Cyanobacterial community patterns as water quality Bioindicators

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Abstract

The main goal of this study was to examine the use of cyanobacteria for evaluating the quality of running water. Accordingly epilithic cyanobacterial communities were collected in Dez River and Ojeyreb drain in south of Iran. Samples were collected in two seasons: autumn and spring. Effective physical and chemical factors on the structure of cyanobacterial communities and the dispersion of the species in relation with them were determined using PCA and CCA analyses. The Shannon-wiener biodiversity index was used to define the species diversity. The concentration of Nitrate as main nutrient had significant increase in Drain stations. A decline in species richness was observed associated with these increases in nutrient load in both seasons in different cyanobacterial community structure. The results indicated that order Oscillatoriales had higher proportion of cyanobacteria species at Drain. The species *Oscillatoria chlorina*, *Chroococcus minor*, *Phormidium tenue* and *Lyngbya kuetzingii* S had the most positive correlation with nutrient factor. Species *Lyngbya infixa* and *Lyngbya mesotrichia* had the most negative correlation with nitrate. Our results confirm the using of cyanobacteria species as indicators for monitoring eutrophication in rivers and define them as water eutrophication bioindicators.

Keywords: Cyanobacteria, Drainage, Eutrophication, River, Water quality, PCA

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Introduction

Monitoring of eutrophication in running waters caused by anthropogenic activities in these ecosystems, is the first step to define an efficient water management program for preservation of these ecosystems. The analysis of microflora community structure and defined bioindicators of water quality is an important tool in environmental monitoring programs. First record of using biological methods to monitor river water quality was introduced by Kolkwitz and Marsson (1908). Many of methods have been defined to monitor rivers water quality include employing algae, bryophytes, macro invertebrates and fishes (Whitton and Kelly, 1995). However, more recent investigations have demonstrated that water quality and nutrient concentration in the river waters had the greater effect on the photosynthetic organisms as algae (Kelly and Whitton, 1998). With this respect algae such as diatom communities have been widely used as bioindicators (Whitton and Rott, 1996; Prygiel et al., 1999), and defined as biotic indices such as IBD index (based on diatoms) in the rivers water quality monitoring programs. It has been found that in special ecosystem of some rivers, cyanobacterial dominance and cyanotoxic effect on the studied communities would have lower values than expected (Aboal et al., 2002). These results highlight the necessity of incorporating corrections in biotic indices definitions and monitoring programs and also to consider the cyanobacterial communities. In recent studies it is shown that the structure of cyanobacterial communities have changed from upstream to downstream of the rivers with physical and chemical conditions and nutrient load in the water (Perona et al., 1998; Aboal et al., 2002; Douterelo et al., 2004; Maldonado et al., 2011). Such investigations led to define the cyanobacteria as water quality bioindicators.

In this study cyanobacterial community's structure in Dez River and Ojeyreb Drain, in Khuzestan province (Iran) is described in two seasons: autumn and spring. Although the quality of Dez river are measured in a few papers (Khodaie et al., 2006; Khodaie and Shahsavari, 2003) this study is the first record on Iranian river epilithic cyanobacterial communities and the relationship between the diversity of cyanobacterial species and physical and chemical conditions of water to determine water quality indicators and compared with universal patterns.

Materials and methods

Sampling sites were selected in order to include locations above and below human settlements and agricultural centers. The research was carried out on seven sites, including the first site at Dez river branch (Dez108) and the other six sites along Ojeyreb drain at the end of Dez river to downstream point of this drain nearby the Persian Gulf (Dez103-Dez107, Fig. 1). Samples were collected two times in 2009, including the end of autumn and the end of spring. Dissolved oxygen, temperature, pH and electric conductivity (EC) were measured in situ in each sampling site

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using portable Multi 350i WTW (Germany).

Water samples were taken in each station. Two polyethylene bottles of water per station were considered for chemical analysis. Nutrient chemical concentrations (NO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}) were analyzed according to Standard Methods For Examination of Water and Wastewater, (APHA) (1995); by chromatography technique methods, respectively.

