

## A Biosystematics Study of *Microcystis* (Cyanobacteria), A Bloom-Forming Cyanobacterium from Aras Reservoir (North-West Iran)

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### Abstract

Today, harmful cyanobacterial bloom is considered as a main problem in freshwater ecosystems all over the world. The *Microcystis* is an important genus in cyanobacteria composed of about 23 species. This genus forms various shaped colonies including spherical or oblong cells which whole colony is surrounded by a mucilage sheath. Many populations and species of the genus produce severe blooms and often toxins in eutrophic freshwaters. The genus *Microcystis* has been defined based on morphological, morphometrical and genetical criteria, however, its classification under the genus level is still indistinct and presence of traditional morphological species within the genus is doubtful. Therefore, determining of the natural diversity under the genus level in *Microcystis* has a crucial importance. The purpose of this study was to determine the species of *Microcystis* in Aras reservoir. The samples were collected from 6 sampling sites on 18 August 2013. Morphological and morphometrical studies of *Microcystis* was carried out by Nikon 50i microscope equipped with Nikon DS-Fi camera and Nikon monitor with length measurement software. Principal Component Analysis (PCA) and two-way Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by PAleontological STatistics (PAST) version 3.04

were performed to determine biosystematics variations between *Microcystis* populations. The results indicated that *Microcystis* from Aras reservoir was composed of two distinct groups based on the shape and size of cells, colony form, presence and absence of gelatinous sheath around the colonies, etc). These two groups may be considered as two morphospecies of *M. botrys* which are separated due to different ecological factors.

**Keywords:** *Microcystis*, Morphology, Morphometry, Aras reservoir.

### Introduction

Cyanobacteria (blue-green algae) are the most ancient photoautotrophic living organisms on the earth. They changed severely the biosphere by producing oxygen which resulted in evolution of higher plants and animals on the land during Precambrian era (Schopf, 2000).

Today, cyanobacteria indicated ecophysiological strategies which have permitted them to benefit from anthropogenic changes, particularly high eutrophication of water ecosystems. One obvious ecological problem in aquatic systems is the overgrowth of some cyanobacteria species that is accompanied by oxygen level drop and sometimes releasing of toxins and fish death. Bacterial de-

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composition of dead cyanobacteria may result in oxygen depletion and fish death (Chorus and Bartram, 1999).

Cyanobacterial harmful bloom threatens the stability and integration of aquatic ecosystems with various applications such as drinking, irrigation, fisheries and entertainment.

*Microcystis* is one of the most important bloom-forming cyanobacteria genus in aquatic systems. Various populations of the genus have been distributed in freshwater ecosystems and several species can produce toxins (Chorus and Bartram, 1999; Komárek and Komárková, 2002). This genus is characterized by irregular colonies with spherical cells and colorless gelatinous sheath. *Microcystis* colonies range from microscopic to macroscopic sizes and live as plankton in freshwater; they form various morphological stages during vegetative cycle of the genus (Reynolds et al., 1981; Bittencourt and Oliveira, 2000). Cells of *Microcystis* were located in the colorless and often planktonic mucilage and there is no mucilage in the periphery of single cells. They have often very densely agglomerated. Cell division occurs in all directions, but in oblong cell division usually takes place in latitudinal direction. Gas vacuoles can be observed in cells (Desikachary, 1959).

In many cases *Microcystis* produce large colonies which are visible with naked eyes. These colonies float on the surface water and may occupy a wide area of an aquatic ecosystem.

The genus *Microcystis* has been defined genetically (16S rRNA molecular sequences), but its classification under the genus level is still obscure and the presence of classical morphologic species in the genus is doubtful (Komárek and Komárková, 2002). Therefore, determining of the natural di-

versity under the genus level in *Microcystis* has a crucial importance. This study was performed to determine the species of the *Microcystis* in Aras reservoir by morphological and morphometrical criteria as important methods in algal biosystematics.

