

[Research]

Phytoplankton as bio-indicator of water quality in Sefid Rud River, Iran (South of Caspian Sea)

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ABSTRACT

Phytoplankton are the first bio- indicators of pollution in aquatic ecosystems. Phytoplankton assemblage and aquatic ecosystems are always influenced by environmental factors therefore these environmental changes and threats must be understood in any ecosystem. Phytoplankton are inexpensive and readily available bio- indicators. In the present study, phytoplankton were used to study the Sefid Rud River (the south Caspian Sea), in Iran. Three sampling sites were selected up to 25- 30 km from the River estuary. Samplings were carried out from June through September 2009. Phytoplankton samples were collected by phytoplankton sampler net with mesh size of 25µm. Water parameters including transparency, temperature, water velocity and pH were measured during the survey. Mean water temperature was 26.9 ± 7.7 °C, velocity 0.13 ± 0.01 m/s, Secchi disk transparency and pH were 45cm and 7.4, respectively. Phytoplankton assemblage comprised *Bacillariophyceae* (61.2%), *Chlorophyceae* (31.6%), *Euglenophyceae* (5.0%) and *Cyanophyceae* (2.23%). Significant differences were observed in diversity and abundance of four main groups of phytoplankton between sampling stages. Significant positive correlations were detected between transparency and abundance of Chlorophyta, while negative correlations were found between transparency and *Euglenophyceae* abundances. The highest abundance of *Euglena* and *Phacus* (*Euglenophyceae*) occurred at high turbidity while maximum abundance of *Pediastrum* and *Scenedesmus* (*Chlorophyceae*) correlated with high water transparencies. Genus *Oscillatoria* (*Cyanophyceae*) was observed (abundance 18%) only in station 2 and influenced by agricultural activities. The study showed that phytoplankton are suitable bio- indicators of environmental changes which may threaten the Sefid Rud River and the Caspian Sea.

Keywords: Bio-indicators, phytoplankton, pollution, Sefid Rud River.

INTRODUCTION

Phytoplankton plays a central role in the structure and functioning of freshwater ecosystems. They are a significant component of water ecosystems as primary producers (Ligeza & Wilk-Woźniak, 2011). Phytoplankton populations are well-known to be influenced by space-time variations in hydro-chemical and physical parameters (UNESCO, 1981; Cloern *et al.* 1989). Most of aquatic ecosystems receive several types of inputs including; urban, industrial and thermal effluents and agricultural run-off (Perin, 1975; Collavini *et al.* 2001). Therefore, Continuous monitoring of aquatic ecosystems is essential to distinguish the effects of human-induced and stressors from natural

patterns of ecologic variation. Sefid Rud River is the largest Iranian river of the South Caspian Sea (CS) and the Sea is the largest inland water body in the world (Nasrollahzadeh Saravi *et al.* 2008). In the CS, the input of nutrients is very much limited to biotransformation and vertical transport due to the minimum suspended solid as well as low river discharge and atmospheric precipitation (Leonov & Stygar, 2001). Dumont (1995) reported that the CS suffers from anthropogenic disturbances including nutrient enrichment (especially in the North CS). In the Europe Water Framework Directive (WFD) (2000/60/EC, EU, 2000), phytoplankton is one of the five biological quality elements

(including; phytoplankton, macrophytes, phytobenthos, macro-invertebrates and fish) which require ecological status assessment in surface waters. However, algal indicators have some advantages over other biotic assemblage indicators of water quality. Unlike fish and macro-invertebrates, algal communities are usually present before disturbance and generally persist in some form after most disturbances. Phytoplankton assemblages are generally more sensitive to pollution than other assemblages (Whitton *et al.* 1995). Therefore, they are the best biological indicators of pollution in the aquatic habitat (Javed, 2006). According to the WFD, the phytoplankton based assessment should include: composition, abundance or biomass, the frequency and intensity of phytoplankton blooms. The algal bloom is an effect of the synergistic action from a complex of environmental factors influencing its development, some of which, for example include the bioavailability of specific nutrients, which have been previously related to this phenomenon (Donnelly *et al.* 1998). The quality and quantity of the phytoplankton depends in part on the nutrient load. They respond to low dissolved oxygen levels, high nutrient levels, toxic contaminants. Therefore, the water quality assessment systems have been based on the number (Thunmark 1945 ; Nygaard 1949 ; Kangro *et al.* 2005) or diversity of taxa from different algal groups (dominant and indicator species).

