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Growth, Pigmentation, Photosynthesis and Morphological Characterization of Crude Oil degrading Cyanobacterium *Phormidium* sp. ISC104

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Abstract

The marine environment is highly susceptible to pollution by petroleum, so it is important to understand how microorganisms degrade hydrocarbons. In this research the effects of crude oil on morphological and physiological **c**haracterization of the cyanobacterium *Phormidium* sp**.** ISC104 were investigated. With respect to the physiological responses, cyanobacterium optimum growth was observed in 1% crude oil. The most of chlorophyll content was observed in control sample. Phycobiliproteins had the highest rate in 1% crude oil. According to biodegradation, the results suggested that *Phormidium* sp. ISC104 can reduce oil content by 24.54 and 43.82% after 14 and 28 days respectively. In morphological point of view, dimensions of cells were not significantly impressed, although a slightly decrease was observed in 5 and 7% crude oil in comparison to control. This study demonstrated that crude oil doesn't have destructive effect on cyanobacterium *Phormidium* sp. ISC104 up to 1%, indicating the potential of this cyanbacteriumin biodegrading this pollutant.

Keywords: Biodegradation, Morphology, Oil pollution, *Phormidium*, Physiology.

Introduction

One of the most dangerous pollutants contaminating the environment is oil, which comprises almost 3000 ingredients. Having entered the soil, oil and oil products exert a toxic effect on plants and animals; inhibit the activity of the soil microbiota. The phototrophic microbial complexes are perceived as integrities of the soil microorganisms including algae and cyanobacteria and the microbiota composed of bacteria, micromycetes, and the representatives of the microfauna (Kireeva et al., 2011).

In fact, there is evidence that microbial communities dominated by cyanobacteria can be actively involved in oil degradation (Abed et al., 2002). Some algae that exist in polluted water and soils are being used as indicators of pollution, and some of the selective types of algae make or play the role in the degradation of industrial pollutants (El-Sheekh et al., 2012).

Biodegradation of crude oil to carbon dioxide and water is the major process by which hydrocarbon contaminated environments are re-

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mediated (McGenity et al., 2012).

A wide variety of microbes, particularly bacteria, filamentous, fungi, yeasts, and cyanobacteria are known to be important hydrocarbon degraders in the sea (Raghukumar et al., 2001). Identification of these key organisms is important for understanding and evaluating bioremediation strategies (Harayama et al., 2004). Members of Family Oscillatoriaceae (cyanobacteria) have shown the potential of oil pollutant biodegradation such as naphthalene and biphenyl (Sanchez et al., 2005). *Oscillatoria salina*, degraded crude oil when grown in artificial medium and natural seawater (Raghukumar et al., 2001) and *Oscillatoria* associated aerobic heterotrophic bacteria were responsible for the biodegradation of n-alkanes (Abed and Koster, 2005).

Although cyanobacteria are important members of the microbial community in both aquatic and terrestrial ecosystems, reports are scarce regarding their involvement in hydrocarbon biodegradation (Das and Chandran, 2011). But recently, the ability of cyanobacteria as cosmopolitan oxygenic prototrophs to degrade the PAHs is the centre of considerable research attention (Al Bader et al., 2012).

The plasticity of morphological features, such as morphology of filament, cellular shapes and sizes used for morphospecies identification was studied under varied experimental conditions (Amirlatifi et al., 2013; Zapomelova et al., 2010; Pan et al., 2008). General responses to environmental stress include changes in both cellular morphology and physiology. Environmental stress influences the organisms to inhibit or enhance the functioning of some physiologically important metabolic pathways (Singh et al., 2012).

We wanted to draw attention to morphological, physiologicaland potential of oil biodegradation manner of cyanobacterium *Phormidium* sp. isolated from oil polluted regions of Iran at elevated concentrations of petroleum.

Material and Methods

The strain *Phormidium* sp. ISC104 was isolated from southern polluted soils of Iran. Isolation and purification carried out by solid culture (Andersen, 2005) and afterward liquid medium was used for cultivating. Carbonless BG11 was used as media culture. Temperature was maintained in 30± 2ºC and cultures were bubbled with air under a constant light intensity of 60µmol photon $m^{-2}s^{-1}$. Treatments were including 0 (control), 1, 2.5, 5 and 7% crude oil.

Growth rate was calculated as dry weight (Soltani et al., 2006). Chlorophyll content determined by extraction of cells with pure methanol for 24 hours at 4°C and determination spectrophotometrically at 665 nm according to Marker (1972). Phycobiliproteins were extracted after osmotic shock and measured spectrophotometrically at 652, 615 and 562nm (Bermejo et al., 2002). O_2 evolution was measured with a Clark-type O_2 electrode (Shokravi and Soltani, 2012).

GC analysis had been operated by GC 6890 N, AGILENT to investigate the TPH of treatments. GC analysis was carried out in control sample (without *Phormidium* sp.) and treated sample (containing 1% crude oil), in days 14 and 28 after adding crude oil into the *Phormidium* sp. ISC104 culture (Chaillan et al., 2006). Data were statistically analyzed using a one-

way ANOVA followed by Duncan's new multiple range test using SPSS version 16.0. Three independent variables were considered for each experiment.

To extract DNA from *Phormidium*, a fresh biomass was obtained by centrifuging at 12000 rpm and using Fermentas kit (k0512). The applied PCR condition has been described by Nübel et al. (2000). PCR amplification, cloning and sequence analysis of 16S DNA content was first extracted from the cyanobactrium, and then PCR was applied using two set of primers. Sequences were amplified using the primers 16s-27F (AGA GTT TGA TCC TGG CTC AG) as forward and $16S^{-1}$492R (GGT TAC CTT ACG ACT T), amplify a ~2000bp region of the 16S rRNA gene. PCR products were obtained by electrophoresis in a 1% (w/v) agarose gel using TBE buffer containing DNA set stain.

The sequence was determined by the Genfanavaran Company with the primers. The sequence data was analyzed using a similarity search by using the BLAST through the website of the NCBI.

In order to morphological studies solid medium was prepared and maintained in the same condition as liquid, following inoculation. Semipermanent slides prepared every other day and used by light and florescence microscopes. The following parameters were selected to describe the morphology of the studied strain: Biometrical characteristics; dimensions of vegetative cells, shape of filament, terminal cell and its aggregation in colonies (pan et al., 2008).

Regarding to study of ultrastructure by scanning electron microscopy (SEM), samples were fixed in 2.5% glutaraldehyde for 4 hours and washed in buffer phosphate (PBS). Then samples were centrifuged and dehydrated in successively increasing concentrations of alcohol (10%, 30%, 50%, 70%, 90% and 100%). Finally, all samples were mounted on metal stubs and coated with a layer of gold (Diestra et al., 2007).

Results

Evidence that cyanobacteria are capable of hydrocarbon degradation is tentative, but preliminary studies indicate that some strains are capable of oxidizing aromatic and aliphatic oil constituents (Radwan and Al-Hasan, 2000). Present study focused on both morphological and physiological behavior fluctuations of *Phormidium* sp. ISC104 in elevated crude oil concentrations. This information is interesting, not only for basic science but also for the application point of view. Cyanobacteria and eukaryotic microalgae were capable of bio transforming naphthalene to more water soluble phenol, 1-naphthol. The algae could use nitro and aminosubstituents, from amino naphthalenes, and amino- and nitrobenzoates as nitrogen sources, and chlorobenzoates could be dehalogenated and the chloride being accumulated by the cells (El-Sheekh et al., 2012).

According to physiological results the presence of the crude oil didn't abolish the growth (Fig. 1). The highest growth rate was observed in control sample and 1% crude oil, which was significantly different with other treatments (ANOVA, >0.05). The culture with 1% oil concentration attained a maximum standing biomass of about 6.92 $mgml^{-1}$ at the 12 day, while the minimum one was belonged to 2.5% oil concentration (4.98 $mgml^{-1}$). As can be seen in Figure 1, the optimal growth occurred in the

presence of light and 1% crude oil under aerobic conditions.

The photosynthetic pigments concentrations are shown in different crude oil treatments (Table 1). The crude oil did not affect on some photosynthetic pigments. In the case of chlorophyll content, remarkable difference in crude oil treatments was seen, and the highest amount of chlorophyll, 1.33µgmgdw^{-1}, belonged to control. Nevertheless the highest amount of phycobiliprotein was distinguished in 1% crude oil and it decreased in control and other treatments significantly. No significant difference was detected in PC and PE, but the maximum amount of APC was observed in control and minimum in 1% crude oil.

Extra information was achieved by physiological experiments. Numerous reports of effects of oil hydrocarbons on cyanobacteria have been published (Babaei et al., 2013; Amirlatifi et al., 2013; Diestra et al., 2007). Cyanobacteria have long history of adaptation to survive in extreme or variable environments by developing specific regulatory systems in addition to more general mechanisms equivalent to those of other prokaryotes or photosynthetic eukaryotes (Singh et al., 2012).The results imply physiological flexibility of this strain. The treatment of algal culture with 1% crude oil led to prolongation the growth phase as well as high algal biomass production. Singh et al. (2012) explained the reason of changes phycobilisome rod pigmentation *Oscillatoria* sp. as exposed to various condition. Acclimation includes synthesis of specific polypeptides of light harvesting antenna complex or phycobilisomes. In *Phormidium* sp. ISC104, PC is the main component of phycobiliproteins, so the changes on total PBP mostly reflect the changes in PC. Total PBP and PC were affected by oil concentrations. This strategy is similar to other exteme conditions and confirms the results of Soltani et al. (2006); but Babaei et al., (2013) did not show any increasing of PBP in 1% crude oil of *Anabaena* sp. ISC 55. Decreasing pattern with increasing oil concentration is similar in both researches.

