*, 40(**1**), 75-86.

Adebiyi, A.P., Jin, D., Ogawa, T. and Muramoto, K., 2005. Acid hydrolysis of protein in a microcapillary tube for the recovery of tryptophan. *Bioscience, Biotechnology, and Biochemistry*, 69(**1**), 255-257. DOI:10.1271/bbb.69.255

Ahmed, S., 2024. Algae as Bioindicators of Aquatic Pollution. *Journal of Advanced Research in Alternative Energy, Environment and Ecology,* 11(**1**), 1-17.

Amer, R., Shehawy, R., El-Dien, S.S., Abu Serie, M. and Shaker, K., 2013. Isolation and Characterization of Cyanobacterial Community Including a Microcystin-Producing *Nostoc* sp. Strain in the Nile River, Egypt. *Advances in Microbiology*, 3(**6A**), 38-46. DOI:10.4236/aim.2013.36A005

Aravind, J., Saranya, T., Sudha, G. and Kanmani, P.A., 2016. Mini Review on Cyanophycin: Production, Analysis and Its Applications. In: Prashanthi M., Sundaram R. (eds) Integrated Waste Management in India. Environmental Science and Engineering. Springer, Cham. pp 49–58. DOI:10.1007/978-3-319-27228-3_5

Atıcı, T., 2020. Production and collection of microalgae isolated from freshwater reserves in Central Anatolia, Turkey. *Türler ve Habitatlar*, 1(**1**), 14-21.

Botes, L., 2003. Phytoplankton Identification Catalogue: Saldanha Bay, South Africa, GloBallast Monograph Series No. 7. IMO, London, UK. pp 88.

Burnat, M., Herrero, A. and Flores, E., 2014. Compartmentalized cyanophycin

[Downloaded from jifro.ir on 2026-11-24]

metabolism in the diazotrophic filaments of a heterocyst-forming cyanobacterium. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 3823-3828. DOI:10.1073/pnas.1318564111

Canizales, S., Hilderink, L., Apkhazava, M., Temmink, H., Wijffels, R.H. and Janssen, M. 2023. Cyanophycin production in turbidostat cultivation of cyanobacteria under phosphorus limitation onsynthetic urine media. *Algal Research*, 103143. DOI:10.1016/j.algal.2023.103143

Chen, X., Wang, J., Wang, Q., Yuan, T., Lei, Z., Zhang, Z., Shimizu, K. and Lee, D.J., 2022. Simultaneous recovery of phosphorus and alginate-like exopolysaccharides from two types of aerobic granular sludge. *Bioresource Technology*, 346, 126411. DOI:10.1016/j.biortech.2021.126411

Cheraghpour, J., Etemadifar, Z., Afsharzadeh, S. and Bahador, N., 2020. Assessment of bioremediation potential of *Microcystis aeruginosa* for removal of cadmium and lead ions from aqueous matrices. *Iranian Journal of Fisheries Sciences*, 19(**4**), 1994-2009. DOI:10.22092/ijfs.2018.119887.

Du, J., Li, L. and Zhou, S., 2019. Microbial production of cyanophycin: From enzymes to biopolymers. *Biotechnology Advances*, 37(**7**), 107400. DOI:10.1016/j.biotechadv.2019.05.006

Edler, L., 1979. Recommendations on methods for marine biological studies in the Baltic sea: phytoplankton and chlorophyll. Baltic Marine Biologists, University of Stockholm, Lund, Sweden. 5, 1-38.