In the present study phytoplankton were used as bio-indicators to study the Sefid Rud River- Iran.

MATERIALS AND METHODS

The study was carried out in Sefid Rud River. Three sampling sites, with 1 km distance, were selected up to 25-30 km from the River estuary (South Caspian Sea), at 37° 16'N, 49° 56'E (Fig. 1). Samplings were carried out from June through September 2009. Sampling were conducted in 8 intervals (N=24). Different parameters including temperature, pH and water velocity were measured using thermometer, a HI 9813 pH meter, a velocity meter, respectively. Water transparency was measured using a 30 cm diameter secchi disk. Phytoplankton samples were collected by phytoplankton sampler net with mesh size of 25 μ m and a 100 ml collection chamber. The filamentous algae were also collected. Algae were placed separately in 100 ml containers and were preserved in 1% formalin. Number of phytoplankton cells (cell.L⁻¹) were calculated for each genus according to the following formula (Bianchi *et al.* 2003); $x = n \times v_3 / v_2 \times v_1$ where x = Number of phytoplankton cell per litre, n =Number of phytoplankton cell in a subsample of 1 ml taken from the original 10 ml samples, v_1 = the filtrated water (2 L), v_2 = condensed water volume (100 ml), v_3 = water volume used for calculation (10 ml).

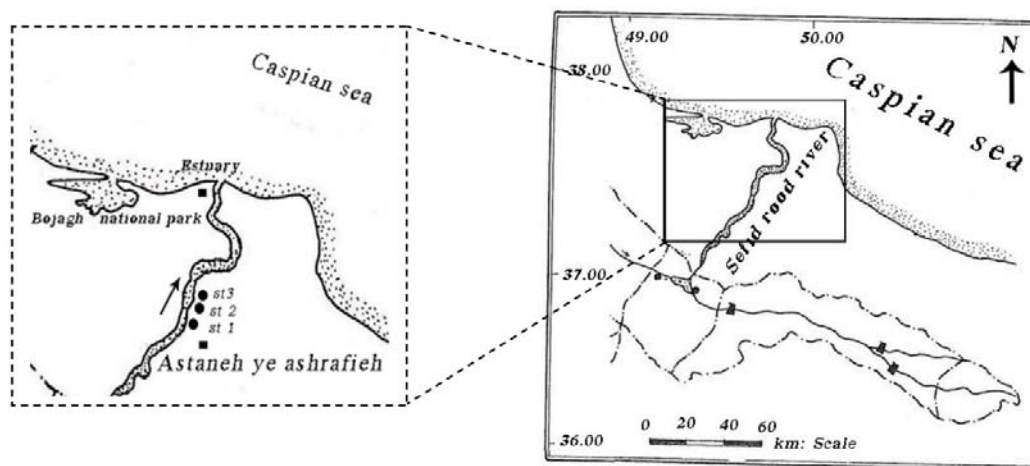


Fig 1. Sefid Rud River

In order to enumerate filamentous and multiple cell algae (e.g. *Oscillatoria* and *Scenedesmus*), 20 random samples were selected and the number of cells and respective mean values in each filament or colony were calculated. Identifications of phytoplankton were carried out following Tomas (1997) identification key with the aid of light microscopy. Two - Way ANOVA was used to test the differences in number and diversity of phytoplankton between sites and sampling efforts. One - Way ANOVA was used to test the differences in water parameters between sampling efforts. Tukey's post hoc test was used to determine differences between sampling efforts at 95% confidence level. In order to highlight relationships between environmental parameters and phytoplankton populations, a principal components analysis (PCA) was performed.

