 SOME NEW RECORDS OF OSCILLATORIAN CYANOPHYTA FROM PADDY FIELDS OF GOLESTEN PROVINCE

R. Siahbalaei, S. Afsharzadeh, Sh. Shokravi & Sh. Nekouei

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Cyanophyta of some paddy fields of Golestan Province (North of Iran and near Caspian Sea) have been studied between autumn 2006 and summer 2007. Lyngbya perelegans, L. rubida, L. laxespiralis, L. sordida, L. putealis, L. dendrobia, L. scotii, L. polysiphoniae, L. erugino-coerulea, L. cryptovaginata, L. spiralis, L. nigra, L. kashyapii are new records for Golestan province and Iran. Results showed that Lyngbya rubida, in spring and winter, L. perelegans in summer, L. kashyapii and L. dendrobia in autumn were dominant species in all stations. Morphological characteristics of these species and some information about their ecological distribution are given.

Roghieh Siahbalaei (correspondence < e-mail: m.siahbala@gmail.com>), Saeid Afsharzadeh and Shokuh Nekouei, Department of Biology, Isfahan University, Isfahan, Iran. -Shademan Shokravi, Department of Biology, Islamic Azad University, Gorgan Branch, Gorgan, Iran.

Key words. Cyanophyta, Iran, Golestan province, paddy field, taxonomy.


Introduction
Cyanophyta are present abundantly in rice-fields and are important in helping to maintain rice-fields fertility through nitrogen fixation, nitrogenous and non-nitrogenous compounds liberation and excretion (Shokravi et al. 2007). In addition some potent bioactive compound that extracted from this group, draw clear landscape for pharmacological industries (Ghasemi et al. 2003; Soltani et al. 2006). It has been showed that many rice-fields of Asian soils contain a high density of Cyanophyta and over 50% of Cyanophyta genera that are in existence in rice-paddy fields belong to heterocystous filamentous form (Jeong–Dong & Choul–Gyun 2008). In spite of this Cyanophyta of paddy fields of Iran and specially Golestan province seems to be relatively unknown. Golestan province is located between the longitudes of 54°30’ and 56°30’ E and at a latitude of 38°15’ N in Iran. The paddy fields were containing nearly 62000 hectares of total cultivation fields in last year. It seems
that in north paddy fields of Iran especially Golestan province, some strains of Cyanophyta especially is common (Shokravi et al. 2006), but there is no clear report about their morphological characterization and taxonomic situations and most of the early investigations have had physiologically or ecophysiological approaches.

Oscillatorian Cyanophyta (Order Oscillatoriales (Bold & Wyne 1985) or Family Oscillatoriaceae (John & al. 2002) are usually common members of the paddy fields. They are predominately filamentous, prokaryotic and photosynthetic Cyanophyta. It has been estimated that the number of living species of Oscillatorian Cyanophyta may be 265 (Jhon et al. 2002) and more than 24% may belong to the genus Lyngbya. Traditional identification of this genus is according to morphology of filaments, life cycle, trichome structure, and type of trichome disintegration (Anagnostidis & Komarek 1990). Until now, approximately 66 species of the blue-green algal genus Lyngbya has been recorded from marine and fresh waters as free floating or benthic forms and from terrestrial habitats like soils and artificial substrates as epiphytic, endophytic and epilithic communities (Ullmann & Budel 2001).

Under natural conditions in rice fields, members of Cyanophyta are exposed to the combined influences of several factors such as pH, irradiance and dissolved inorganic carbon fluctuations, which varied both daily and over the crop cycle (Quesada & al. 1995, 1998). Growth, biochemical and physiological characteristics of Cyanophyta are influenced by environmental factors (Grossman & al. 1993; Islam & Whitton 1992; Fernández-Valiente & Leganés 1989). pH is a basic factor, which clearly affects the distribution of Cyanophyta. Most members of Cyanophyta grow in environments that are neutral to alkaline and in laboratory cultures the optimal pH ranges from 7.5 to 10. Generally, a wide range of adaptation to pH has been observed not only among different genera but also between different populations of the same species. In rice fields, the pH of floodwater varies during the day and during the growth of the crop due to the photosynthetic activity of Cyanophyta, algae and other macrophytes (Fernández-Valiente & Leganés 1989).