Also the growth rate in different concentrations of crude oil demonstrated the ability of consumption of both CO_2 and hydrocarbons by *Phormidium* sp. ISC104. Of course different types of crude oil have distinct effects on cyanobacteria (Diestra et al., 2007). Raghukumar et al. (2001) showed mixed culture of *Oscillatoria* sp. has good growth in the pres ence of crude oil and also degraded it substantially. These cultures formed excellent cyanobacterial mats when grown in mixed cultures, and thus have the potential for use in mitigating oil pollution on seashores, either individually or in combination. Our results are in agreement with Ibrahim and Gamila et al. (2004) who studied the effect of 0.1% crude oil on algal growth. Gaur and Singh (1990) carried out the growth experiment under crude oil treatments. They concluded that oil inhibited the growth of the studied strain. As the cyanobacterial cultures were grown photoautotrophically, the degradation of the crude oil is a means of detoxification of the environment (Narro et al., 1992).

Results did not show significant differences among the size of phycobilisomes which is usually represented by the ratio (PE+PC)/APC (Wyman and Fay, 1986) (Table 2).

The ratios of PBP/Chlorophyll are used to show the relationship between photosystem II

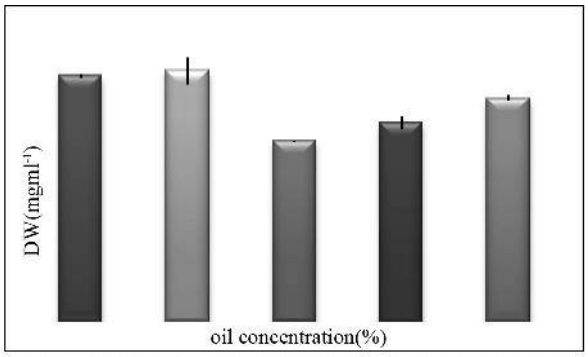

Fig. 1. Dry weight amounts in different crude oil concentrations.

Table 1. Photosynthetic pigments amount in different crude oil concentrations in *Phormidium* sp. ISC104 grown under the above conditions. Data shows X±SE.

Crude oil (%)	APC	PC	PE ($\mu gmgdw^{-1}$)	Chl	PBP
0	1.69±0.21 ^{b}	3.80±0.53 ^{a}	0.08±0.05 ^{a}	1.33±0.19 ^{b}	37.88±2.98 ^{a,b}
1	0.29±0.25 ^{a}	5.99±2.15 ^{a}	1.67±2.90 ^{a}	0.85±0.13 ^{a}	55.05±5.86 ^{c}
2.5	1.04±0.35 ^{a,b}	5.93±0.90 ^{a}	1.66±0.17 ^{a}	1.06± 0.17 ^{a,b}	42.94±2.74 ^{b,c}
5	1.44±0.64^{a,b}	3.15±1.56 ^{a}	2.40±0.08 ^{a}	0.85±0.11 ^{a}	38.18±5.25 ^{a,b}
7	0.65± 0.74 ^{a,b}	2.16±1.46 ^{a}	1.11±0.73 ^{a}	0.76±0.12 ^{a}	24.20±7.79 ^{a}

Chl: Chlorophyll PBP: Phycobiliproteins APC: Allophycocyanin PC: Phycocyanin PE: Phycoerythrin

and photosystem I (Poza- Carrión et al., 2001). Significant difference was observed between control and other treatments. Considering the results of the pigments, the rate of photosynthesis is more or less related and explainable. No significant difference was detected in photosynthesis and the highest rate was 2.93± 2.34 nmol O_2 μgdw^{-1} min^{-1} in 2.5% crude oil (Table 2). Results of photosynthesis rate are compatible with the Sundaram and Soumya (2011). We concluded that adding crude oil can not affected on photosynthesis rate significantly. This result is in conflict with Cohen (2002).

The GC results of 1% crude oil showed that the TPH of treatment in day 14 decreased 24.54%; however, this rate was reduced to 43.82% in day 28 (Data not shown).

Cyanobacteria are able to metabolize natural aromatic hydrocarbons and xenobiotics. Since cyanobacteria utilize crude oil and individual n-alkanes as carbon and energy sources, microbial cyanobacterial mats participate in decontamination of oil-polluted water (Koksharova, 2010). Degradation of polycyclic and heterocyclic aromatic compounds by algae seems to be related to the molecular structure of the compound. The compound degradation is related to the physiological metabolism of the algae. The pathway was believed to be common in all microorganisms involved in aromatic metabolism, including denitrifers, sulfate reducers, and fermenters.

According to the morphological variation due to flexibility of cyanobacteria under oil treatment, cell morphology and colonization in liquid cultures were examined by light and electron scanning microscope.

The morphological characteristics of the *Phormidium* sp. ISC 104 are summarized in (Table 3). This strain had straight or curved trichomes which were solitary (Table 1, Fig. 2). The sample was non-heterocystous and vegetative cells

were more Square or rectangle. The terminal cell of strain was crooked tip (conical or completely tapered), that clearly distinguishable from vegetative cells. In the treatments distance between cells or cell wall compression increased (wall constricted). As seen in the Table 1, the width of vegetative cells decreased significantly with enhancing the oil concentration but the length of vegetative cells did not change significantly. The maximum width was observed in 1% crude oil and minimum in 5 and 7% crude oil. These results covered the physiological observations.

The images obtained by SEM indicated that in 7% oil a slightly decrease of length of the cells and also the relative degeneration of walls was observed. Also the hyaline mucilaginous sheath was observed clearly in control and treatments. The fact that draw attention to the morphological studies is that all environmental variables were shown to be important and influence the cyanobacterial behavior (Jezberova and Komarkova, 2007). Identification of these genera is based on morphological features such as morphology of the filament, vegetative cells. The form of the colony, shape of terminal cells, presence of sheath and as well as life cycle, are additional features used for the identification of some genera (Pan et al., 2008; Sili et al., 2011). The cell dimensions of the strain studied

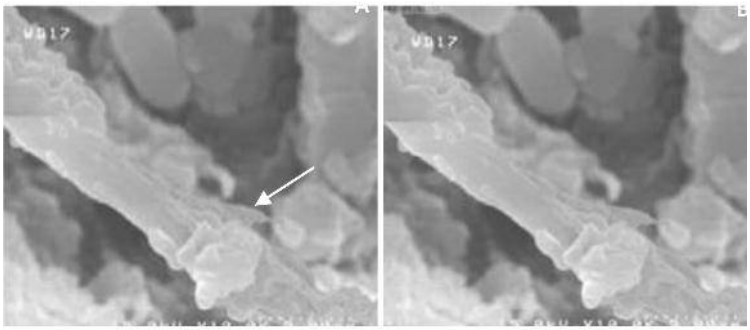

Fig. 2. Scanning electron microscopy of control *Phormidium* sp. ISC104 filaments (Left) and treated with 7% crude oil (Right). Arrows show the filament tip.

Table 3. The morphological characteristics of *Phormidium* sp.ISC104 grown under the above conditions. Data shows X±SE.

morphological characteristics	Crude oil (%)				
	Control (0)	1	2.5	5	7
Morphology of filaments	Solitary, curved	Solitary, slightly curved	Solitary, Curved	Solitary or bundled, curved	Solitary or bundled, curved
Mucilage sheath	+	+	+	+	+
Terminal cell	crooked tip	crooked tip	crooked tip	crooked tip	crooked tip
Vegetative cells shape	Cylindrical	cylindrical	Cylindrical	Cylindrical	Cylindrical
Vegetative Width (µm)	5.68±0.42 ^{b§}	6.61±0.36 ^{b}	5.82±0.60 ^{b}	3.96±0.82 ^{a}	3.97±0.41^{a}
Vegetative Length (µm)	4.06±0.30 ^{a}	3.97±0.84 ^{a}	4.17±0.90 ^{a}	3.14±0.44 ^{a}	3.77±1.02 ^{a}

§ dissimilar characters show significant difference between means (ANOVA<0.05).

turned out to be very variable. Although cell elongation was described as a feature adapted to extreme conditions, we observed maximum elongation under 1% crude oil. Detailed biometrical observations of this strain showed no remarkable change in vegetative length. These results confirm the appropriate conditions of treatments for *Phormidium* sp. ISC104. In addition, electron microscopy techniques (SEM) supported these structures.

This research was enriched with molecular techniques to confirm the morphological identification of *Phormidium* sp. ISC104. Phylogenetic analysis was carried out based on the 16S rRNA gene sequence for strain *Phormidium* sp. ISC104. The sequences were compared with those of representative cyanobacteria available in GenBank (http://www.ncbi.nlm.nih.gov/Blast).

The 16S rRNA sequences were combined with other *Phormidium* species available in the database (Casamatta et al. 2003). The nucleotide sequences described in this study have been submitted to the NCBI under the accession number NCBI: KF443074.

Discussion

In conclusion, recent reports have demonstrated that photosynthetic microorganisms, particularly cyanobacteria, may play a direct or indirect role in the metabolism and degradation of hydrocarbons. Our results demonstrated the ability of *Phormidium* sp. ISC104 not only to retain its survival in the presence of crude oil but also to biodegrade of it. This potential has been shown in other cyanobacteria such as *Anabaena cylindrical*, *P. faveolarum*, *Oscillatoria* sp. strain JCM, and *Agmenellum quadruplicatum*, all of which can degrade different aromatic compounds (Cohen, 2002). Cyanobacteria are present in association with oil-degrading bacteria and prevent them from being washed out, by immobilizing them in their extracellular polysaccharide. In addition, cyanobacteria also supply these bacteria with oxygen and fixed nitrogen. This indirect role of cyanobacteria can be important to the overall success of the biodegradation process (Abed et al., 2002).

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Screening of Thermophilic Cyanobacteria from some hot Springs of Iran

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Abstract

Hot springs have been a subject of intense discussion for biologists in the last decades. The polyphasic approach is the most progressive system that has been suggested for distinguishing and phylogenetically classifying cyanobacteria. In a revision of the cyanobacteria in hot springs of Iran, four hot springs of Iran were investigated. We developed a combined molecular and morphological approach to identification of cyanobacteria. In this study seventeen populations and 13 morphological characters were analyzed. Molecular study based on 16S rRNA gene sequence does not disrupt morphological information and it confirms the separation of studied taxa according to morphological characters. Based on their growth characteristics; seven species were selected for the production of antimicrobial agents. The results of this study showed that the methanol extracts exhibited high antimicrobial activity against some gram positive bacteria, moderate antibacterial activity against some gram negative organisms and moderate antifungal activity against some fungi. Four species were selected for salinity tolerance. The results showed high tolerance of these taxa to salinity.