RESULTS

Bacillariophyceae, Chlorophyceae, Euglenophyceae and Cyanophyceae, were 4 main phytoplankton groups observed in 25-30 km river reach from the Sefid Rud River estuary respectively. Significant differences were found between sampling efforts and sites ($F = 57.37$, $df = 3$, $p < 0.05$) (Fig. 2). Bacillariophyceae dominated phytoplankton assemblages from June through August. In this period, the most abundant diatoms was *Synedra* (31%) followed by *Navicula* (30%), *Surirella* (19%) and *Pleurosigma* (18%). Chlorophyceae were dominant in September and the most abundant algae were *Pediastrum* sp. and *Scenedesmus* sp. Chlorophyta algae were presented mostly with: *Scenedesmus* sp., *Pediastrum* sp., *Pandorina* sp., *Cosmarium* sp. and *Closterium* sp., during the study. Water temperatures ranged between 31°C in June and 21 °C in September (mean 26.9 ± 7.7 °C). Significant differences were observed between sampling efforts ($F = 15.51$, $df = 7$, $P < 0.05$) (Fig. 3). Mean water velocity was 0.13 ± 0.01 m/s and significant differences were found between sampling efforts ($F = 8.4$, $df = 7$, $P < 0.05$)

(Fig. 3). Maximum Secchi disk water transparency was 130 ± 23.7 cm in mid September and minimum was 18.3 ± 2.7 cm in midsummer and significant differences were found between sampling efforts ($F = 35.3$, $df = 7$, $P < 0.05$) (Fig. 3). Mean pH value was 7.4 and there was no significant differences between sampling sites and efforts. Totally, 21 genera of phytoplankton were identified as bio-indicator, presented in Table 3. To understand the significance of various environmental variables on phytoplankton distribution and abundance, a principal components analysis (PCA) was performed, resulting in a series of eigenvalues, of which the first two components explained more than 70% of the total variance—a highly significant percentage, considering that biological variables also occurred in the examined system. The contribution of each variable is shown in Table 1 where in the first component, Bacillariophyceae, pH and temperature had a negative and transparency and velocity positive influences; while in the second component, the impacts of Chlorophyceae and Euglenophyceae was positive and Cyanophyceae was negative. These variables fell in the plane of the first two principal components, identifying two groups: (1) Chlorophyceae, Euglenophyceae, Turbidity and Velocity; (2) pH, Temperature, Bacillariophyceae and Cyanophyceae (Fig. 4). These results indicate that velocity and transparency are the main factors contributing to the distribution of Chlorophyceae and Euglenophyceae in Sefid Rud River. Although, Transparency is linked to velocity; and pH and temperature are main factors contributing to the distribution of Bacillariophyceae and Cyanophyceae in this study. Among Phytoplankton species *Scenedesmus* sp., *Pediastrum* sp. (Chlorophyta), *Phacus* sp. and *Euglena* Sp. (Euglenophyta) indicated direct relationships with water transparency (Fig. 5). A ridge regression analysis was performed and the results are presented in Table 2. A statistically significant linear correlation was recorded between Bacillariophyceae and

Temperature ($r = 0.932$, $N=24$, $p<0.05$). There was a significant positive correlation ($r = 0.690$, $N = 24$, $p<0.05$) between Chlorophyceae with velocity

and transparency while Euglenophyceae is negatively correlated ($r = -0.739$, $N = 24$, $p<0.05$) with transparency.