## Materials and Methods

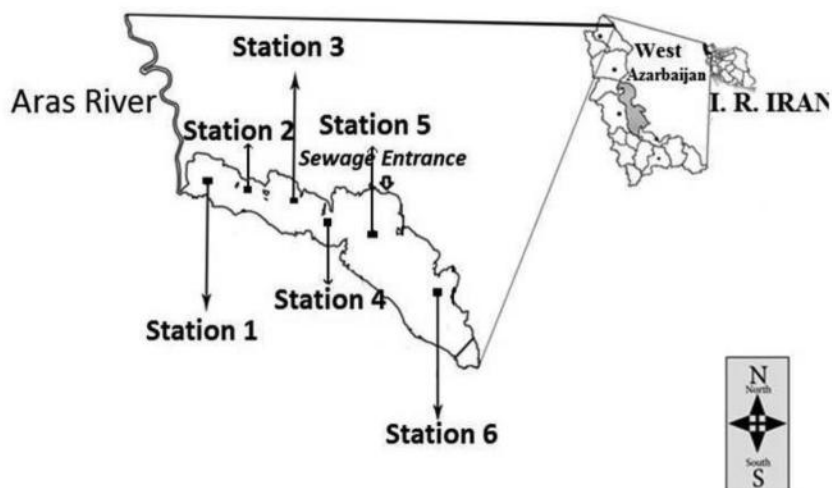
Aras Reservoir (maximum capacity = 1350 × 10<sup>6</sup> m<sup>3</sup>,

**Table 1.** Morphometric and hydrologic characteristics of Aras reservoir

Characteristics	Value
Basin surface area	102000 km <sup>2</sup>
Maximum Capacity	1350 × 10 <sup>6</sup> m <sup>3</sup>
Usable Capacity	1150 × 10 <sup>6</sup>
Maximum Area	153 km <sup>2</sup>
Maximum Depth	27.5 m
Mean Depth	20 m
Crown Height	36 m

maximum area = 153 km<sup>2</sup>, mean depth = 20 m, Crown height = 36 m, Table 1) is a significant water resource constructed on this river in 1970 for the purpose of hydropower production (Aliyev et al., 2013). The reservoir also provides irrigation water for about 400000 ha of arable lands in Iran and Azerbaijan (Filipuzzi and Faramarzi, 2007). In addition, it plays an important economic role as a large fisheries resource and drinking water supply in the region. Sampling for biosystematics study of the genus *Microcystis* was performed from 6 sampling sites along the Aras reservoir main body (Fig. 1). In each station two samples were collected: one from surface, second sample from 1 m depth. Sampling from two different depths was performed to determine probable differences of biosystematics characterizes of the genus between various depths.

The samples were filtered through a 150μ mesh



**Fig. 1.** Location of study area and sampling sites on Aras reservoir for Biosystematics study of *Microcystis*

size filter in order to eliminate zooplankton. About 100L water was filtered in each sampling site. These samples were labeled and transferred to the laboratory in cool boxes.

*Microcystis* samples were analyzed based on morphological characteristics of classical species. Morphological and morphometrical studies of *Microcystis* was carried out by Nikon 50i microscope equipped with Nikon DS-Fi 1 camera and Nikon monitor with length measurement software.

The following characteristics were used to determine morpho-species of *Microcystis*:

- Colony forms (with holes, flattened, lobed or composed of dense sub colonies)
- Mucilage structure (width of mucilaginous margin around colonies, delimited or diffuse)
- Cells diameter (minimum, maximum, mean)
- Density and organization of cells within the colonies (three dimensional, in rows, very densely and irregularly arranged or constantly scarcely)
- Life cycle (presence or absence of particular stages, dormant stages, morphological variability limits)

The size and shape of colonies were analyzed and the results were recorded.

Seventy cells as random samples were analyzed biometrically. Thirty five cells from each depth were evaluated from cell shape and diameter point of view. The shape of cells were assessed and determined carefully.