Key words: Cyanobacteria, Hot Spring, Morphological Diversity, 16S rRNA, Polyphasic study.

Introduction

The high temperatures associated with geothermal activity frequently result in surface and subsurface geothermal springs, commonly known as “hot springs” (Ghozzi et al., 2013). In general, these ecosystems inhabit with population of microorganisms with great commercial importance and interest to the researchers and industry working on enzymes, sugars, compatible solutes and antibiotics (Zakaria and Abdulrahman, 2007). Several investigations of microalgae and cyanobacteria flora for many sources of springs have reported a dominance of prokaryotic organisms (Brock, 1978; Castenholz, 2001; Ward et al., 2000; Sompong et al., 2005), and small eukaryotes (e.g., diatoms, unicellular algae) (Jonathan and John, 2009). Despite the possibility of the presence of novel taxa with high economic and industrial use very few reports are available on the cyanobacterial diversity of hot springs from Iran.

The polyphasic taxonomy, which includes a combination of morphological, ultra structural, ecophysiological, biochemical, and molecular characters, was the most progressive system that has been suggested for distinguishing

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and phylogenetically classifying cyanobacteria (Stoyanov et al., 2014). Cultivation-dependent studies are valuable for isolating novel organisms and exploring their properties. The study was designed to investigate cyanobacterial mats by using morphological and molecular approach and a physiological study of hot spring cyanobacterial samples. The findings revealed the presence of a large number of cyanobacterial taxa and test extracts from various cyanobacteria isolated from hot spring against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and fungi to evaluate its antibacterial and antifungal activities. The widespread distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as temperature, pH, desiccation, etc. Salt stress is one the limiting factors on the growth and productivity of microorganisms. They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors (Soltani et al., 2007). In this research we showed the effect of salinity on growth some cyanobacteria isolated. Interest in antimicrobial and physiological activity of cyanobacteria isolated from hot springs of Iran under salinity stress has been studied in this work.

Materials and Methods

Sampling and Morphological study

Water samples were collected from four hot springs in Geno, Khamir, Chahahmad and Ramsar (Table 1). Samples were streaking on agar nitrate BG-11 medium (Stanier et al., 1971), and incubated in a culture chamber at 25°C and a 12/12 h light-dark cycle in artificial illumination (37- 46 $\mu molm^{-2}s^{-1}$) for two weeks. Isolation involved from removing colonies that developed in medium and observed under light microscope. Taxonomic determination was carried out by light microscopy (Olympus, Model BM-2) and based on Komarek and Anagnostidis (1989, 1998, 2005). Identification was carried out by morphometric method. Seventeen morphological characters and numerical taxonomic studies were used for classifying the various species of cyanobacteria. Taxonomic analysis was performed with cluster analysis and principal component analysis. A data matrix based on the coded multiple states of characters were used in this study (Table 3). Cluster analysis using the UPGMA method (unweighted pair-group method with arithmetical averages) was carried out. Phenetic relationships among the species were constructed. All analyses were carried out using SPSS (Ver.16).

DNA extraction, PCR amplification and sequencing

The bulk of cyanobacterial culture isolation was extracted by genomic DNA extraction kit AccuPrep (Bioneer). 25 mg of each sample in a 1.5ml tube with 200µl lysis bufferand 20µl of ProteinaseK were homogenized. The tubes were incubated for 1 h at 60°C. 200 µl Binding Buffer was added. After mixing gently for 10 minutes, the samples were incubated at 60°C. 100 µl Isopropanol was added. The supernatant was then transferred to a 2 ml tube. After a final gentle mixing centrifugation for 5 min at 8,000 rpm, 150 µl elution buffer was added. Extracted DNA was harvested at -20°C temperature.

For DNA amplification, the 16S rRNA gene regions, approximately 600 bp in length, were

amplified by PCR using the A2 (5- AGAGTTTGATCCTGGCTCAG-3) and S8 (5-TCTACGCATTTCACCGCTAC-3) primers (Giovannoni et al., 1988). Each reaction contained 2.8 µl $MgCl_2$, 150 mMd NTPs, 0.5 µM of each primer, approximately 50 ng template DNA, 3 µl Taq polymerase in a total volume of 100 µl. For PCR amplification cycle using the cyano

Table 1. Some physio-chemical parameters of four hot springs of Iran.

Parameters	Geno	Chahahmad	Khamir	Ramsar
Location	Hormozgan province, Bandar Abbas city	Hormozgan province, Khamir city	Hormozgan province, Khamir city	Mazandaran province, Ramsar city
Latiude/Longitude	26° 56′ 55″ N 55° 32′ 27″ E	26° 56′ 31″ N 55° 26′ 17″ E	26° 56′ 46″ N 55° 33′ 23″ E	36° 55′ 20″ N 50° 39′ 30″ E
Temp (°C)	40	39	37	50
Ph	7/2	6/9	7	6/8
EC ($ms\ cm^{-1}$)	14/02	20	20	13.3
Na^{+} ($mg\ L^{-1}$)	1600	2400	2200	410
K^{+} ($mg\ L^{-1}$)	580	400	200	240
Ca^{++} ($mg\ L^{-1}$)	800	3000	1440	1100
Mg^{++} ($mg\ L^{-1}$)	475	950	710	1100
Cl^{-} ($mg\ L^{-1}$)	4560	18520	9210	3504
PO_4^{-3} ($mg\ L^{-1}$)	14/02	300	400	400
SO_4^{-2} ($mg\ L^{-1}$)	1600	152	1374	1800
NO_3^{-} ($mg\ L^{-1}$)	0.7-1.0	1.1- 1.3	1.3-1.4	0.8-13.6
Total alkalinity ($mg\ L^{-1}$)	135	150	160	244

Table 2. Total variance of factors according to principal component analysis of morphological characters.

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	%of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.144	39.566	39.566	5.144	39.566	39.566	3.256	25.045	25.045
2	2.131	16.392	55.958	2.131	16.392	55.958	3.172	24.401	49.446
3	1.696	13.046	69.004	1.696	13.046	69.004	1.730	13.305	62.751
4	1.372	10.557	79.561	1.372	10.557	79.561	1.690	12.997	75.748
5	1.135	8.734	88.295	1.135	8.734	88.295	1.631	12.547	88.295
6	.875	6.730	95.025						
7	.282	2.167	97.192						
8	.230	1.770	98.962						
9	.086	.659	99.622						
10	.041	.316	99.938						
11	.007	.055	99.993						
12	.001	.007	100.000						
13	-7.371E-16	-5.670E-15	100.000						

Extraction Method: Principal Component Analysis.

bacterial primers was 4 min at 95°C, then 30 cycles of 1 min denaturation at 95°C, 1 min annealing at 59°C, 2 min extension at 72°C, and a final extension of 8 min at 72°C.

Phylogenetic analysis

Multiple alignments were created with reference to the selected GenBank sequences using BioEdit which implements the Clustal W multiple alignment algorithm. Sequences were aligned with the software MEGA (version 5). The Neighbor-joining method was used to compute evolutionary distances in present study. In this program, bootstrap analysis was used to evaluate the tree topologies by performing 1000 resamplings. The tree was rooted using the *Bacillus subtilis* 16S rRNA sequence as an out-group.

Experiments for physiological characteristics

Six different salinities (0%, 3%, 6%, 9%, 12% and 15%,) were obtained by preparing BG-11 medium in various ratios of NaCl in distilled water. Four cyanobacterial isolation *Oscillatoria subbrevies, Chroococcus minimus, Synechocystis aquatilis, Synechococcus* elongates were selected and were cultured in different salinities media. All cultures for salinity tolerance were incubated in a culture chamber. The growth rate was measured over a period of five days.

Microbial Study

The antimicrobial activity of cyanobacteria extracts isolated from hot spring were individually tested against a panel of microorganisms, including; *Bacillus subtilis* (ATCC 465), *Enterococcus fecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 85327), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763). Bacterial strains were cultured activity. Overnight at 37°C in Mueller Hinton agar (MHA). Yeast Data on antimicrobial activity in terms of inhibition was cultured overnight at 30°C in Sabouraud's dextrose zones exhibited by the cyanobacteria methanol extract agar (SDA). Antimicrobial screening by the Disk Diffusion Method was applied for determination of antimicrobial activity of the prepared methanol extracts.

Extracts were dissolved in dimethyl sulfoxide (DMSO). A suspension of tested microorganism (0.1 ml of 108 cells/ml) was spread over surface of agar plates (MHA and SDA). Filter papers having a diameter of 6 mm, soaked with 25µl of each extracts were placed on the inoculated agar plates. then all Petri dishes were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for yeast. The diameters of the inhibition zones were measured in millimeters. Minimum Inhibitory Concentrations (MIC) were determined by broth micro dilution assay recommended by the [NCCLS].

Statistical Analysis

The statistical analysis was performed with stratis graph (Centurion XV) and Excel software. The multi-factorial ANOVA analysis was followed by the Tukey multiple comparison tests for statistical comparisons. P-value of less than 0.05 was assumed for significant differences.

Results

In morphological study, 43 distinct morphospecies of cyanobacteria were character-

Table 3. Rotated component matrix according to PCA analysis of morphological characters.

	Component				
	1	2	3	4	5
VAR00001	.267	.309	.844	-.027	.045
VAR00002	.872	.367	.110	-.009	.210
VAR00003	-.072	.030	-.110	.938	.004
VAR00004	.943	.203	.178	.035	.148
VAR00005	.092	.973	.051	-.030	.076
VAR00006	.685	.599	.181	.097	.345
VAR00007	-.041	-.345	.660	.335	.370
VAR00008	.097	.898	.105	.275	.156
VAR00009	-.045	.075	.169	-.179	.694
VAR00010	.836	-.463	.064	-.061	-.175
VAR00011	.459	.625	.598	.001	-.081
VAR00012	.101	.181	.291	.755	-.174
VAR00013	.347	.157	-.064	.061	.854

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 9 iterations.