Elbahloul, Y., Krehenbrink, M., Reichelt, R. and Steinbüchel, A., 2005. Physiological conditions conducive to high cyanophycin content in biomass of *Acinetobacter calcoaceticus* strain ADP1. *Applied and Environmental Microbiology*, 71, 858-866. DOI:10.1128/AEM.71.2.858-866.2005

Elrefaey, A.A., El-Gamal, A.D. and El-Belely, E.F., 2017. Seasonal dynamics of phytoplanktonic groups in Damietta branch, River Nile, Egypt. *Egyptian Journal of Phycology*, 18, 1-20

Flores, E., Arévalo, S. and Burnat, M., 2019. Cyanophycin and arginine metabolism in cyanobacteria. *Algal Research*, 42, 101577. DOI:10.1016/j.algal.2019.101577

Frommeyer, M., Wiefel, L. and Steinbüchel, A., 2016. Features of the biotechnologically relevant polyamide family "cyanophycins" and their biosynthesis in prokaryotes and eukaryotes. *Critical Reviews in Biotechnology*, 36(**1**), 153-164. DOI:10.3109/07388551.2014.946467

Füser, G. and Steinbüchel, A., 2007. Analysis of genome sequences for genes of cyanophycin metabolism: identifying putative cyanophycin metabolizing prokaryotes. *Macromolecular Bioscience*, 7(**3**), 278-296. DOI:10.1002/mabi.200600207

Gao, X. and Song, J., 2005. Phytoplankton distributions and their relationship with the environment in the Changjiang Estuary, China. *Marine Pollution Bulletin*, 50, 327-335.

Guiry, M.D., 2013. Algae Base. World-wide electronic publication, National University of Ireland, Galway.

[Downloaded from jifro.ir on 2026-11-24]

Hai, T., Oppermann-Sanio, F.B. and Steinbüchel, A., 1999. Purification and characterization of cyanophycin and cyanophycin synthetase from the thermophilic *Synechococcus* sp. MA19. *FEMS Microbiology Letters*, 181(**2**), 229-236. DOI:10.1111/j.1574-6968.1999.tb08849.x

Hakkoum, Z., Minaoui, F., Chabili, A., Douma, M., Mouhri, K. and Loudiki, M., 2025. Biofertilizing effect of soil cyanobacterium *Anabaena cylindrica*–based formulations on wheat growth, physiology, and soil fertility. *Agriculture,* 15(**2**), 189. DOI:10.3390/agriculture15020189

Khare, P., Singh, A., Prabha, C. and Kumari, S., 2014. Study of Cyanobacterial Biodiversity in Rice Fields of Central Bihar. 3rd World Conference on Applied Sciences, Engineering & Technology, Kathmandu, Nepal.

Krammer, K. and Lange-Bertalot, H., 1986. Bacillariophyceae 1. Teil:Naviculaceae. Naviculaceae In: Ettl, H., J. Gerloff, H. Heynig and D. Mollenhauer (eds.) Süsswasserflora von Mitteleuropa, Bd. 2, Gustav Fischer Verlag, Jena, Germany. pp. 876

Krehenbrink, M., Oppermann-Sanio, F.B. and Steinbüchel, A., 2002. Evaluation of non-cyanobacterial genome sequences for occurrence of genes encoding proteins homologous to cyanophycin synthetase and cloning of an active cyanophycin synthetase from *Acinetobacter* sp. strain DSM 587. *Archives of Microbiology*, 177(**5**), 371-380. DOI:10.1007/s00203-001-0396-9

Kumar, J. and Amit, P., 2012. Water quality monitoring of Ken river of Banda District Uttar Pradesh, India. *Elixir Pollution*, 42, 6360-6364.

Magana-Arachchi, D. and Wanigatunge, R., 2013. First report of genus Chroococcidiopsis (cyanobacteria) from Sri Lanka: a potential threat to human health. *Journal of the National Science Foundation of Sri Lanka*, 41(**1**), 65-68. DOI:10.4038/jnsfsr.v41i1.5334

Nausch, H., Dorn, M., Frolov, A., Hoedtke, S., Wolf, P. and Broer, I., 2020. Direct Delivery of Health Promoting β-Asp-Arg Dipeptides via Stable Co-expression of Cyanophycin and the Cyanophycinase CphE241 in Tobacco Plants. *Frontiers in Plant Science*, 11, 842. DOI:10.3389/fpls.2020.00842

Nawaz, T., Gu, L., Gibbons, J., Hu, Z. and Zhou, R., 2024. Bridging Nature and Engineering: Protein-Derived Materials for Bio-Inspired Applications. *Biomimetics (Basel)*, 9(**6**), 373.