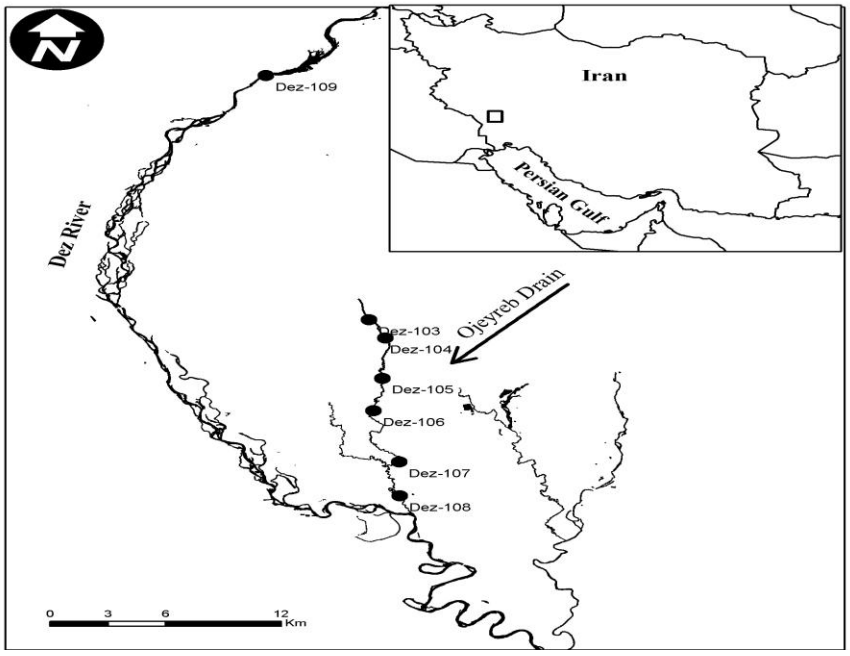

Figure 1: Geographical position of sampling sites

For sampling of cyanobacterial communities in each sampling sites two stones were collected from a submerged part of the river bank. The attached algae were removed by brushing in 5 ml of medium. Aliquots were transferred on Petri dishes with different solid culture media (BBM, BG11 and Chu; Andersen, 2006). Another aliquot was fixed with formaldehyde 4% for microscopic examination. Cyanobacteria taxonomy and nomenclature is primarily classified based on the methods of Prescott (1962), Desikachary (1959), John et al. (2003). Their abundance in epilithon samples was evaluated by counting the presence of each species (as cells in filament or equal number of individual cells) in alternate transects in 16 cm^2 surface on semipermanent slides by light microscopy. The Shannon-Wiener biodiversity index (Margalef, 1957) was used to define the species diversity of algae inhabiting different types of habitats.

Data were subjected to multivariate statistical analysis. Environment data were ordinated using a correlation - based principal component analysis (PCA). Relationship between cyanobacterial abundance and environmental variables of sampling periods were analyzed with canonical correspondence analysis (CCA). Statistical analysis performed with Past and SPSS 16 software. In addition, cluster

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analysis was carried out by ward's method at Euclidian scale by PAST software.

Results

OD and pH in Dez River are higher than drain sites water. No significant difference between different drain sampling sites was found. However, EC had no significant difference in all sites, but it had higher level in the autumn. Greater amount of Cl^- were detected in the river than drain sites especially in the autumn. Amount of sulfate at the autumn was same in all sites but in the spring it shows high decrease in river water. Phosphate had very low amount in all sampling sites (not shown). These results show high variation in physical condition and nutrient content in the river water and drain sites (Table 1). Table 2 describes the occurance of species in different sampling sites. The dominant community of cyanobacteria of these stations is created by 35 species belonging to 13 genera. *Oscillatoria* (7 species), *Lyngbya* (5 species), *Phormidium* (3 species), *Chroococcus* (2 species) and *Anabaena*, *Aphanocapsa*, *Merismopedia* , *Microcoleus, Nostoc, Plectonema* each with one species were present in sampling sites. Dominant species in first sampling were *Phormidium angustissimum* West *et* G.S. West, *Homeothrix juliana* and *Oscillatoria annae* in Dez 108, 107 and 106 sites, respectively, *L.mesotrichia* in Dez 105, 104, 103 sites and *Oscillatoria limentica* in Dez 109 sampling site. The dominant species in the spring changed to *Microcoleus chthonoplastes* (Vaucher) Gomont, *Oscillatoria subbrevis* Schmidle and *L. mesotrichia* in Dez 108,107 and 106, *Lyngbya limnetica* Lemmermann in Dez 105 and 103, *L.infixa* in Dez104 and *L.mesotrichia* in Dez 109 (Fig. 2). In other view the dominant filamentous cyanobacteria species in two sampling seasons were *L.mesotrichia, P.angustissimum, O.annae, O.limnetica, O.subbrevis, H.juliana, L.infixa and M.chthonoplastese* that the dominance of two species *O.limnetica* and *L.mesotrichia* were observed in Dez River and the others were in Ojeyreb drain sites, also non-filamentous dominant species were *Chroococcus turgidus* (Kuetz.) Naegeli, *Ch.minor* and *Dermocarpa* sp.. The dominance of this species just observed in Drain sampling sites. The most abundant filamentous species were observed is *L.mesotrichia* in Dez109 in the spring and *Dermocarpa* sp. as non-filamentous species in Dez105 at the autumn.