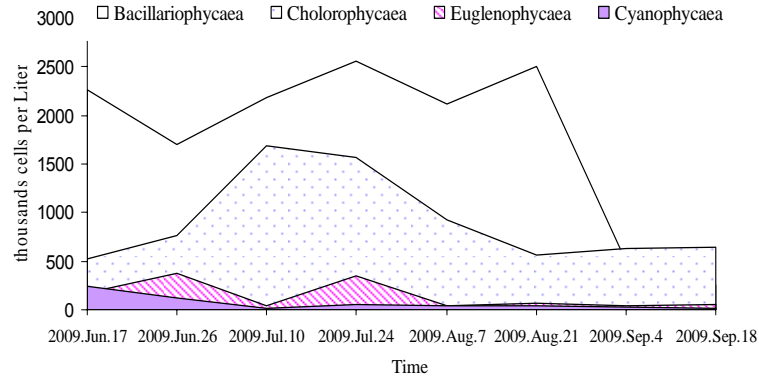


Fig. 2. The Comparison of dominate four Phytoplankton groups in Sefid Rud River

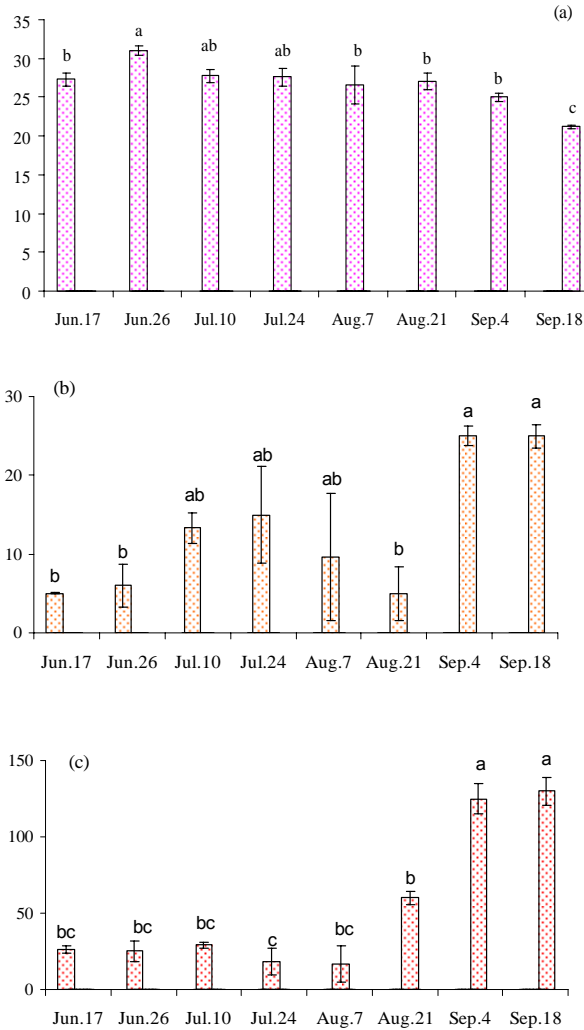


Fig. 3. (a) Water temperature (°C) (b) Water velocity(cm/s), (c) Secchi disk transparency (cm), in sampling stages in Sefid Rud River; One Way ANOVA and Tukey's test at significance level of 5% ($p<0.05$) .

Table 1. Principal Component analysis: Eigenvalues for the first two components

parameters	Component I	Component II
pH	-0.3828	0.1849
Temperature	-0.3902	0.0640
Transparency	0.4105	-0.2628
Velocity	0.3755	0.2893
Bacillariophyceae	-0.3936	0.0805
Choloropyceae	0.2985	0.6271
Euglenophyceae	-0.2736	0.5474
Cyanophyceae	-0.2698	-0.3310

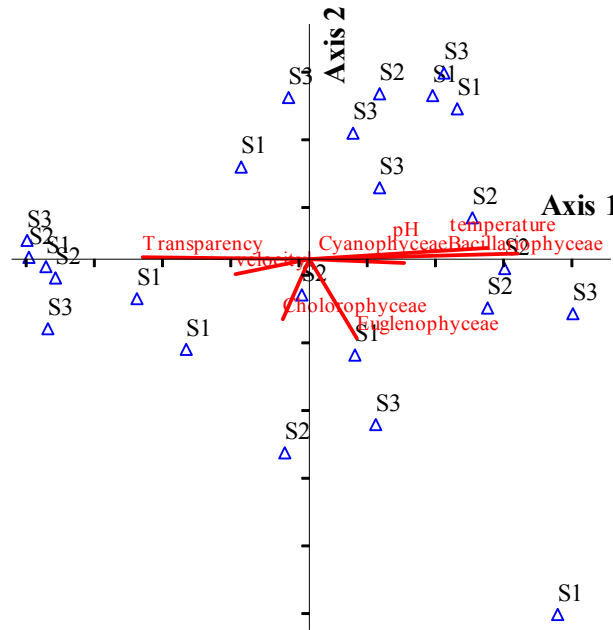


Fig. 4. Principal components analysis: ordination model, with rotation of Varimax axes, of variables and stations, in plane of first two principal components. Two main groups (1, 2) were identified; (1) Choloropyceae, Euglenophyceae, Turbidity and Velocity; (2) pH, Temperature, Bacillariophyceae and Cyanophyceae.