The aim of this paper is to contribute to the knowledge on the algal flora of paddy fields based on morphological investigations of the Cyanophyta of the paddy field in Golestan province and a preliminary investigation of their abundance especially in relation to pH of the paddy fields.

Materials and Methods

Soil samples were obtained from paddy fields of different stations of Golestan province (North of Iran). Five stations were chosen in different areas of the paddy fields. The samples were taken from the surface and 2 cm depth between autumn 2006 and summer 2007. Samples were taken from flooded and non-flooded soils (Kaushik 1987). After collection, samples were brought to the laboratory for culturing, preserving and preparation for study (Thalliophyta laboratory of Isfahan University). pH and EC were measured using digital pH meter (Table 1). The samples cultured in solid BG11 medium (NaNO3, 17.65 mM, mg SO4.7H2O, 0.3 mM, CaCl2.2H2O, 0.25 mM, K2HPO4.3H2O, 0/18Mm, Mg EDTA, 0/003 mM, citrate ferric ammonium, 0/02 mM Acid citric, 0/029 mM Na2CO3, 0/188 mM, microelements lml 1)-1. The cultivation was done under illumination 1500–1600 lux, pH 7.2 and temperature 25 ± 2°C. After colonization and isolation, samples were purified by several subculturing (Kaushik 1987). Identification of samples was carried out by usual microscopy in addition of fluorescence and phase-contrast microscopy. Determination and nomenclature accomplished according to famous keys and manuals (John & al. 2002, Anagnostidis & Komarek 1990, Prescott 1962 and Desikachary 1959). Photographs were taken with Nikon microscope, equipped with a Canon model camera. Axenity were checked daily using microscopic observations.

Results

A total of 17 species belonging to the genus Lyngbya were identified, 13 species are new records for Golestan Province and Iran. They ranged from 6-9 species in spring, 1-6 species in summer, 5-11 species in autumn and 3-6 species in winter, between stations. List of new records are given below and their photographs are presented in Fig. 1. Identified species and their distribution on the paddy field of Iran are listed in Table 2.

<table>
<thead>
<tr>
<th>station</th>
<th>site</th>
<th>pH</th>
<th>conductivity (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kordkoy</td>
<td>1-2-3</td>
<td>7.2-7.4-8.2</td>
<td>0.56-0.69-0.81</td>
</tr>
<tr>
<td>Gorgan</td>
<td>4-5-6</td>
<td>7.4-7.9-8.2</td>
<td>0.51-0.62-0.55</td>
</tr>
<tr>
<td>Aliabad</td>
<td>7-8-9</td>
<td>7.4-7.7-7.5</td>
<td>1.18-1.25-1.50</td>
</tr>
<tr>
<td>Azadshahr</td>
<td>10-11-12</td>
<td>7.4-8-8</td>
<td>0.55-0.56-0.76</td>
</tr>
<tr>
<td>Manudasht</td>
<td>13-14-15</td>
<td>6.9-7.2-7.8</td>
<td>1.36-1.12-0.72</td>
</tr>
</tbody>
</table>

Table 1: physical-chemical properties of surface soil samples from monitoring sites in the paddy fields of Iran.
Table 2: *Lyngbya* species and relative frequencies of occurrence on the paddy fields of Iran. Stations: 1-Aliabad. 2-Kordkoy. 3-Minodasht. 4-Azadshahr. 5-Gorgan. D=Dominant (75-100%); A=Abundant (50-75%); F=Frequent (25-50%); R=Rare (<25%).