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Table 1: Variation of the chemical and physical variables associated with the quality of waters in two seasons (ANOVA, p<0.001)

Sampling site	Coordinates	OD		EC (µS/cm)		T (°C)		pH		NO_3^- (mg /L)		SO_4^{2-} (mg /L)		Cl^- (mg /L)	
		Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Dez109	X=256649 Y=3588618	8.60 a	11.20 a	614 a	820 a	17.1 a	20.5 a	8.00 a	8.40a	5 a	14.0 a	135 a	60.0 a	66 a	89.5 a
Dez108	X=263598 Y=3555420	8.60 a	7.50 a	614 a	868 a	17.1 a	21.0 a	8.00 a	7.60 b	16 a	15.0 a	172 a	50.4 a	66 a	39.0 b
Dez107	X=263556 Y=3558111	7.50 b	7.03 a	723 a	917 a	24.2 b	21.5 a	7.60 b	7.70 b	16 a	19.9 a	617 b	49.0 a	69 a	44.7 b
Dez106	X=262227 Y=3562155	7.12 b	6.20 b	749 a	920 a	24.3 b	22.0 a	7.55 b	7.80 b	19 a	28.9 a	104 a	71.0 a	69 a	71.5 a
Dez105	X=262701 Y=3564710	6.60 b	6.94 b	787 a	937 a	24.3 b	22.6 a	7.54 b	7.70 b	22 a	19.2 a	94 a	50.8 a	77 a	38.8 b
Dez104	X=262855 Y=3567886	7.89 b	7.33 a	724 a	888 a	23.4 b	22.6 a	7.43 b	7.60 b	25 a	25.0 a	243 a	49.6 a	51 b	31.6 b
Dez103	X=262004 Y=3569334	5.86 b	6.38 b	743 a	904 a	22.7 b	21.1 a	7.38 b	7.45 b	28 a	25.3 a	560 b	49.4 a	48 b	31.9 b

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Table 2: Occurrence of cyanobacteria species in different site sampling

Sampling site	Dez103		Dez104		Dez105		Dez106		Dez107		Dez108		Dez109	
Species	**S**	**A**	**S**	**A**	**S**	**A**	**S**	**A**	**S**	**A**	**S**	**A**	**S**	**A**
***Anabaena* sp.**	-	-	-	-	+	-	-	-	+	-	-	-	+	-
Aphanocapsa greviellii	-	-	-	-	-	-	-	-	+	+	-	+	-	-
***Calothrix* sp.**	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Chroococcus minor	+	+	-	-	-	+	-	-	-	-	-	-	-	-
Chroococcus turgidus	-	-	+	+	-	-	-	-	-	-	-	-	-	-
***Dermocarpa* sp.**	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Homeoethrix juliana	-	-	-	+	-	-	+	+	-	-	-	-	+	+
Lyngbya cryptovaginata	-	-	+	+	-	-	-	-	-	-	-	-	-	+
Lyngbya infixa	-	-	+	+	-	-	-	-	-	-	-	-	+	+
Lyngbya kuetzingii	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Lyngbya limnetica	-	-	-	+	-	-	+	-	+	-	-	-	-	-
Lyngbya mesotricha	-	-	+	+	-	+	+	+	-	-	+	+	+	+
***Merismopedia* sp.**	-	-	-	-	-		-	-	-	-	-	-	-	+
Microcoleus chthonoplastes	-	-	-	-	+	-	-	-	-	-	+	-	-	-
***Nostoc* sp.**	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Oscillatoria annae	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Oscillatoria calcuttensis	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Oscillatoria chlorine	-	-	-	-	+	-	-	-	+	+	+	-	-	-
Oscillatoria limnetica	+	+	-	+	+	-	-	-	-	-	-	+	+	+
Oscillatoria rubescens	-	-	-	-	-	-	-	-	+	+	-	+	-	-
Oscillatoria subtilisima	-	-	-	-	+	-	-	-	-	-	-	+	-	-
Oscillatoria subbrevis	-	-	-	-	-	-	-	+	+	+	-	+	-	+
Phormidium angustissimum	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Phormidium fragile	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Phormidium tenue	+	+	-	-	-	-	+	+	+	-	-	+	-	-
Plectonema notatum	-	+	-	-	-	-	-	-	-	-	-	-	-	-