Table 2. Regression analysis

Dependent variable	Variables related with dependent ones			
Bacillariophyceae F=31.58 , df=4 , p≤0.0001	pH 0.657	Temperature 0.910*	Transparency -0.847	Velocity -0.709
Choloropyceae F=4.316 , df=4 , p≤0.05	-0.503	-0.524	0.422*	0.654*
Euglenophyceae F=4.731, df=4 , p≤0.05	0.364	0.625	-0.642*	-0.34
Cyanophyceae F=3.316, df=4 , p≤0.05	0.296	0.396	-0.474	-0.541

F -test values, degrees of freedom (df) and significance levels (p) shown for each dependent variable (left). *: variables with coefficient values significantly correlated with respective dependent variables.

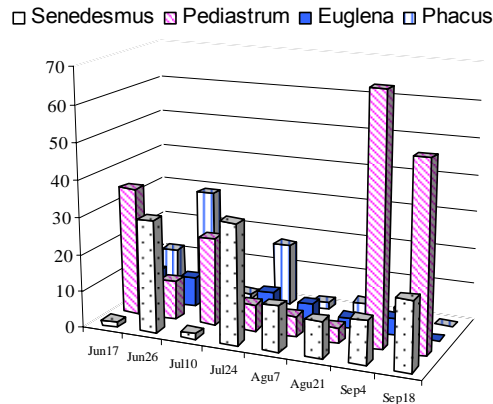


Fig. 5. Four main algae in Sefid Rud River, which indicated direct relationships with the intensity of water transparency

DISCUSSION

The use of phytoplankton for water quality assessment has a long history. Classification schemes based on phytoplankton biomass were developed from more than 20 years ago (Heinonen 1980; OECD 1982; Hillbricht-Ilkowska & Kajak 1986). The water ecosystems are consisting of hydrochemical and biological variables, which are extremely complex, partly due to the strong influence of the many different sources of pollution such as industrial, thermal, urban and agricultural (Bianchi *et al.* 2003). In this frame, chemical parameters, considered independently, may not be sufficient to furnish a complete picture of the environment. Therefore, examination of biological components, in this case phytoplankton, may supply further elements determining the quality of Sefid Rud River.

Since, the set of variables making up environmental characterization often do not act independently, generating self-correlation phenomena: to avoid this, some authors recommend applying a ridge regression analysis to be performed (Draper & Smith, 1981; Bianchi *et al.* 2003).

The obtained results show that high turbidity in stations are influenced by "urban inputs" and "run-off waters" from surrounding land, mainly under agricultural activities with high concentrations of dissolved nutrients and organic particles. Turbidity plays major role in phytoplankton dynamic (Bianchi *et al.* 2003). A key factor which influenced abundance and diversity of phytoplankton was turbidity. Genus *Oscillatoria*

(Cyanophyceae) indicated direct relationships with inputs of agricultural run-off. The genus was observed only in sampling sites and efforts, (station 2 ; in 17 June) influenced by agricultural activities and drainage, with abundance of 18 % (Table 3). Cyanobacteria have a bloom when organic load is low (20 m³/ day) (Amengual-Morro *et al.* 2011). The highest abundance of genus *Anabaena* sp. occurred in late July, in Station 2, synchronized with highest water pollution. Most of the time, a surface scum characterized the water ecosystem when the cyanobacteria are dominant (Kotut *et al.* 2010).

Turbidity of the Sefid Rud River is basically due to heavy erosion in its watershed and flushing of Manjil Dam constructed on this river. The sediments released from the Dam are concentrated and remained in the bottom of the River for several years and delay the recovery of the River. Flooding events suspends this sediment in water column and increases water turbidity. Bacillariophyceae, Chlorophyceae, Euglenophyceae and Cyanophyceae were the dominant phytoplankton group in 25- 30 km river reach from the Sefid Rud River estuary, while in a previous study on planktonic community structure of the Sefid Rud River estuary (Rahimi bashar *et al.* 2011) Bacillariophytes, Cyanophytes, Chlorophyceae, Pyrrophytes and Euglenophytes were the dominant phytoplankton groups.