Presence/absence of mucilaginous sheath around the colonies were determined by microscope. If present, its width was measured (Fig. 2).

Besides, some *Microcystis* samples were sent to Prof. Komárek for confirmation.

UPGMA clustering method was used to evaluate relationship between morphological characters between *Microcystis* samples from different stations and depths. PCA was used to reduce data to limited taxa which were statistically significant and their distribution pattern produced the total variance in data (Beaver et al., 2013).

PCA and two way clustering were both performed with Paleontological statistics (PAST) version 3.04 software (Hammer et al., 2001).

## Result

Morphological and morphometrical characteristics of *Microcystis* samples from Aras reservoir are shown in table 2.

Both UPGMA and PCA (Figs 3 and 4) recovered four major groups: Group 1 included all specimens of sampling sites 2 and 3 (surface and deep); Group 2 specimens of sampling site 1 (surface and deep), sampling site 4 (deep) and 5 (surface); group 3 specimens of sampling sites 4 (surface), 5

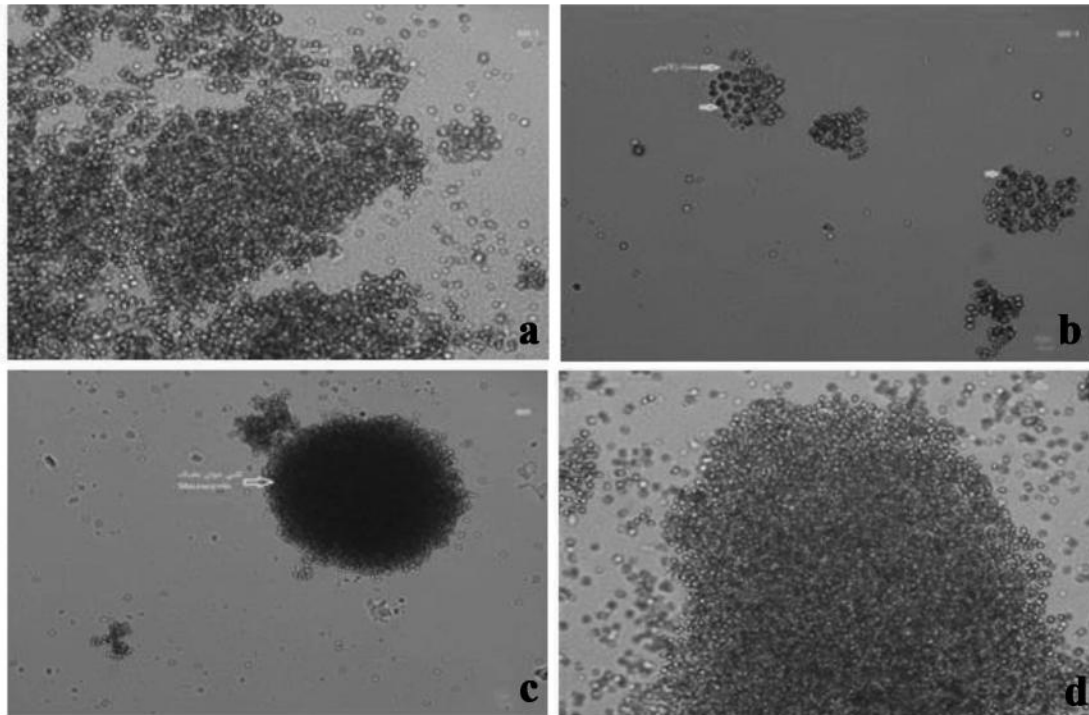
(deep) and 6 (surface); finally group 4 was formed from just deep specimen of sampling site 6 which was separated from all other samples significantly. However, there are some insignificant variations between two analyses.

**Table 2.** Morphological and morphometrical characteristics of the genus *Microcystis* in sampling stations of Aras reservoir .