A: Autumn, S: Spring

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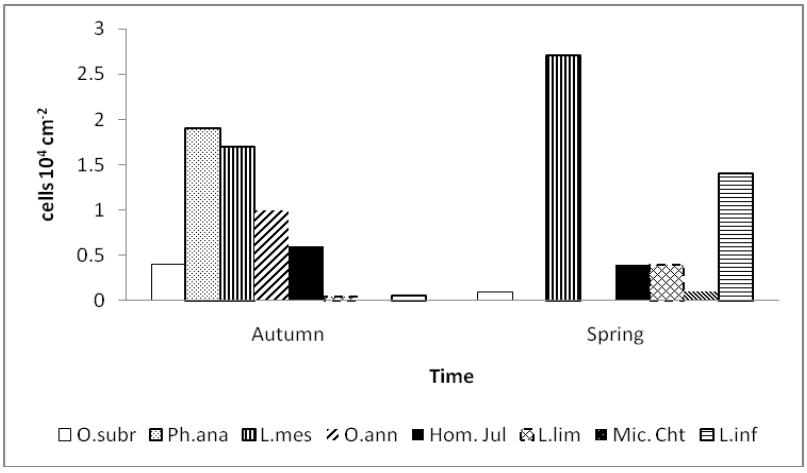

Figure2: Variability of the abundunce of dominant cyanobacteria species in two sampling seasons.

Table 3: Shannon-Wiener and Dominance indices

Site	Shanon-Wiener indices	
	spring	Autumn
Dez103	0.346	0.479
Dez104	0.430	0.664
Dez105	0.368	0.185
Dez106	0.964	0.309
Dez107	0.178	0.756
Dez108	0.732	0.322
Dez109	0.221	0.273

Maximum species diversity was observed in autumn in Dez 106 and 108 sampling sites and so the minimum diversity was in Dez 107 in spring. Increases in the diversity Ordination of environmental data by PCA showed a clear pattern. In the autumn sampling the first two components explained 98% of the data variance (pc1=3.5, pc 2=0. 03) on component 1, high positive values were associated to EC (then SO_4^{2-}) and pH, OD (then temperature) were related to high negative values. Positive and negative values on second component were associated to NO_3^- and Cl^- concentrations, respectively. At the spring sampling as above, the first two components explained 96% of the data variance (pc1=3.9, pc2= 0.08). Positive values on first component were associated to EC and SO_4^{2-} and negative values were associated to pH, OD and NO_3^- concentration. Cl^- concentration is associated to negative values on the second component (Fig. 3).