Table 4. GenBank code of samples.

	Sample	**GenBank code**
1	*Synechocystis sp.*	HQ900669.1
2	*Synechocystis sp.*	AB039001.1
3	*Synechocystis sp.*	HQ900668.1
4	*Synechococcuselongatus str. Ramsar*	JQ771323.1
5	*Synechococcus sp.*	AF448077.1
6	*Oscillatoria sp.*	EF150796.1
7	*Phormidiumirriguum f. minor*	FN813342.1
8	*Lyngbya sp.*	AY049752.1
9	*Lyngbya sp.*	AY049751.1
10	*Spirulina sp.*	DQ058861.1
11	*Phormidium sp.*	HM217057.1

ized using light microscopy. The lowest species diversity was observed in Nostocales and the highest species diversity belonged to Oscillatoriales. Among the genera were identified, *Oscillatoria*, *Phormidium*, *Chroococcus* and *Spirulina* showed the highest number of species. *Oscillatoria subbrevis*, *Jaaginema metaphyticum* (*Syn.: Oscillatoria angusta*) and *Spirulina subsalsa* was observed in 3 stations of each hot spring. In total 17 morphospecies of cyanobacteria from 4 hot springs by using 13 most stable quantitative and qualitative characters used for clustering analysis (Table 3). Results of this

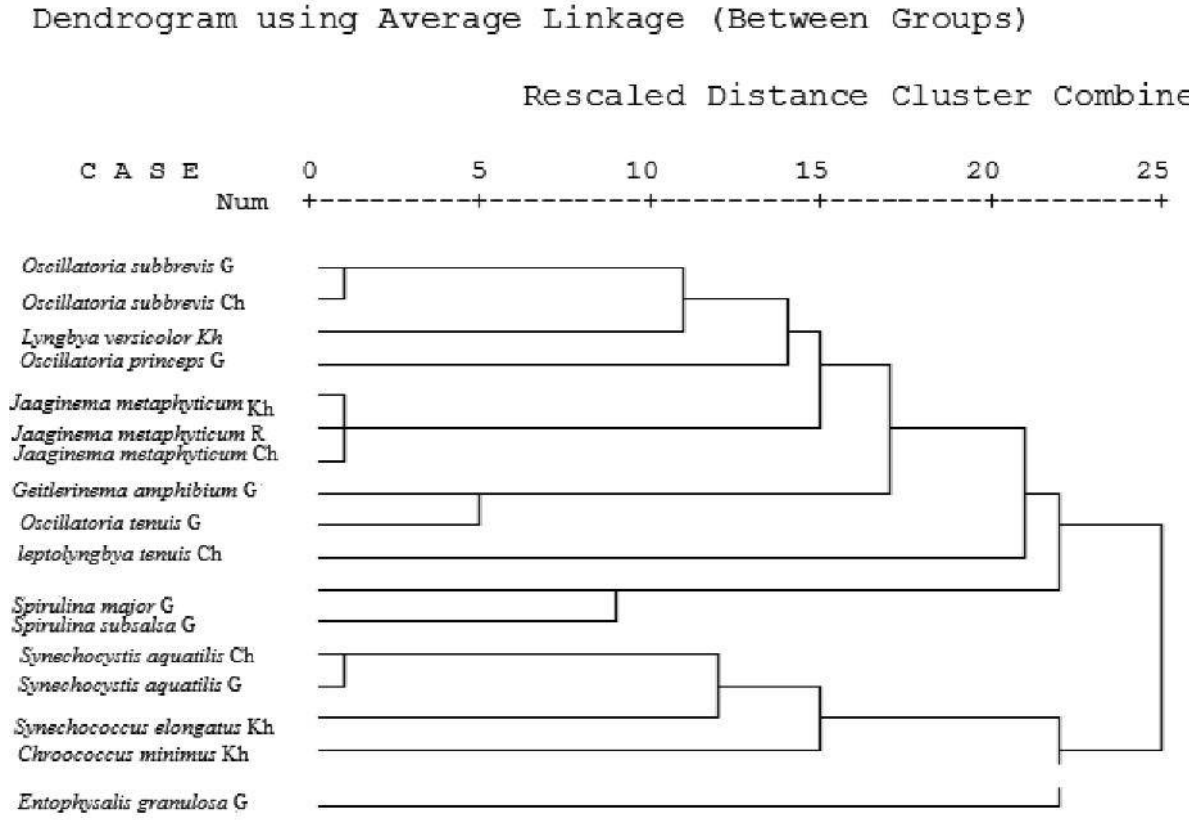

Fig. 1. Hierarchical cluster analysis dendrogram of cyanobacteria taxa based on morphological characters u UPGMA method.

Table 5. Antimicrobial activity of cyanobacteria methanol extract isolated from hot springs of Iran.

	OS.SUB		OS.TEN		OS.LIM		OS.ANG		OS.ARTI		SYN.AQU		SYN.CER	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
B.SUB	20	3.75	18	3.75	19	3.75	16	7.5	19	3.75	22	3.75	20	3.75
B.PUM	22	3.75	20	3.75	18	3.75	17	7.5	20	3.75	20	3.75	19	3.75
E.FAE	14	15	10	15	11	15	0	-	15	7.5	16	7.5	14	7.5
S.AU	16	7.5	12	7.5	14	7.5	12	15	15	7.5	17	3.75	15	7.5
S.EP	24	3.75	20	3.75	17	3.75	14	7.5	19	3.75	23	3.75	22	3.75
E.coli	15	7.5	11	15	14	15	10	>15	14	15	17	7.5	15	3.75
P.AER	0	-	0	-	0	-	0	-	0	-	0	-	0	-
K.PNE	0	-	0	-	0	-	0	-	0	-	0	-	0	-
CAN	0	-	0	-	0	-	0	-	0	-	12	15	0	-
SAC	12	15	0	-	10	>15	0		12	15	16	7.5	14	7.5

[a] Inhibition Zone includes diameter of disc (6 mm). [b]Minimum inhibitory concentration values as mg/ml.
abbreviations: OS. SUB (*Oscillatoria subbrevis*), OS. TEN (*O. tenuis*),OS.LIM (*O. limentica*), OS. ANG (*O. angusta*), OS. ARTI (*O. articulate*), SYN. AQU (*Synechocystis aquatilis*), SYN. CER (*Synechococus cerdorum*). B. SUB(*Bacillus subtilis*), B. PUM (*B. pumulis*), E.FAE (*Enterococcus faecalis*), S.AU (*Staphylococcus aureus*), S.EP (*S. epidermidis*), E. COLI (*Escherichia coli*), P. AER (*Pseudomonas aeruginosa*), K. PNE (*Klebsiella pneumonia*), CAN (*Candida albicans*) and SAC (*Saccharomyces cerevisiae*).Inactive (-); moderately active (7 - 14); highly active (> 14); nt, not tested. Values are given as mean + standard deviation.

study showed that the selective morphological characters separate genera but they did not separate them in species rank. Although, single cell strains were separated completely from filamentous forms. Some of filamentous samples due to their morphological similarities were not completely separated from each other. In numerical taxonomic study according to moropphological char acters, it was considered that the species *Jaaginema metaphyticum* (Syn: *Oscillatoria angusta*) and *Geitlerinema amphibium* (Syn: *Oscillatoria amphibian*) are grouped to their pervious genus *Oscillatoria* (Fig. 1).

To discover the most variable characters among the several morphological features, PCA carried out. The analysis revealed that the first five factors comprise about 88.2% of total variance. In the first factor with about 25% of total variance, characters of trichome, vegetative cell, form of spiral and kind of proliferation possessed the highest positive correlation. In the second factor with about 24% of total variance, characters of apical cell shape and filament width possessed the highest positive correlation (Table 5). In phylogenetically analysis, for drawing cladogram by using the analysis of data of sequence of genome in 16S rRNA region, the genome sequences of 22 samples were used. Among the studied samples, 11 samples belonged to cyanobacteria of hot springs from four regions of Geno, Chahahmad, Khamir and Ramsar. In the phylogenetic tree by algorithm neighbor-joining based on gene sequence 16S rRNA the distinctive primary clustering of the sample of out-group from taxa of photosynthetic prokaryotes from cyanobacteria were analyzed. In clusters, the cluster containing all of the analyzed cyanobacteria, two minor groups are recognizable. In one of minor clusters, all the unicellular samples belonged to *Synechoccoccus, Synechocystis, Chroococcus* with the bootstrap value of 99% placed in a unique group, and in other cluster *Spirulina* and *Oscillatoria* were presented (Fig. 2).

Based on cyanobacteria growth characteristics; seven species namely *Oscillatoria subbrevis*, *O. tenuis*, *O. limentica O. angusta*, *O. articulate*, *Synechocystis aquatilis* and *Synechoccous cerdorum* were selected for antimicrobial activity. Data on antimicrobial activity in terms of inhibition zones exhibited by the cyanobacteria methanol extract were shown in Table 5. Result showed that all cyanobacteria extracts were found to have high activity against *B. subtilis*, *S. epidermidis* and *B. pumullis P. aeruginosa* and *K. pneumoniae* were resistant to all tested algal extracts. All extracts inhibited slightly the growth of *E. coli*, *S. aureus* and *E. fecalis* (Table 5).

Results obtained from disc diffusion method, followed by measurements of MIC, indicated that *S. epidermidi*s was the most sensitive among tested organisms, since the methanol extract of cyanobacteria showed lowest MIC value (3.75 mg/ml) (Table 5).

Results of salinity tolerance showed despite *Oscillatoria subbrevies* peresence in all of the hot springs but have best growth rate in culture media without salinity. Three unicellular cyanobacterial samples in 3% and 6% salinity have better growth rate than without salinity culture media. Moreover, the results indicated that unicellular cyanobacterial isolated (*Synechoccoccus elongates, Synechocystis aquatilis*) with higher growth rates in NaCl treatments, have the maximum level of antimicrobial activity.