Maximum load of pollution via "urban inputs" and high turbidity were observed in late July, in sampling site 1. In this period, the highest abundance and bloom

of *Euglena* sp. and *Phacus* sp. (Euglenophyceae) occurred. In another study, the relationships between bloom events and environmental conditions have been reported for Euglenoids (Duttagupt *et al.* 2004 ; Ligez *et al.* 2011). The Euglenophyta are mixotrophic and can

alternate the carbon source, so in higher pollution loads they are more competitive. *Euglena* have the advantage to keep a double ecological niche (Reynolds, 2006), and they can feed from primary production or from particulate organic matter present on water (Amengual-Morro *et al.* 2011).

Table 3. Phytoplankton Genus list, as bio-indicator, for three stations and eight stages in Sefid Rud River (+: Observed)

Stage	1			2			3			4		
Date	2009.Jun.17			2009.Jun.26			2009.Jul.10			2009.Jul. 24		
Station	1	2	3	1	2	3	1	2	3	1	2	3
Bacillariophyceae												
<i>Cyclotella</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cymatoplura</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cymbella</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Gyrosigma</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Nacicula</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Nitzschia</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Surirella</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Thalassiosira</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Synedra</i>	+	+	+	+	+	+	+	+	+	+	+	+
Chlorophyceae												
<i>Binuclearia</i>										+	+	+
<i>Cladophora</i>												
<i>Closterium</i>										+		+
<i>Cosmarium</i>					+	+	+	+		+	+	+
<i>Pandorina</i>				+	+	+	+	+	+	+	+	+
<i>Pediasterum</i>				+	+	+	+	+		+	+	+
<i>Scenedesmus</i>	+	+		+	+	+	+	+	+	+	+	+
Euglenophyceae												
<i>Euglena</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Phacus</i>	+	+	+	+	+	+	+	+		+	+	+
Cyanophyceae												
<i>Anabaena</i>											+	+
<i>Merismopedia</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Oscillatoria</i>		+	+	+	+	+					+	
Stage	5			6			7			8		
Date	2009.Aug.7			2009.Aug.21			2009.Sep.4			2009.Sep.18		
Station	1	2	3	1	2	3	1	2	3	1	2	3
Bacillariophyceae												
<i>Cyclotella</i>	+	+	+	+	+	+	+		+	+	+	
<i>Cymatoplura</i>	+	+	+	+	+	+	+		+		+	
<i>Cymbella</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Gyrosigma</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Nacicula</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Nitzschia</i>	+	+	+	+	+	+	+	+				+
<i>Surirella</i>	+	+	+	+	+	+	+	+				+
<i>Synedra</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Thalassiosira</i>	+	+	+	+	+	+	+	+	+	+	+	+
Chlorophyceae												
<i>Binuclearia</i>												
<i>Cladophora</i>							+	+	+	+	+	+
<i>Closterium</i>				+	+	+	+	+	+			
<i>Cosmarium</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pandorina</i>	+	+	+	+	+	+	+	+	+			
<i>Pediasterum</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Scenedesmus</i>	+	+	+	+	+	+	+	+	+	+	+	+
Euglenophyceae												
<i>Euglena</i>	+	+	+	+	+				+			
<i>Phacus</i>	+	+	+	+	+							
Cyanophyceae												
<i>Anabaena</i>		+	+									
<i>Merismopedia</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Oscillatoria</i>							+					

In the present study, *Merismopedia* sp., was observed in the all samplings with highest abundance coinciding with high turbidity (late July). Chlorophyceae dominated in September and most abundant genera were *Senedesmus* sp. and *Pediastrum* sp. Synchronized with maximum depth, transparency, velocity and consequently reduced pollution load in Sefid Rud River due to rainfall. The highest abundance of *Cladophora* sp., *Pandorina* sp., *Cosmarium* sp. and *Closterium* sp. were found in early September with high water transparency and velocity. In studies on Metal contamination levels in plankton and their role as biological indicator of water pollution in the River Ravi, these genera were indicated direct relationships with the intensity of pollution as these genera were almost absent in highly polluted tributaries (Javed, 2006). Sometimes, when dominated by green algae, the Rivers water exhibited a dark green appearance, which is indicative of a healthy algal population (Mara and Pearson, 1998; Amengual-Morro *et al.* 2011).