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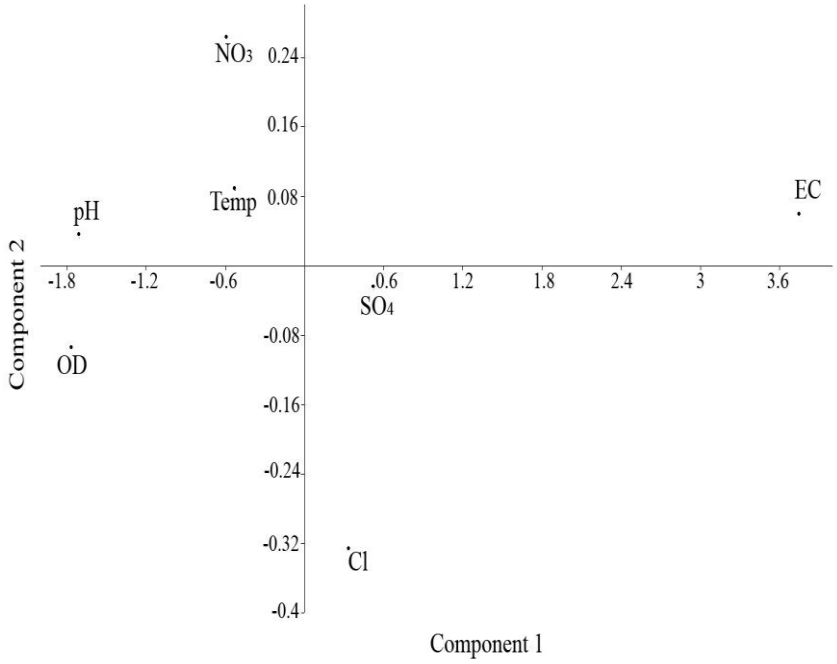
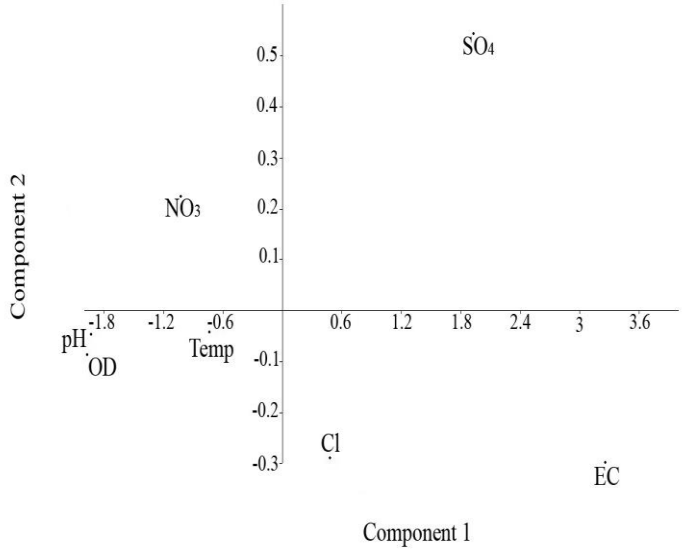


(a)



(b)

Figure 3: PCA analysis of environmental data obtained in (a) autumn and (b) spring

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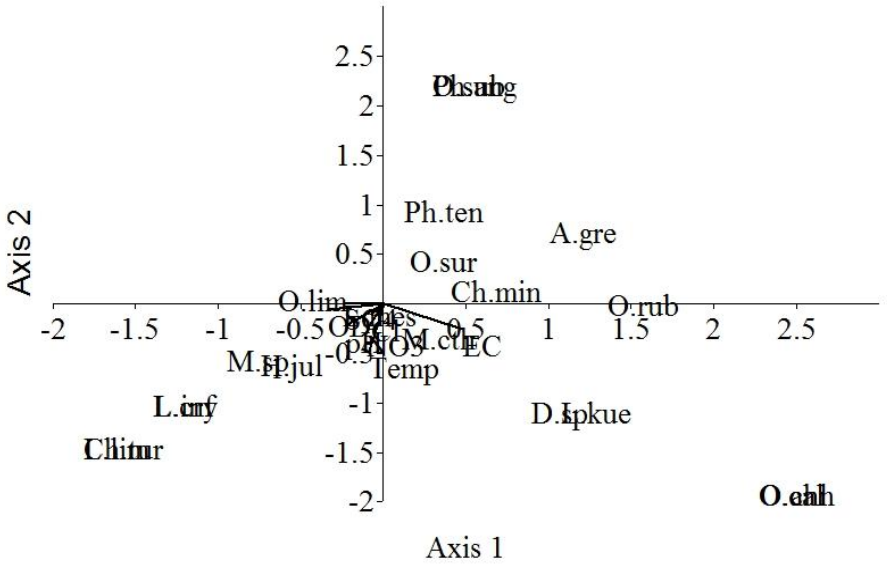