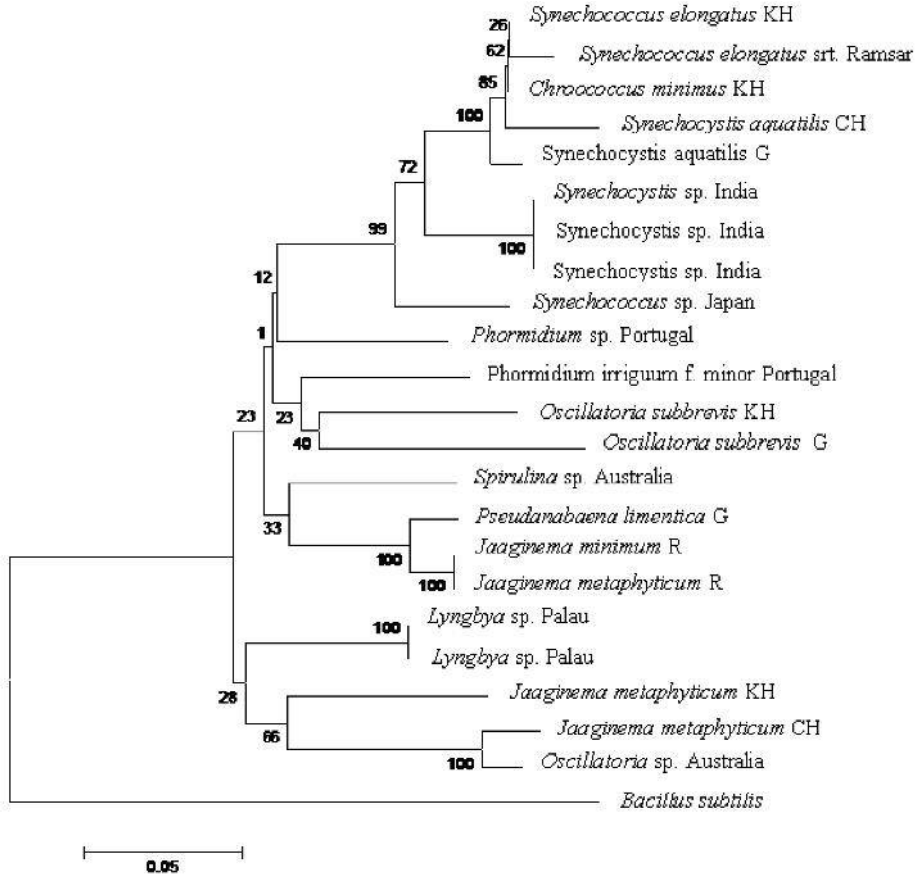

Fig. 2. Phylogenetic relationship for the cyanobacteria taxa was constructed using partial 16S rRNA gene sequences.

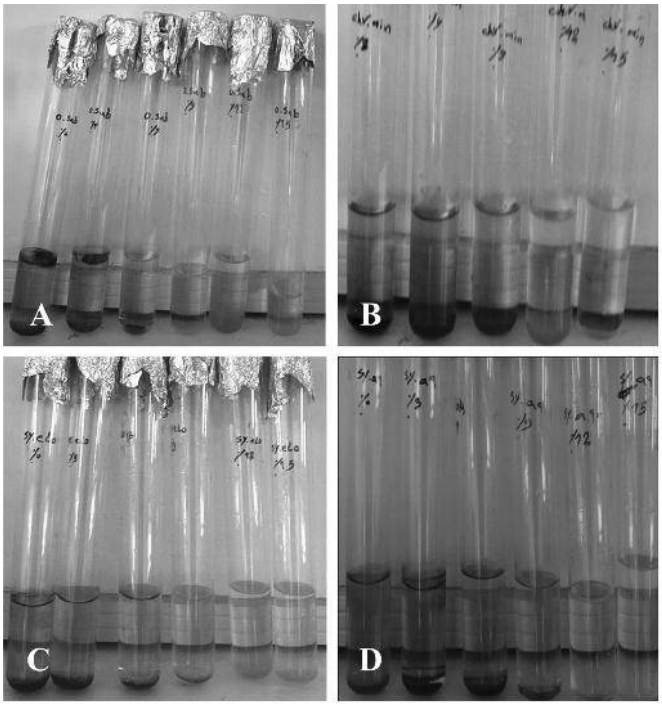

Fig. 3. A: *Oscillatoria subbrevies*, B: *Chroococcus minimum*, C: *Synechococcus elongates*, D: *Synechocystis aquatilis*.

Discussion

Temperature is one of the most important parameter for cyanobacterial species diversity in microbial mat of hot springs. The studies revealed that cyanobacterial diversity and complexity decreased with increasing temperature

(Ferris, 1996; Ferris and Ward, 1997; Hongmei et al., 2005). Skimisdottir (2000) showed that in thermal gradients from 50˚C to 75˚C, the layered mats are characterized by the presence of unicellular forms like *Synechococcus*. The cyanobacterial mats occurring at the lower end of thermophily (40-50˚C) are often dominated by morphologically defined filamentous cyanobacteria like *Phormidium, Oscillatoria, Pseudanabaena, Calothrix* and *Fischerella* (Ward and *Castenholz,* 2000; Marteinsson et al., 2001; Sompong et al., 2008). However, Norris et al. (2002) reported that cyanobacteria such as *Synechococcous* also co-occurs with other unicellular and filamentous forms at lower temperature. In the present study, *Synechococcous* have been found in the mats that grow between 30-40˚C. Some workers also focus on the role of pH and combined nitrogen (especially ammonium), on the species distribution in cyanobacterial mat community below 60˚C (Ward and Castenholz, 2002; Sompong et al., 2005). Results of this study also support the previous studies. Diazo trophic cyanobacteria are able to colonize in the springs where nitrogen levels are lower than proper condition for the other taxa. Conversely, they may be out-competed by non diazotrophic cyanobacteria in spring with sufficient combined nitrogen (Ward and Castenholz, 2002).

According to this fact that the grouping of taxa according to morphological characters is not sufficient, especially in complex taxa such as *Jaaginema metaphyticum*, 16S rRNA gene sequencing was used for better sample recognition.

In conclusion, we report here that the molecular study confirmed results of morphological classification. Genomic sequences also don't provide the necessary information for separation of taxa from pervious groups (*Oscillatoria angusta, Oscillatoria amphibian, Oscillatoria limentica* and *Phormidium tenue*) and the separated taxa remain close to their pervious groups.

It can be concluded from this study that the extract obtained from some cyanobacteria strains isolated from the hot spring showed antimicrobial activity against the pathogens used in the present investigation. Regarding physiological responses of cyanobacteria isolated to NaCl as shown in Figure 3, the growth rate decreased with increase in salinity though it continued in NaCl 6%. These data were also seen in *Nostoc* sp. (Sekar and Subramanian, 1999). Further researches should be made to identify and purify natural products from these cyanobacteria with antimicrobial activity and high salinity tolerance.

Acknowledgments

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Dried Seaweed (*Sargassum ilicifolium*) as an Adsorbent for Phosphorous Removal from Aqueous Solutions

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Abstract

Aquaculture is a source of significant amounts of wastes, which generally leads to deterioration of water quality. Removal of phosphorous (P) from aquaculture wastewater is an important environmental challenge. In the present study, efficacy of dry sea weed (*Sargassum ilicifolium*) to remove water P was investigated under laboratory conditions. Several levels of medium pH (3.5-10), initial P concentration (0.015-0.45mgl^{-1}), contact time (7-60min), particle size (0.5-5mm) and the sea weed particle concentration (10-40gl^{-1}) have been monitored. The results showed a high efficiency of the sea weed to remove water P under different conditions (83.1-97.7% P removal). Among the tested pH, 3.5 had the lowest P removal. P removal linearly increased along with time progress. The lowest P removal was observed in the lowest initial P concentration (0.015 mg/l), however, there was no significant difference among the groups with initial P concentration of 0.15-0.45 mg/l. P removal in 10 g/l sea weed concentration was significantly lower than those of 20 and 40 g/l. P removal significantly increased with decrease in sea weed particle size. Regression analysis showed that the weight of factors to remove P from the medium was as follow: particle size (β = -0.659)> particle concentration (β = 0.427)> time (β = 0.227)> initial P concentration (β = 0.190)> medium pH (β = 0.113). In conclusion, dry *S. ilicifolium* is capable to efficiently remove P from wastewater at aquaculture-relevant concentration. The P removal capability of the seaweed markedly increases by decrease in particle size and increase in particle concentration in medium.

Keywords: Uptake, Phosphorus, Wastewater, Seaweed, Adsorption.

Introduction

The rapid expansion of aquaculture has contributed to the excessive increase of nutrients, especially phosphorous (P), in aquatic ecosystems (Beveridge, 2008). Waste phosphorous in aquaculture come from fish fecal wastes due to high dietary P and/or low P digestibility (Rodehutscord et al., 1994). On the other hand, earthen pond fertilizers increases effluent P considering low P solubility under aquaculture condition (oxidative state of the pond water) (Boyd and Tucker, 2012). Excess P in effluents of aquaculture systems leads to eutrophication

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and a consequent change in the aquatic ecosystem (Jahan et al., 2003). Reducing of farms effluent P is considered to be a key element for the long-term sustainability of aquaculture around the world (Cho and Bureau, 1997). In this sense, the major challenge has been to develop non-polluting strategies that minimize the negative effects of this activity. The most practical and economical approach to reduce the concentration of P in aquaculture areas is to treat the effluents before entering the receiving water.