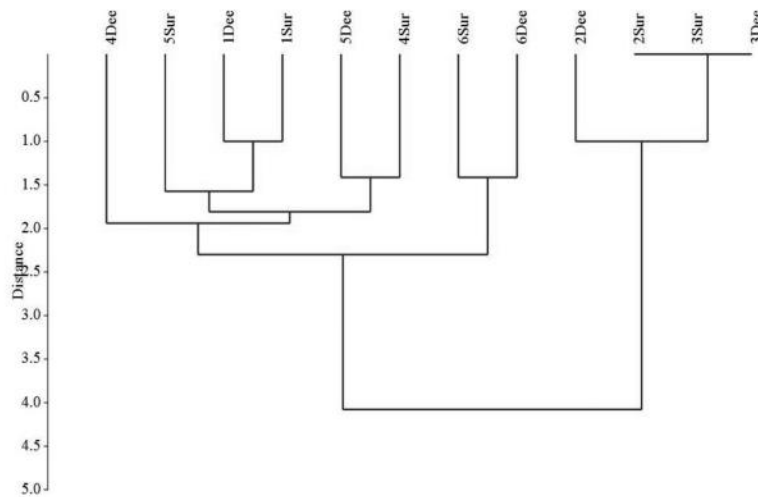
station	depth	Colony form	Cells shape	Cell diameter (μ)	Cell diameter (μ) (min)	Cell diameter (μ) (max)	Cells org in the colony	Mucilage sheath	Sheath width (μ)	Sheath form
1	Surface	Irregular-	Spheric	4<	2.89	6.15	Densely	Obvious	2-3	Radial
	deep	Irregular-	spherical	<4	3.66	6.56	Densely	obviou	2-3	Radial
2	Surface	With small	Spheric	4>	2.16	5.69	Densely	Indistinc	—	—
	deep	With	spherical	4>	2.44	4.99	Densely	indistic	—	—
3	Surface	With small	Spheric	4>	2.41	5.32	Densely	Indistinc	—	—
	deep	With	spherical	4>	2.82	5.74	Densely	indistic	—	—
4	Surface	Irregular-	Spheric	4<	3.66	6.98	Densely	distinct	2-3	No
	deep	Irregular-	Spherical	4<	3.24	6.15	Dense-	indistin	2-3	With
5	Surface	Irregular-	Spheric	4<	2.82	5.32	Densely	distinct	2-3	With
	deep	Irregular-	Spherical	4<	3.25	5.32	Densely	distinct	2-3.5	Without
6	Surface	Irregular-	Spheric	4<	4.90	6.40	Densely	distinct	2-3.5	Without
	deep	Irregular- without hole	spherical	4<	7.82	4.07	Densely organized	distinct	2.5-3.7	With radial structure

**Table 3.** Numerical status of morphological and morphometrical characteristics of the genus *Microcystis* in sampling stations of Aras reservoir