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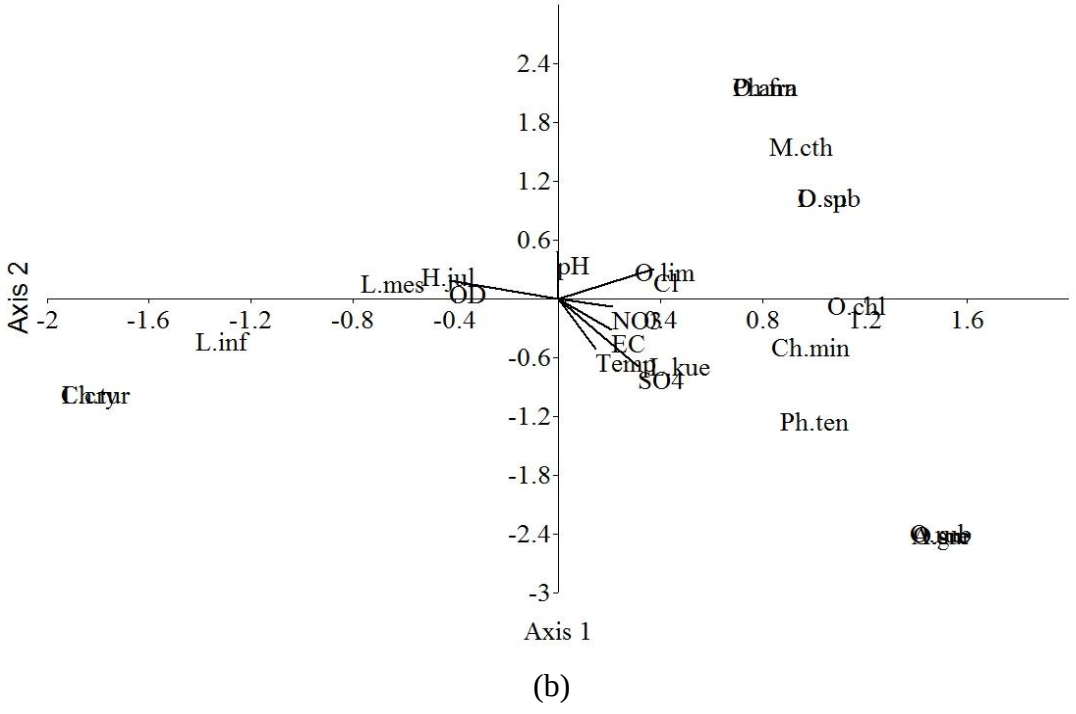

(b)

Figure 4: CCA ordination plot in (a) autumn and spring (b)

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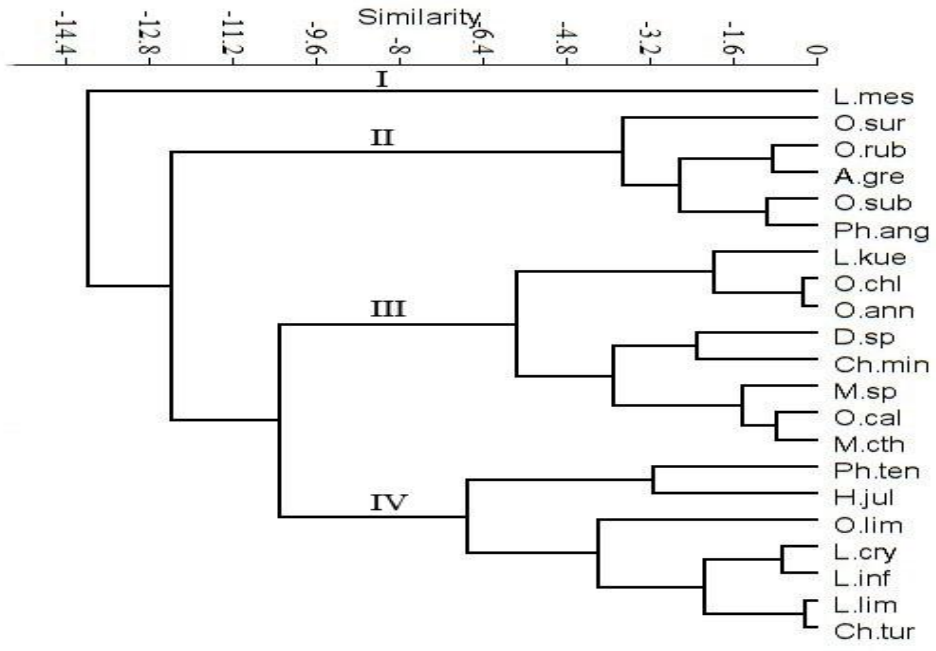
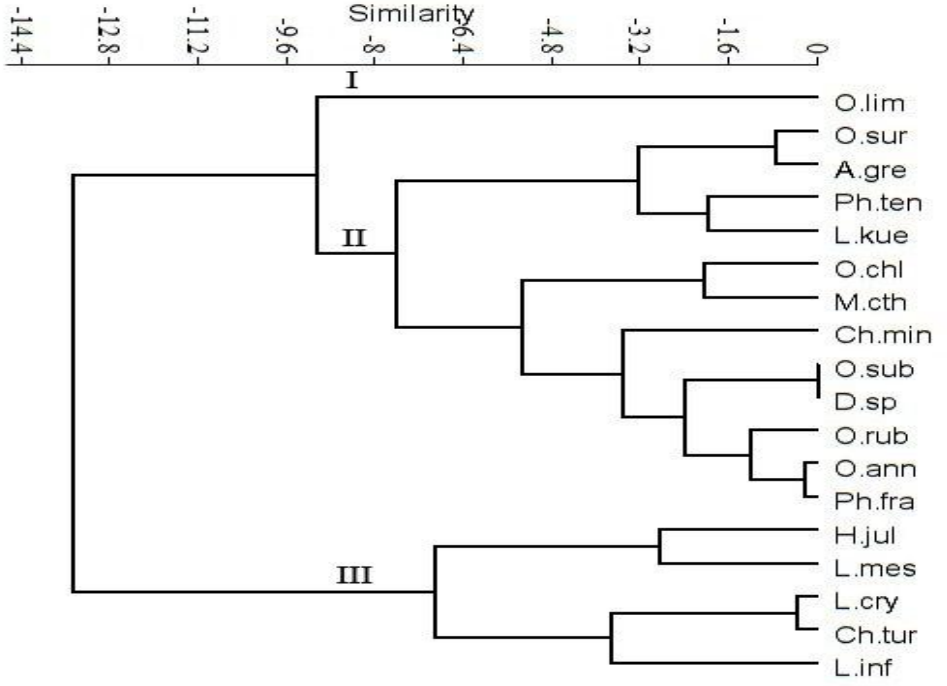