There are different methods for P removal from aquaculture effluent. Constructing a wetland with different macrophyte plants found to efficiently suppress effluent P concentration in farms of different species including milkfish, *Chanoschanos* (Lin et al., 2002) and rainbow trout, *Oncorhynchus mykiss* (Comeau et al., 2001; Schulz et al., 2003). Using live microalgae is another efficient method to treat wastewater leading to biomass production, for example *Scenedesmus obliquus* was found to efficiently remove P from urban wastewater (Martinez et al., 2000). Although both methods are efficient in P removal, they have some limitations. For instance, their efficiency relays on P loading rate and one fail to increase the system efficiency at moment. On the other hand, as live plant and algae are used in these methods, one should have knowledge about their maintenance conditions. Using non-live materials is a promising method to treat water P content. P has a great affinity to be adsorbed on suspended particle surface, thus such materials may be efficient in P removal from aqueous medium. In this regard, mineral materials such as alum residual and iron oxide particles were found to efficiently suppress wastewater p concentration (Kang et al., 2003; Mortula and Gagnon, 2007). On the other hand, organic materials may be suitable form P removal by surface adsorption theory. There is no study on P adsorption by organic non-live materials. However, these materials, for example lignocelluloses and dried microalgae, were found to efficiently suppress aqueous metals through the surface adsorption theory (Rezaei, 2016; Hosseini et al., 2017). It is worthy to illustrate efficiency of non-live organic materials in P removal from aqueous medium. In this study, dried particles of sea weed *Sargassum ilicifolium* were test for P removal under experimental condition. The seaweed is very abundant in south sea coasts of Iran. The plant is bring to the coast line by sea waves, thus is very cheap and available; and if it is capable to remove P, it is a cheap and practical method.

Materials and Methods

Preparation and characteristics of biosorbent

Healthy and fresh samples of brown seaweed *S. ilicifolium* were collected from coastal line of Chabahar Bay, Sistan and Baluchistan province, Iran (25°21’40’’N 60°36’27’’E). After washing, the seaweed were dried and crashed to appropriate size (Esteves et al., 2000). After crashing, the seaweed particles were passed through meshes with appropriate sizes to obtain particle size in three classes: 0.5, 2 and 5mm. In order to determine the functional groups responsible for biosorbent, IR spectroscopy was performed. For this reseon, about 0.1 g of the seaweed was mixed with KBr for FT-IR spectra analysis (Shimadzu, Mode l8400). Scanning electron microscope (SEM, JEOL,

JSM-6360A) was used to investigate the morphology of the biosorbent.

The isotherm studies were performed in the solution with the initial concentrations ranging from 0.015 to 0.45mg/l of Pat optimum pH values for ions (pH=6). After shaking the flask containing the mixture of biomass (120 rpm, 25ºC) and solution for 60 min, the amount of P in the filtrated solution were analyzed. The biosorption equilibrium uptake capacity for each sample was calculated according to mass balance on the ions expressed in this equation: where V is the sample volume (L), C_0 is the initial ion concentration (mg/l), C_e is the quilibrium or final concentration (mg/l), M is the biomass dry weight (g), and q_e is the biomass biosorption equilibrium ions uptake capacity (mg/g). Langmuir and Freundlich isotherms, the two classical adsorption models, were used to describe the equilibrium between adsorbed ions on the biomass cell (q_e, q) and ions in the solution (C_e,q) in this study.

equation: $q_e = \frac{(C_0 - C_e)}{M} * V$

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That after arranging we have:

Langmuir isotherm model: $q_e = \frac{q_{max} C_e b}{1 + C_e b}$

$$\frac{C_e}{q_e} = \frac{1}{q_{max} b} + \frac{C_e}{q_{max}}$$

These values q_{max} and b (where b, is the adsorption equilibrium constant) can be obtained from the slopes and the intercepts of the linear plots, respectively, where experimental data of C_e/q_e as the function of C_e.

The empirical Freundlich equation based on sorption on a heterogeneous surface, on the other hand, is as follows:

$q_e = k_f (c_e)^{1/n}$

K and n: An experimental constant, K is an indication of the adsorption capacity of the adsorbent; n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The equation can be linearized in the following logarithmic form:

Ln q_e= Ln K_f+1/n Ln C_e

These values n and K_f can be obtained from the slopes and the intercepts of the linear plots, respectively, where experimental data of Ln q_e as the function of Ln Ce.

The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter R_L, which is defined by the equation:

$R_L = 1/(1+bC_0)$

Where Co is the initial adsorbent concentration (mg/l) and b is the Langmuir constant (l/mg). The parameter indicates the shape of the isotherm as follows (Table 1).The R_L values at different initial adsorbate concentrations indicate favorable adsorption for all the adsorbents and adsorbates studied (Rezaei, 2016).

Experiment setup on adsorption of nutrients by seaweeds

Table 1. Type of isotherm for various R_L

R_L	$R_L>1$	$R_L=1$	$0<R_L<1$	$R_L=0$
Type of isotherm	Un favorable	Linear	Favorable	Irreversible

In the present study, the effects of several levels of pH (3.5, 6, 7.5, 9 and 10), initial P concentration (0.015, 0.15, 0.30 and 0.45 mg/l), contact time (7, 15, 30 and 60 min), particle size (0.5, 2 and 5mm) and the sea weed particle concentration (10, 20 and 40 g/l) were studied. Potassium phosphate salt was used to prepare different concentration of P in distilled water. The media pH was adjusted using NH_4OH and HCl.Seaweed particles were added (different particle sizes and amounts) to medium (100 ml) in 250-mL flasks. Then the flasks were maintained at 30°C in shaker for various time intervals of 7-60 min at 200 rpm. At the end of the experiment, the fluid was separated from the seaweed by filtration through Whatman filter paper (47 nm) for P content analysis. Percentage P removal was determined as follow:

Removal (%) = 100 x (IC-FC)/IC

Where, IC is initial P concentration and FC is final P concentration.

Water pH was measured using a digital portable apparatus (pH meter, WTW, Germany). Water P concentration was measured using standard method (O'Dell, 1993). Briefly, ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Statistical analysis

Data normal distribution was confirmed by Shapiro-Wilk test. Percentile data were arcsin transformed before ANOVA analysis. Significant difference in mean P removal percentages was analyzed with Duncan test. To determine the statistical weight of pH, initial P concentration, contact time, particle size and the seaweed particle concentration in P removal, regression analysis was performed and beta coefficient was used to determine the factors' weight. Data are presented as mean ±SD. all analyses were performed in SPSS v. 22.

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SEM analysis

The micrographs of SEM analysis of seaweed before and after adsorption are presented in Figure 2. The micrograph clearly revealed the surface texture and morphology of the biosorbent with large surface area.

Equilibrium

The equilibrium experimental results of P have been fitted in the Langmuir and Freundlich models, respectively (Fig. 3 and 4). The results indicate that Freundlich model adequately describe the experimental data of the adsorption

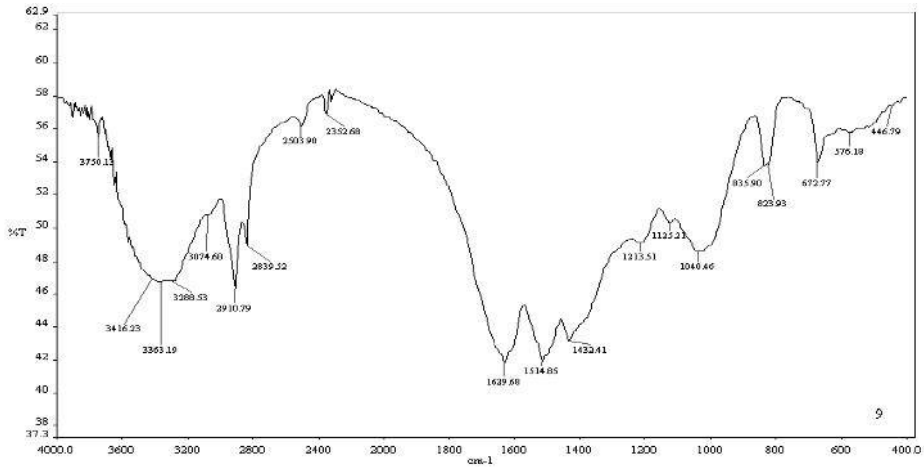

Fig. 1. FTIR spectrum of the seaweed (*S. ilicifolium*)

Table 2. Assignment of bands to functional groups on the surface of the seaweed observed from FTIR spectroscopy

Wave Numbers (Cm^{-1})	Assignment
3363.19	O-H Stretching / N-H Stretching
2910.79	C-H Stretching Aliphatic
2839.52	C-H Stretching
1629.68	C=O Stretching vibration / N-H Stretching vibration
1514.85	CH_2Bonding vibration
1432.41	CH_2Bonding vibration
1040.46	C-O Stretching
Below 1000	Fingerprint

of P. Most of the P was sequestered very fast from the solutions. Table 3 describes summaries of linear regression data for Langmuir and Freundlich isotherms for P adsorption using seaweed biomass. Langmuir and Freundlich constant k were obtained from the linear equations of both models.

Equilibrium parameter R_L

The R_L values at different initial adsorbate concentrations indicate favorable adsorption for all the adsorbents and adsorbents studied.