<table>
<thead>
<tr>
<th>Species</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td><em>L. perelegans</em></td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td><em>L. rubida</em></td>
<td>F</td>
<td>R</td>
<td>R</td>
<td>F</td>
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<tr>
<td><em>L. sordida</em></td>
<td>-</td>
<td>-</td>
<td>F</td>
<td>-</td>
</tr>
<tr>
<td><em>L. kuetzingii</em></td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>L. kashyapii</em></td>
<td>-</td>
<td>-</td>
<td>F</td>
<td>-</td>
</tr>
<tr>
<td><em>L. erugineo-coerulea</em></td>
<td>F</td>
<td>R</td>
<td>F</td>
<td>-</td>
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<tr>
<td><em>L. lemnetica</em></td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
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<tr>
<td><em>L. nigra</em></td>
<td>-</td>
<td>-</td>
<td>R</td>
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<td><em>L. aestuarii</em></td>
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<td><em>L. spiralis</em></td>
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<tr>
<td><em>L. dendrobia</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>-</td>
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<tr>
<td><em>L. laxespiralis</em></td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>F</td>
</tr>
<tr>
<td><em>L. contorta</em></td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
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<td><em>L., scotii</em></td>
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<tr>
<td><em>L. polysiphoniae</em></td>
<td>-</td>
<td>R</td>
<td>R</td>
<td>-</td>
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<tr>
<td><em>L. cryptovaginata</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>R</td>
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<tr>
<td><em>L. putealis</em></td>
<td>F</td>
<td>R</td>
<td>-</td>
<td>R</td>
</tr>
</tbody>
</table>

Filaments straight or curved, pale green, 2-2.5 μm broad; sheath very thin, hyaline; trichomes 2 μm broad, not constricted at the cross walls; cells 7-8 μm long and 1.9-2 μm broad, granulate; end cell rounded, not capitate, not calyptrate.

*L. rubida* Fremy
Thallus more of less expanded; filaments straight, 5-6 μm broad, loosely intricate; sheath very thin, not lamellate, at first colourless, later reddish, trichome brown, not constricted at the cross wall; cross wall not visible, not granulated; cells granulate and rounded; calyptra absent.

*L. laxespiralis* Skuja
Filaments inter mixed with algae, sometimes irregularly bent of more or less straight 11 μm broad; sheath thin, smooth, hyaline, 1.5 μm broad; trichome 9 μm broad, at the cross wall not constricted, granulate; cells granulate and rounded; calyptra absent.

*L. sordida* Gomont
Thallus caespitose; filaments more of less straight, 10-12 μm broad; sheath thin, 1.5-2 μm broad; trichomes dull green, when dried dark violet, distinctly constricted at the cross wall, not granulated, 9-10 μm broad, 8.5-9.5 μm broad, granulate; end cell rounded with calyptras, in another specimen end cell rostrate. (Fig. 1n).

*L. putealis* Mont. ex Gomont
Filaments curved or nearly straight, 12-14 μm broad; sheath thin; trichome dull green, 11-13 μm broad, at the cross wall constricted, slightly attenuated at the apex; cells 3-4 μm long and 10 -12 μm broad, not granulated; end cell rounded.

*L. dendrobia* Bruhl & Biswas
Syn.: *Porphyrosiphon notarii* Kutz
Filament long and flexible, 10 μm broad; sheath usually thin, smooth, hyaline, 1.5 μm broad; trichome 9 μm broad, at the cross wall not constricted, granulated; cells 5-6 μm broad and 7-8 μm long, in various shades of brown, not granulate; end cell rounded.

*L. scotii* Fritsch
Filament straight, 4-4.5 μm broad; sheath smooth, hyaline, lamellate, 2 μm broad; trichome 2.5 μm broad, attenuated at the end, at the cross wall not constricted; end cell prominently conical; cells as long as broad, granulate, no calyptra.

*L. polysiphoniae* Fremy
Filament straight or curved, single, 11 μm broad; sheath thin, colorless; trichome 10 μm broad, at the cross wall constricted, not attenuated at the apex; cells 4-5 μm long and 2 times as long as broad, not granulated; cross wall visible; end cell convex, rounded.

*L. kashyapii* Ghose
Filament straight, more or less curved, 2.5-3 μm broad; sheath very thin, pink or purple, smooth; trichome 2.5 μm broad, at the cross wall not constricted; cross wall not visible; cells granulated; end cell in some of the specimens rounded and not attenuated, and in the other specimens bent and a little attenuated.

*L. erugineo-coerulea* Gomont
Filament single or flexuous, 10 μ broad; sheath thin, smooth, not lamellate; trichome 8-9.5 μ broad, at the cross wall not constricted; cells often granulated, end not attenuated, no calyptra.

*L. cryptovaginata* Schkorbatow
Filament single, straight, 8 μ broad; sheath thin, not lamellated, 2 μ broad; trichome 6 μ broad, at the cross wall constricted; cells nearly quadrate, 2 μ long, 5-6.5 μ broad, not granulate; end cell attenuated, rounded.