(a)



(b)

Figure 5: Dendrograms of the Cyanobacterial communities groups based on environmental factors of sampling sites (a) autumn, (b) spring

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The preliminary cyanobacteria CCA analysis was carried out on the basis of seven variables in two sampling seasons: autumn and spring, shown in table 1, to show the species- environment correlation. *Aphanocapsa greviellii* (Hassall) Rabenhorst, *O.rubescens* (de Candol) Gomont, *O.subbrevis* and *Ch.minor* in autumn showed the highest direct correlation to EC and another hand the species *L.mesotrichia*, *M.*sp., *H.juliana* and *O.limnetica* have the highest correlation to OD and SO_4^{2-}, and *L.mesotrichia* and *H.galiana* in the spring samplings showed the highest direct correlation to OD and SO_4^{2-}. *O.chlorina*, *C.minor*, *L.kuetzingii*, *P.tenue* have the highest positive and *L.mesotricha*, *C.turgidus* and *H.juliana* have the highest negative association to NO_3^- and SO_4^{2-} as nutrient water ions (Fig. 4). Cluster analysis was performed to separate the species to distinct groups based on environmental factors, with the help of CCA analysis results (Fig. 5).In autumn sampling, 4 groups of species could be distinguished from the dendrogram based on hierarchical cluster:

I. A species with highest direct correlation just to OD.
II. Species with direct positive correlation to EC and negative correlation to temperature.
III. Species with direct positive correlation to EC and temperature
IV. species with direct positive correlation to OD, SO_4^{-2} and pH

The dissimilarity between groups was higher than -9.6. The CCA analysis results revealed that EC, OD and SO_4^{-2} are the main factors influencing the cyanobacterial species communities in the autumn. Three groups of species could be distinguished from the dendrogram of the cluster analysis the spring sampling:

I. A species with highest positive correlation to Cl^{-2}.
II. Species with positive direct correlation to NO_3^- , EC and SO_4^{-2}.
III. Species with highest positive direct correlation to OD.

The dissimilarity between groups was higher than -8.0 the CCA analysis results confirmed that EC, SO_4^{-2}, NO_3^- and OD are the main factors influencing the cyanobacterial species communities in the spring.

Discussion

Our results revealed great difference in physical condition and nutrient content between river and Drain water, that led to different cyanobacterial community structures in this locations, so we can define two different zones: The fresh water of the river and also second station in the

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Drain water where subjected to a particularly wide variety of perturbations as domestic waters and due to agricultural practices. In describing of water chemical content a distinction can be drawn between conservative chemical factors as pH, SO_4^{2-}, Cl^-, etc. that closely associated with lithological composition of river basin and determine the mineralization of the water and non-conservative component such as nutrient whose concentrations depend on local conditions (Sabater et al., 1990). There are many records confirmed that the cyanobacteria community responds to changes in fresh water quality and there is not always a general response for all cyanobacteria as group, since the responses of single species can be quite different. So changes in cyanobacterial communities or single species can be used as water quality bioindicators (Douterelo et al., 2004).