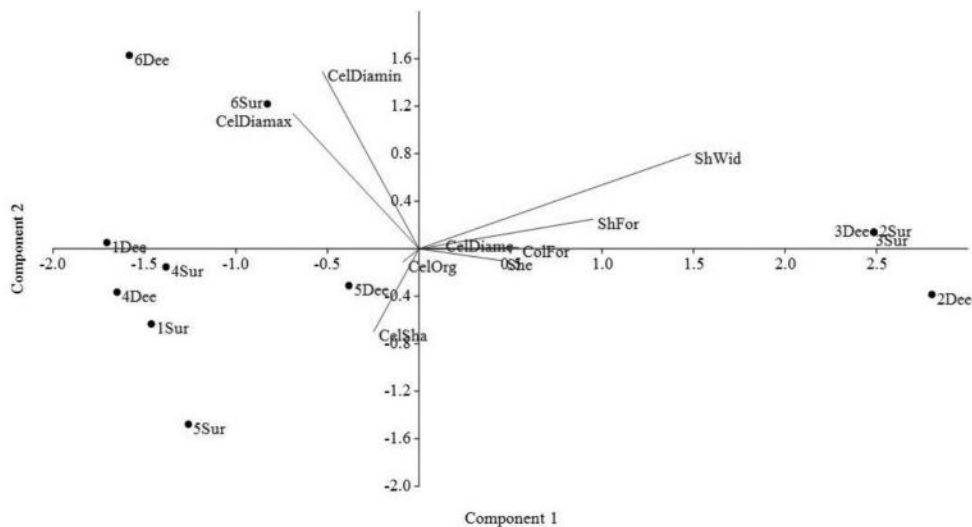
Station	Depth(surface=1,deep=2)	Colony form (irregular-without holes=1, with small holes=2)	Cell shape (spherical=1, spherical-ellipsoid=2)	Cell diameter (μ) (4<=1, 4>=2)	Cell diameter(μ) (min) (3>=1, 4>=2, 5>=3)	Cell diameter(μ) (max)(4<=1, 5<=2, 6<=3, 7<=4)	Cells organization in the colony (dense=1, discrete=2)	Sheath(distinct=1, indistinct=2)	Sheath width(μ) (2-3=1, 2-3.5=2, 2.5-3.7=3, indistinct=4)	Sheath form (with radial structure=1, without radial structure=2, indistinct=3)
1	1	1	1	1	1	3	1	1	1	1
	2	1	1	1	2	3	1	1	1	1
2	1	2	1	2	1	2	1	2	4	3
	2	2	1	2	1	1	1	2	4	3
3	1	2	1	2	1	2	1	2	4	3
	2	2	1	2	1	2	1	2	4	3
4	1	1	2	1	2	3	1	1	1	2
	2	1	2	1	2	3	2	2	1	1
5	1	1	2	1	1	2	1	1	1	1
	2	1	2	1	2	2	1	1	2	2
6	1	1	1	1	3	3	1	1	2	2
	2	1	1	1	3	4	1	1	2	1



**Fig. 2.** Biometrical analysis of *Microcystis* samples; b. Young colonies of *Microcys* (note the gelatinous sheath around the colony); c. spherical colony of *Microcystis*; d. Part of a *Microcystis* mature colony collected from Aras reservoir.



**Fig. 3.** Two way UPGMA analysis of sampling sites in Aras reservoir for biosystematics study of the genus *Microcystis*. Numbers refer to sampling sites and (Sur = Surface, Dee = Deep).



**Fig. 4.** Biplot of Principal Component Analysis (PCA) of sampling sites in Aras reservoir for biosystematics study of the genus *Microcystis*. Numbers refer to sampling sites; (Sur= Surface, Dee= Deep). Abbreviations for morphological characteristics: CelSha= Cell Shape; CelOrg= Cell Organization; CelDiam= Cell Diameter; CelDiamin= Cell Diameter in minimum; CelDiamax= Cell Diameter in maximum; CoFor= colony Form; ShFor= Sheath Form; ShWid= Sheath Width

**Table 4.** Factor loadings of all components of PCA

Axis	Eigenvalue	%
1	45.656	52.761
2	17.772	20.538
3	7.71	8.91
4	3.4393	3.9746
5	2.0489	2.3678
6	1.5343	1.7731
7	0.37866	0.4376
8	0.18863	0.21799
9	0.00095	0.0011
10	-2.0696E-15	-2.391E-15
11	-0.53575	-0.61913

For instance, in clustering analysis, specimens of sampling site 4 (surface) formed a cluster with specimens of sampling site 5 (deep), whereas in PCA these two samples were considerably distinct. As shown in Table 4, two first components of PCA accounted for more than 73% of total variation between sampling sites. This indicated that the main patterns of variation were captured by two first axis of PCA in the original variables. Crow (1923) studied some water samples of Cylon freshwater which

was collected by Prof. Fritsch in (1903). He showed that the members of the genus *Microcystis* were probably an important part of the phytoplankton in these ecosystems. *Microcystis* species were dominant in many of samples and produced blooms. Geilert defined 23 species from *Microcystis* for first time in (1932). These included both species with vesicles and without vesicles. Then, Stainer et al (1971) indicated that only cells that have gas vesicles should be considered of the genus *Microcystis*.