Padisak, J., Crossetti, L.O. and Naselli-Flores, L., 2009. Use and Misuse in the Application of the Phytoplankton Functional Classification: A Critical Review with Updates. *Hyrdrobiologia*, 621, 1-19.

Parveen, B., Ravet, V., Djediat, C., Mary, I., Quiblier, C., Debroas, D. and Humbert, J.F., 2013. Bacterial communities associated with Microcystiscolonies differ from free-living communities living in the same ecosystem. *Environmental Microbiology Reports*, 5(**5**), 716-724.

Sarma, U., Hoque, M., Thekkangil, A., Venkatarayappa, N. and Rajagopal,

[Downloaded from jifro.ir on 2026-11-24]

S., 2024. Microalgae in removing heavy metals from wastewater – An advanced green technology for urban wastewater treatment. *Journal of Hazardous Materials Advances*, 15, 100444. DOI:10.1016/j.hazadv.2024.100444

Sharma, P.D., 2002. Practical Manual: Ecology and Environmental. Rastogi Publications, Meerut, India pp 569-572.

Sharon, I. and Schmeing, T.M., 2023. Bioinformatics of cyanophycin metabolism genes and characterization of promiscuous isoaspartyl dipeptidases that catalyze the final step of cyanophycin degradation. *Scientific Reports*, 13, 8314. DOI:10.1038/s41598-023-34587-w

Simon, R.D., 1973. Measurement of cyanophycin granule polypeptide contained in blue-green alga *Anabaena cylindrica*. *Journal of Bacteriology*, 114, 1213-1216. DOI: 10.1128/jb.114.3.1213-1216.1973

Utermohle, H., 1936. Quantitative methods zur untersuchugn des Nannoplanktons. In: Abberhalden's Handbuck der Biologischen. Arbietsmethoden, Berlin, 2, 1879-1937 (In: German).

Watzer, B., Engelbrecht, A., Hauf, W., Stahl, M., Maldener, I. and Forchhammer, K., 2015. Metabolic pathway engineering using the central signal processor PII. *Microbial Cell Factories*, 14, 192. DOI:10.1186/s12934-015-0384-4

Watzer, B. and Forchhammer, K., 2018. Cyanophycin synthesis optimizes nitrogen utilization in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. *Applied and Environmental Microbiology*, 84(20), e01298-18. DOI:10.1128/AEM.01298-18

Wingard, L.L., Miller, S.R., Sellker, J.M.L., Stenn, E., Allen, M.M. and Wood, A.M., 2002. Cyanophycin Production in a Phycoerythrin-Containing Marine *Synechococcus* Strain of Unusual Phylogenetic Affinity. *Applied and Environmental Microbiology*, 68(**4**), 1772-1777. DOI:10.1128/AEM.68.4.1772-1777.2002

Ziegler, K., Deutzmann, R. and Lockau, W., 2002. Cyanophycin synthetase-like enzymes of non-cyanobacterial eubacteria: characterization of the polymer produced by a recombinant synthetase of *Desulfitobacterium hafniense*. *Zeitschrift für Naturforschung C*, 57,522-529. DOI:10.1515/znc-2002-5-621

Zimba, P.V., Huang, I-S., Foley, J.E. and Linton, E.W., 2017. Identification of a new-to-science cyanobacterium, Toxifilum mysidocidagen. nov. & sp. nov. (Cyanobacteria, Cyanophyceae). *Journal of Phycology*, 53, 188-197. DOI:10.1111/jpy.12490

Zou, K., Huang, Y., Feng, B., Qing, T., Zhang, P. and Chen, Y., 2022. Cyanophycin Granule Polypeptide: a Neglected High Value-Added Biopolymer, Synthesized in Activated Sludge on a Large Scale. *Applied and Environmental Microbiology*, 88(**14**), e0074222. DOI:10.1128/aem.00742-22

[Downloaded from jifro.ir on 2026-11-24]