In this study PCA analysis results showed that physical parameters such as OD, EC and pH have the most important effect on describing the patterns of cyanobacterial species diversity that have higher level in the river water than drain sites. There are a few results about the effect of physical water conditions on the cyanobacterial communities.

In previous studies the use of epilithic diatoms as acidity indicators (Coring, 1996) and the negative effect of OD on richness of cyanobacterial communities in high level of dissolved oxygen were shown in fresh waters (Whitton, 1992). Our results showed the highest direct correlation between OD and abundance of two species *L.mesotrichia* and *H.juliana*. Abundance of some species such as *A. greviellii*, *O.rubbescens*, *O.subbrevis* and *O.limnetica* also correlated with this factor.

It has described an increase in prevalence of cyanobacteria in response to different types of fluvial pollutions such as eutrophication (Shaji and Patel, 1991), high level of organic pollution (Necchi et al., 1994) and high relative abundance of filamentous cyanobacteria with increasing fecal coliform concentration or urban wastewater (Vis et al., 1998). At least it had confirmed that epilithic cyanobacterial communities are suitable eutrophication bioindicators in the rivers (Perona et al., 1998). Our results showed less nitrate level in the river than drain water, whereas the phosphate concentration had very low level in both. CCA analysis results showed the highest negative correlation with NO_3^- concentration and species *L.infica* and *L.mesotrichia* abundance. Also *C.turgidus*, *L.mesotrichia* and *H.juliana* have significant decreases in abundance at sites with high level of NO_3^-. *C.minor* has the most positive correlation with NO_3^- concentration. Increase of NO_3^- level in the water has direct effect on the abundance of *O.chlorine*, *L.kuetzingii* and *P.tenue*

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species. In subsequent reviews (Fjerdingstad, 1964; Sladeček, 1973; Rott et al., 1997) several species of genera *Oscillatoria* emerged as organically polluted waters. However, *Oscillatoria* species such as *O.limosa* and *O.boryana* (Rott and Fister, 1988; Aboal, 1989) became more abundant in enriched waters; species belonging to Oscillatoriales were associated with highly eutrophic media. Douterelo (2004) defined greater number of *Oscillatoria* species at the downstream sites of the rivers that had high trophic levels. Also, our study confirmed the relationship between water entrophication and *Oscillatoria* abundance.

In addition, this study suggested that *C.minor* and then *O.chlorina*, *P.tenue* and *L.kuetzingiias* can be as indicators of eutrophic waters. In the other hand, some genera such as *Nostoc* (Sabater 1983; Aboal, 1988), *Chamaespihon* (Sabater, 1983; Aboal, 1988), *Calothrix* and *Scytonema* (Fernandez-pinas et al., 1991) are commonly associated with low level nutrient waters and upstream sites. Our results also suggested the presence of two *Lyngbya* species *L.mesotrichia* and *L.infixa* and other species *H.juliana* and *Chroococcus turgidus* as low influenced environment or unpolluted waters indicators.

Totally, these results showed the replacement of sensitive species in cleaned waters to resistant species in the drain as polluted water in agreement with previous suggestions (Perona et al., 1999). Results also confirm that cyanabacteria community responds to changes in fresh water quality and can be used as bioindicators in environmental studies of quality of these ecosystems.

The present study clearly illustrated the response of cyanobacterial species and structure of their communities to fresh water quality and replacement of sensitive species in cleaned waters to resistant species in the drain as polluted water. In confirmation of previous reports this study showed that most abundant species in eutrophic water of the drain belonged to Family Oscillatoriaceae, besides in this study the frequent presence of *Chroococcus minor*, *O. chlorina*, *P.tenue* and *L.kuetzingii* were reported in the drain water. On the other hand the abundance of species *L.mesotrichia*, *L.infixa*, *H.juliana* and *Chroococcus turgidus* in the river indicates how influenced or unpolluted waters. So these species can be considered as bioindicators in the environmental monitoring programs. The results of such studies could be led to define a universal freshwater quality index based on cyanobacterial community structures.

Acknowledgements

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