In the present study, *Binuclearia lauterbornii* was observed synchronous with flooding of river and low alkalinity in late August (2009. Jul. 27; Stations 1, 2 and 3). In studies on Norwegian rivers using non-diatomaceous benthic algae, to establish a quantitative relationship between algal communities and mean annual pH, an indicator value (IV) of 5.57 was estimated for *Binuclearia tectorum* (Schneider *et al.* 2009).

In conclusion, our findings suggested that phytoplankton of Sefid Rud River could be considered as bio-indicators of water quality in several areas subjected to anthropogenic disturbance. The study showed that spatial and temporal variations in hydro-chemical (inputs of urban and agricultural run-off) and physical parameters (rainfall and flooding of the River, flushing of Manjil Dam) are significant sources of variation and fluctuations in densities of phytoplankton genera, in this Rive and phytoplankton are suitable bio- indicators for environmental changes which may threaten the River and the Caspian Sea.

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فیتوپلانکتون ها به عنوان شاخص زیستی کیفیت آب در رودخانه

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چکیده

فیتوپلانکتون ها نخستین شاخص زیستی آلودگی در اکوسیستم های آبی هستند. اجتماعات فیتوپلانکتونی و اکوسیستم های آبی همواره تحت تاثیر فاکتورهای زیست محیطی قرار دارند. لذا این تغییرات و تهدیدات زیست محیطی در هر اکوسیستمی باید شناخته شوند. فیتوپلانکتون ها از جمله شاخص های زیستی کم هزینه و در دسترس به شمار می روند. در این مقاله، فیتوپلانکتون ها جهت مطالعه رودخانه سفیدرود (جنوب دریای خزر) در ایران مورد استفاده قرار گرفتند. تعداد 3 ایستگاه نمونه برداری در فاصله 25 تا 30 کیلومتری مصب رودخانه انتخاب شدند. نمونه برداری از خرداد تا شهریور 1388 انجام شد. نمونه های فیتوپلانکتونی توسط تور پلانکتون با چشمه 25 میکرون جمع آوری شدند. پارامتر های آب شامل درجه حرارت آب، سرعت جریان آب شفافیت، میانگین شفافیت و pH آب در طی مطالعه اندازه گیری شد. میانگین این پارامتر ها به ترتیب °C 26/9 ± 7/7، 0/13 ± 0/01 m/s، 45 و 7/4 ثبت شد. اجتماعات فیتوپلانکتونی شامل دیاتومه ها (61/2 %)، جلبک های سبز (31/6 %)، اوگلنوفیتا (5 %) و جلبک های سبز آبی (2/23 %) بودند. اختلاف معنی داری در تنوع و فراوانی 4 گروه اصلی فیتوپلانکتون ها در بین مراحل مختلف نمونه برداری مشاهده شد. همبستگی مثبت معنی داری بین شفافیت و فراوانی جلبک های سبز بدست آمد در حالی که همبستگی منفی معنی داری بین شفافیت آب و فراوانی اوگلنوفیتا وجود داشت. بالاترین فراوانی جنس های *Euglena* و *Phacus* (Euglenophyta) در کدورت بالا مشاهده شد در حالی که حداکثر فراوانی جنس های *Pediastrum* sp. و *Scenedesmus* sp. (جلبک های سبز) با شفافیت بالای آب در ارتباط بود. جنس *Oscillatoria* sp. (جلبک سبز آبی) با فراوانی 18 % تنها در ایستگاه هایی که تحت تاثیر فعالیت های کشاورزی بودند، مشاهده شد. این مطالعه نشان داد، فیتوپلانکتون ها شاخص زیستی مناسبی جهت مطالعه تغییرات زیست محیطی هستند که ممکن است رودخانه سفیدرود و دریای خزر را تهدید کند.

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