*L. nigra* C. Ag. ex Gomont
Filament straight, 8-9 μ broad; sheath thin, not lamellate; trichome green-brown, 8.5 μ broad, not constricted at the cross wall, not granulated; cells 3 μ long and 7-7.5 μ broad, ends attenuated; end cell conical.

*L. spiralis* Geitler
Filament entirely for major part spirally coiled, distance between two spirals 70-80 μ; sheath delicate and not lamellate; trichome 2.5 μ broad, at the cross wall not
Discussion
Collectively our knowledge about *Cyanophyta* of Golestan province is limited. However, until now, a few reports have been published with the highest degree of consideration on stigonematalean species (Sepehri et al. 2003, Norouzi et al. 2004). About oscillatary strain, the dominant of our knowledge is considerably narrower. A few species of *Oscillatoria* has been recorded by Siahbalaie et al. (2008) and Shokravi et al. (unpublished data). About species of *Lyngbya* unfortunately we have no report yet. Morphological versatility and the narrowing border between such genera and others like *Planktothrix*, *Anagnostidis* & Komarek, *Phormidium*, Kutzin g ex Gomont and rarely *Oscillatoria* Vaucher ex Gomont and *Plectonema* Thuret ex Gomont may be the main reason. However in our study on the algal flora in paddy fields the members of *Cyanophyta* formed the majority with a ratio of 75%, this can be explained by the variety of habitats or may have been the result of physical, chemical or geographical differences between paddy fields. There were 17 taxa in total, four species has been reported from the lakes and rivers for Iran. *L. contorta* (Zarei-Darki 2004), *L. kuetzingii* (Compere 1981). *L. limnetica* (Compere 1981, Hirano 1973, Afsharzadeh 2003, Zarei-Darki 2004) and *L. aestuerii* (Zarei-Darki 2004). Rest of the species are first records for paddy fields of Iran. The flora of algae in paddy fields varied between stations and months. The number of species at each site ranged from 3 to 11 with maximum at Kordkoy, Gorgan and Azadshahr stations, a minimum at Minodasht and Aliabad stations. Dominant species were in all stations *L. rubida* in spring and winter, *L. perelegan* in summer, *L. kashyapii* and *L. dendrobia* in autumn. Many of *Lyngbya* species are useful indicators in paddy field *L. borterti* Lemm and *L. hiermoniusi* Lemm, *L. kuetzingiana* Kirchnert were not observed in this area. However, *L. scottii*, *L. aerugineo–coerulea*, *L. limnetica* are also characteristic species in the paddy field and this study. Results of this survey show that morphological variation of some *Cyanophyta* such as *Lyngbya* in paddy field is very high, that need to improve identification keys. However, based on the results it may be possible to draw a relatively primitive picture of the morphological and taxonomical situation of *Cyanaphita* especially *Lyngbya* species in paddy fields of North of Iran.

In this study, we can attribute to some characteristics of *Lyngbya* species in paddy fields of Iran. Absence of thick and visible sheath, absence of calyptra, absence of constricted and granulated cross wall, are noticeable characters from morphological point of view (exception 3-4 samples), that can be useful for identification *Lyngbya* species.

They are common inhabitants of aquatic and terrestrial surfaces. The widespread distribution of *Cyanophyta* indicates that they can cope with a wide spectrum of global environmental stresses such as temperature, *pH*, desiccation, etc. Salt stress and *pH* are from the most limiting factors on the growth and productivity of microorganisms. The main ecological factors of *pH*, EC affected the development of the algal flora in paddy fields (Whitton & Potts 2000). Lower acidities and higher EC may simply have a delaying effect on the development of the algal flora (Broady 1984). Electric conductivity values ranged from 0.51 to 1.50 ms.cm⁻¹. A maximum value of EC was observed at site 8, 9 and 13 in Aliabad and Minodasht. The *pH* values ranged from 6, 9, 8.2. The *pH* values at site 3, 6 and 11 were higher than values at the other sites. Lowest *pH* and highest EC were measured at Minodasht and Aliabad stations, as they are near the forest but Azadshahr, Gorgan and Kordkoy had lower EC and highest *pH* these stations are near the agricultural fields. Thus, factors *pH* and EC affect on the *Cyanophyta* variations in the paddy fields. Our result is in agreement with Hickman (1978).

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