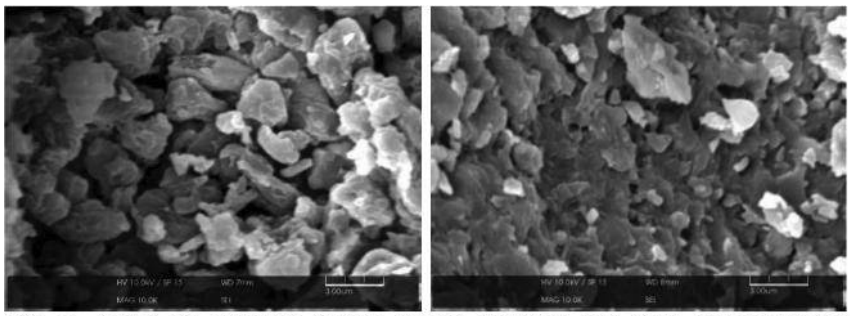

Fig. 2. SEM of seaweed before (upper picture) and after (lower picture) adsorption

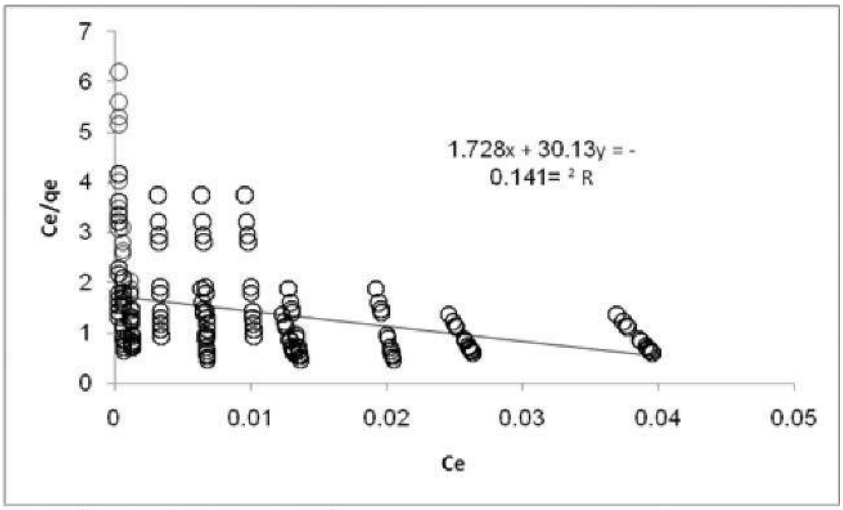

Fig. 3. Langmuir isotherm of P

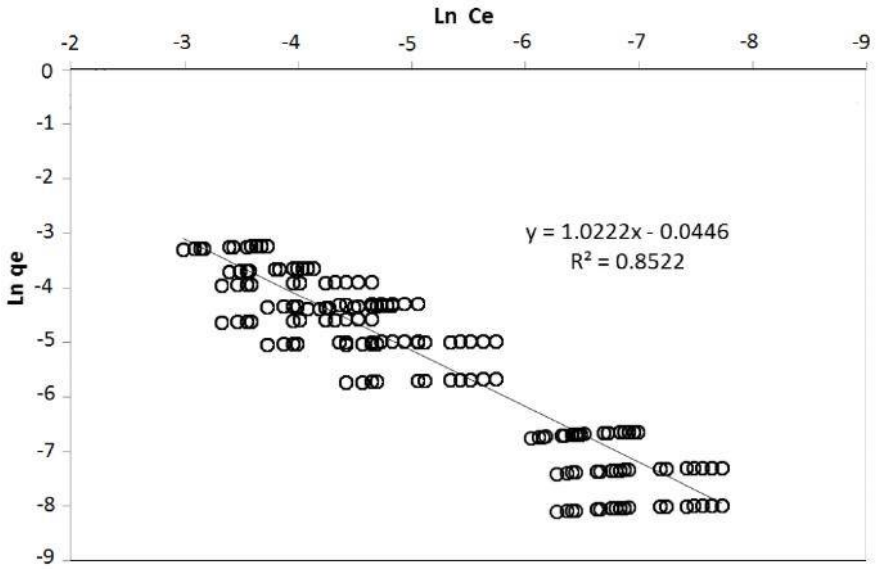

Fig. 4. Froundlich isotherm of P

Table 3. Parameters of isotherm models for the adsorption of P using seaweed

	Langmuir Parameters			Freundlich parameters		
	q_{max} (mg/g)	B (L/mg)	R^2	K_f	N	R^2
P	0.033	1.753	0.141	1.045	0.978	0.8522

Phosphorus removal

pH significantly affected P removal from medium as the lowest removal was related to pH 3.5. However, there was no significant difference in P removal among pH 6-10 (Fig. 5).

P removal significantly increased as time progressed, which there was a significant difference between 7 and 60min (Fig. 6).

Initial P concentration significantly affected P removal from medium as the lowest removal was related to the lowest initial P concentration. However, there was no significant difference in P removal among the other treatments (Fig. 7)

Seaweed amount significantly affected P removal from medium as the lowest removal was related to 10g/l seaweed concentration. How

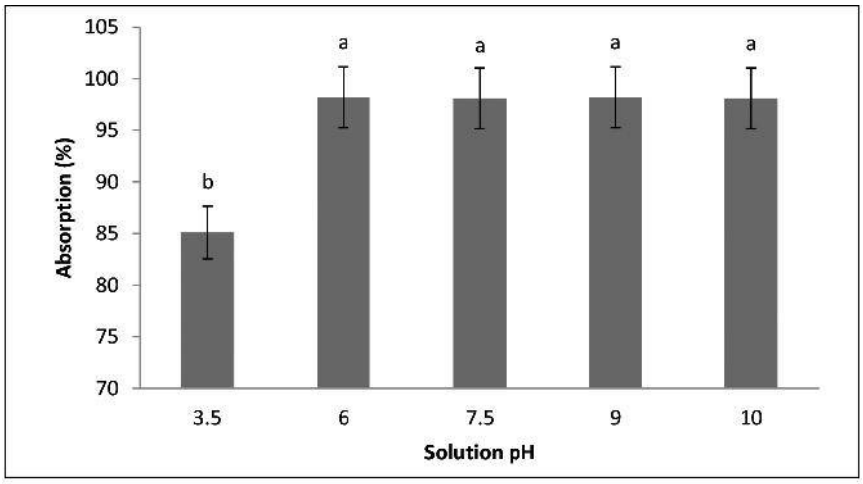

Fig. 5. Effect of initial pH solution on P absorption of seaweed. Different letters mean significant difference among the treatments.

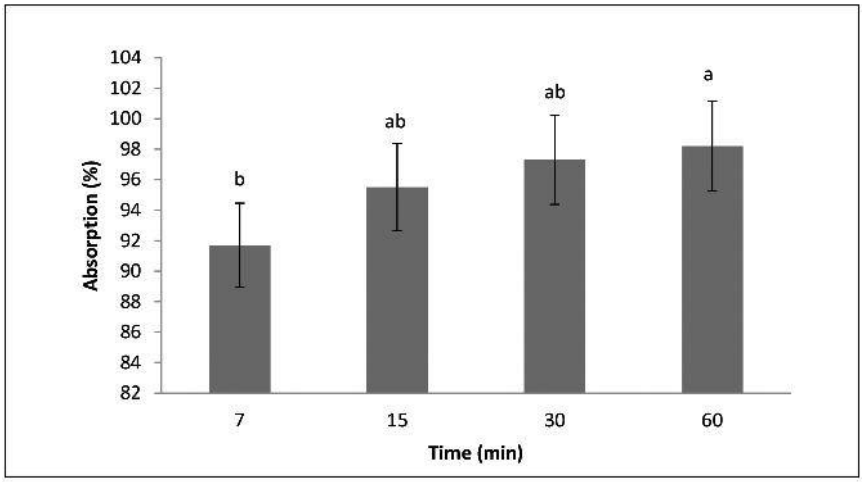

Fig. 6. Effect of contact time on P absorption of seaweed. Different letters mean significant difference among the treatments.

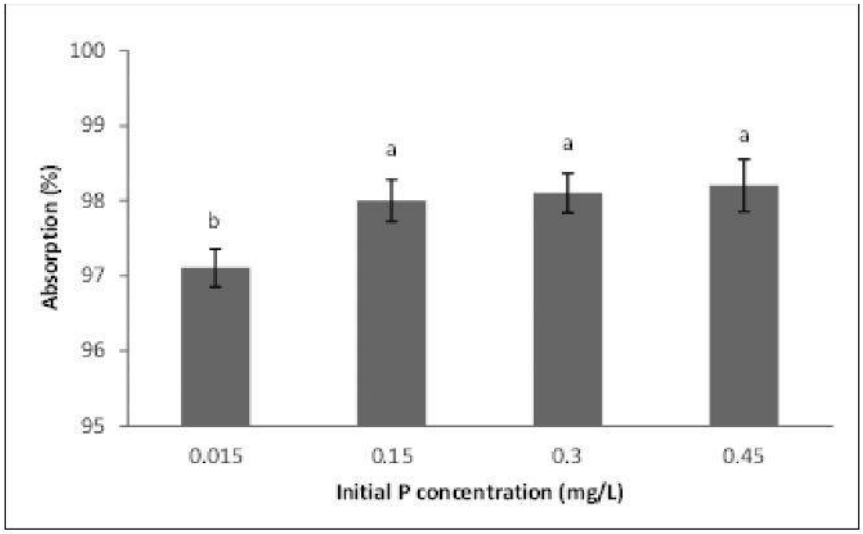

Fig. 7. Effect of initial P concentration on P absorption of seaweed. Different letters mean significant difference among the treatments.

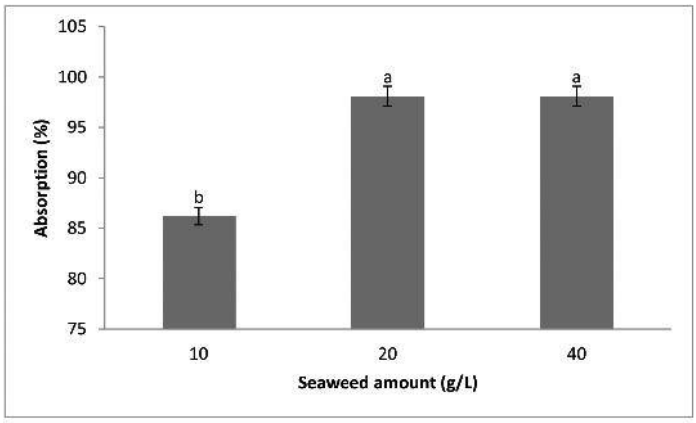

Fig. 8. Effect of seaweed amount on P absorption of seaweed. Different letters mean significant difference among the treatments.