Holt et al (1994) introduced the gas vesicles as an identification criterion for this genus. Therefore, *Microcystis* species were characterized by gas vesicles, spherical cells, and tendency to form colonies and anamorphous mucilage or sheath around the colonies (Holt et al., 1994). Cyanobacteria had been classified as eukaryotic algae, before studies conducted by Gibbons and Murray (1978) and Stainer et al (1978). When cyanobacteria were ruled under the bacteriological code, their collection as monoculture strains instead of herbarium samples which were necessary for plant taxonomy was possible. With diagnose of cyanobacteria as prokaryotic organisms, their evolution were studied with regard to other bacteria and photosynthetic organisms (Doolittle, 1982).

Toxic cyanobacteria density and diversity with emphasizes on the genus *Microcystis* in four recreational reservoirs in Thailand was studied by Somdee et al. (2013). The most important toxic cyanobacteria in these reservoirs were: *Cylindrospermopsis* sp., *Microcystis* sp., *Oscillatoria* sp. and *Pseudoanabaena* sp. Morphological and 16S rRNA gene analysis of *Microcystis* in these reservoirs showed that it was closely related to *M. aeruginosa*. Microcystin is the most important water soluble toxin in drinking water.

As shown in Figures 3 and 4, we may determine two groups or morphospecies of *Microcystis* in Aras reservoir. Samples from stations 1, 2, 3 and 6 are located in one group, while samples from stations 4 and 5 form the second group. However, this separation is not completely distinct and some characteristics overlap with each other.

One- way ANOVA indicated that there was not

a significant difference between samples taken from surface and depth of each station. Aras reservoir sampling stations can be divided to two distinct groups according to *Microcystis* cells shape: first included stations 1, 2, 3 and 6 which had completely spherical cells, second group had spherical and ellipsoid cell shapes which included stations 4 and 5. These two groups can be divided into two relatively distinct groups according to other morphological characteristics such as colony form, cell size and cells arrangement within the colonies. Regarding the above sentences and view of Prof. Komárek, the presence of *M. botrys* in the reservoir was confirmed, however, more studies is necessary to know whether there was another species of *Microcystis* (as second morphological group) in the Aras reservoir. Regarding the various ecological conditions in sampling sites of Aras reservoir, the larger size and different cells and colony forms in *Microcystis* in stations 1, 2 and 3 may be linked to high nutrients input and in station 6 to untreated sewage of Nakhjavan city enters into the reservoir in this site (Fig. 1). If this is the case, it may be concluded that there is only *M. botrys* in Aras reservoir which different morphospecies of it has been created due to ecological variations in different sites.

Morphological characteristics can provide important data about living organisms; however, it is necessary to remember that some organisms cannot be classified just based on morphological characteristics. Observation of the genus *Microcystis* morphospecies under culture condition indicates that their separation is ambiguous or impossible (Otsuko et al., 2000). Otsuko et al. (2000) concluded that the genus *Microcystis*

based on morphological characteristics is not valuable and a strain may have different colony forms. Thus, today, authors use an integrated of morphological and molecular approaches for precise description of *Microcystis* species. Principal component analysis indicated that spatial variations were responsible for the main morphologic and morphometrical variations in the structure of the *Microcystis* community during the present study, so that we may distinguish three major sections across the Aras reservoir main body: the first is the entrance of the reservoir (site 1), the second are sampling sites 2 and 3 and the third comprise the part of the reservoir that is affected by Nakhjoan waste water on the north of the reservoir (sites 5 and 6). In this study, two distinct groups of *Microcystis* was distinguished using UPGMA and PCA methods, based on morphological and morphometrical (shape and size of cells, colony forms, presence or absence of gelatinous sheath around the colonies) characteristics. These two groups are regarded as two morphospecies of *M. botrys* which have been formed by various ecological parameters.

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