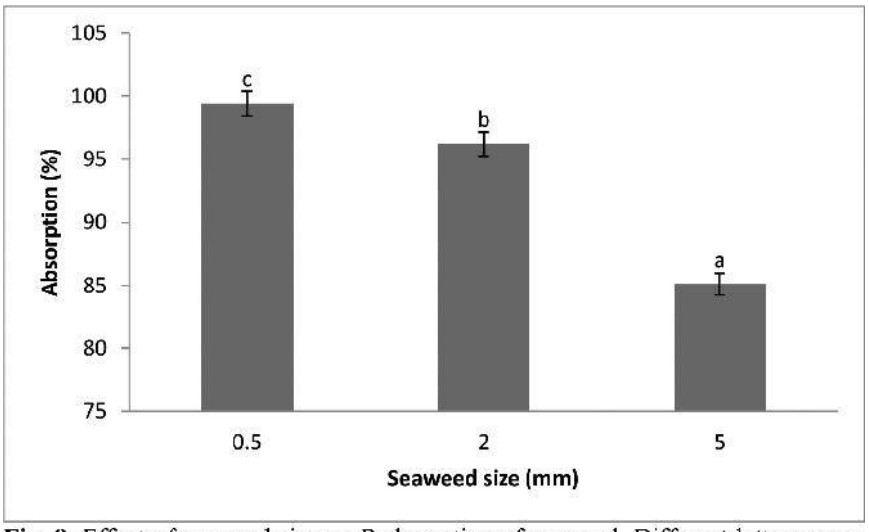

Fig. 9. Effect of seaweed size on P absorption of seaweed. Different letters mean significant difference among the treatments.

ever, there was no significant difference in P removal among 20 and 40g/l seaweed concentrations (Fig. 8).

Seaweed particle size significantly affected P removal as decrease in particle size from 5 to 0.5 mm resulted in increase in P absorption from 85 to 98% (Fig. 9).

Regression analysis results are presented in Table 4. The model was capable to predict P removal using the predictors (R2 = 0.72). The highest weight for P removal was related to sea weed particle size and seaweed concentration. The lowest weight was related to medium pH.

Discussion

Containment and collection of wastes, both solid and dissolved, is very difficult and costly in aquaculture, as the wastes are rapidly dispersed into the surrounding waters (Cho and Bureau, 1997). Seaweeds are known as very good sorbents, because the cell wall of brown algae contains alginate with its carboxyl and hydroxyl groups (Vieira and Volesky, 2000; Davis et al., 2003).

In this study, pH, initial P concentration, contact time, particle size and the seaweed particle concentration significantly affected P removal

Table 4. Regression coefficients for different predictors of P removal.

Predictors	B	Beta	t	P-value
Constant	91.40		622.402	0.0001>
Sea weed concentration	0.980	0.427	37.236	0.0001>
Sea weed particle size	-1.007	-0.659	-57.405	0.0001>
Time	0.032	0.227	19.794	0.0001>
Medium pH	0.141	0.113	9.834	0.0001>
Initial P concentration	3.583	0.190	16.585	0.0001>

percentage. Similarly Mithra et al., (2012) also showed significant differences in nutrient removal under different contact periods and aqueous pH using different seaweed *Caulerpa taxifolia* concentrations under laboratory condition. The concentration of the four nutrients tested (NO_2-N, PO_4-P, NO_3-N and NH_3-N) decreased significantly during the experimental period, indicating that macro algae has large nutrient removal capacity. The concentration of PO_4^{-3} decreased from 0.037µmol/l to 0.002µmol/l which corresponds to about 94.5% of nutrients removal during the five days of study. A considerable amount of reduction in NO_3^- concentration was observed in the present study (Mithra et al., 2012). The nutrient removal ability for the whole period was approximately 54.54%. At the end of the experiment (fifth day) most of the NO_2 was absorbed, corresponding to 92.3% removal efficiency. There was also a considerable NH_3^+ removal (42%) during the experiment. There was a considerable decrease in NO_3^- (12.281µmol/l) during the culturing period (Mithra et al., 2012). This decrease may be related to the mineralization of the organic materials as reported earlier by Jones et al. (2001). Previous studies have also shown that seaweed may be able to remove high concentrations of PO_4^{3-} (Neoriet al., 1998; Troell et al., 1997; Zhou et al., 2006; Jones et al., 2001 and Martinez-Aragon et al., 2002). However the bio filtration capacity of PO_4^{3-} obtained in this experiment was considerably higher.

Conclusion

In conclusion, the present observation inferred that the *S. ilicifolium* showed significant removal in nutrient, nitrate and phosphate. Hence, this species can be considered as potential candidate for bioremediation of domestic and industrial effluent which can cause environmental pollution. It is concluded that the brown seaweed *S. ilicifolium* species not only can be cultivated in shallow coastal ponds used as a bioremediation agent in the effluent treatment plants for conservation of nature and natural resources, specifically marine shrimp and also after harvesting and drying also can be as a good biosorbent. Furthermore, the effluent cultivated seaweeds can be utilized as feedstock for production of biofuel, biofertilizers and additives for animal feed fish aquaculture activities, but which can reduce the cost of wastewater treatment process.

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The Effects of Physico-Chemical Parameters on the Phytoplankton Population of Shahrchai Dam Reservoir (Uramia- Northwest) Iran

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Abstract

The effects of water physico- chemical factors on phytoplankton communities of Shahrchai dam reservoir were studied. 6 sampling sites were chosen along Shahrchai dam and surface and deep layers were sampled during 3 seasons in 2015-2016. In this study, phytoplankton samples were fixed with 4% formalin and transferred to laboratory. Identification and enumeration of the phytoplankton were performed by inverted microscope equipped with 5 ml counting chamber. Totally 34 species of phytoplankton belonged to Green algae, Diatomes, Cyanobacteria and Euglenophyta were determined. Statistical analysis was carried out by PCA and UPGMA methods. The analysis of data by PCA showed that first and second axis created 99% of changes alone. There were 3 completely distinct groups of samples regarding the sampling seasons. The most effective physico- chemical factors influenced the phytoplankton communities were water transparency, EC and water temperature.

Keywords: Shahrchai reservoir, Physico-Chemical factors, Phytoplankton.

Introduction

Beside the social and economic importance, lentic water resources such as dam reservoirs are considered as valuable environmental and ecological resources. Due to high volume of soluble nutrients and load of organic maters from the basin, dam reservoirs are one of the fertilized systems providing nutrients to various plant communities. Phytoplankton are a group of photosynthesized floating algae in the water that play a key role in providing the nutrients, and oxygen to other live organisms, fixation of waste nitrogenous products, and the carbon dioxide fixation. These organisms are primary producers in aquatic ecosystems and were used to determine water pollution level. Algae are very powerful to identify and interpretation of water ecological conditions (Reynolds, 2006; Barone and Naselli, 1994; Stoermer and Smol, 1999).

Therefore, composition and abundance of algal communities are commonly used as a water quality index and trophic status of dam reservoirs and rivers (Mohebbi, 2012; Wetzel, 1983; Trinova, 1998; Reynolds et al., 2002; Mohsenpour et al., 2011; Yerli et al., 2012).

Human activities has increased the algal bloom and accelerated eutrophication of water bodies that has been observed in water systems all over the world (Ma et al., 2014). The over growth of

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cyanobacteria algae has a harmful effects on the use of urban water, industrial, fisheries and recreational activities (Dokulil and Teubner, 2000). Shahrchai dam reservoir was constructed on Shahrchai River, which flows through Uramia city and enters into the lake Uramia. The reservoir provides the drinking water of more than 860000 population of Uramia city and agricultural water of Uramia plain. In spite of high importance of this reservoir, its phytoplankton populations and related water quality indices has not yet been studied. Regarding to drinking water shortage and reduced percipitation in recent years, determination of Shahrchai water quality status by phytoplankton population changes is a useful tool to manage water quality.

In Iran, some studies on phytoplankton populations of reservoir dams and rivers has made earlier (Mohebbi et al., 2012; Mohsenpour et al., 2011; Shams et al., 2012). These studies are mostly carried on one water ecosystem by phytoplankton population changes and water quality status. Also, internationally various work has done on determination of water quality using algae and related ecological variables (Wetzel, 1983; Trifonova, 1998; Reynolds et al., 2002; Mohsenpour et al., 2011; Yerli et al., 2012).

Materials and Methods

Water samplings were performed by a rottener type sampler. Two samples were taken from each site (surface and 1m depth). All samples immediately were fixed by logul solution. After transferring to laboratory, the samples were kept in dark and cool place. Identification and counting of phytoplankton were performed by a TS100 Nikon inverted microscope with resolution 400 and 5ml count chamber by Utermöhl (1958) method. In each sample, at least 50 field of view or 100 individuals from most abundant phytoplankton were counted (Venrick, 1978). In this study the identification keys such as Bellinger (1992); Tiffany and Britton (1971); Prescott (1962) were used to determine phytoplankton taxa. Water temperature, Dissolved Oxygen (DO), EC, pH and water transparency

Fig. 1. Study area and sampling sites in Shahrchai dam reservoir

were measured by Oxymeter WTW320, EC meter WTWLF320, pH meter Teso320, and Secchi disc, respectively. PO_4^{3-}, NO_3^-, Total Nitrogen (TN) were determined with Spectrophotometer PG T80+ according to Greenberg et al. (1992).

Multivariate statistical analysis

Multivariate statistical analysis such as Principal Component Analysis (PCA) are techniques that shows the relationships between each taxon in a population or between population structure and ecological variables. The PCA method is used for decreasing phytoplankton data to limited number of taxon's that statistically significant and their distribution pattern is the cause of total variance of data (Beaver et al., 2013).

In this study grouping was made by dividing to basic components and then the role of studied factors were determined by biplot. Also, PCA analysis and the role of environmental factors were illustrated in PCA loading Table and Figures 1-7. Unweight Pair Group Method with Arithmetic Mean (UPGMA) is a simple hierarchical clustering method. This method is one of the most common techniques in ecology to classify the sampling units based on the amount of similarity of reciprocal pairs in prescribed variants (such as species composition). Algorithm UPGMA confirms a dendrogram that reflexes a shown structure in a similar or non-similar matrix. UPGMA is the simplest method to form a tree. This method basically was performed to make taxonomic phonograms. UPGMA use a consecutive clustering algorithm that in it localized topologic relations were determined based on the rate of similarity and similarity tree was constructed step by step. Two- way clustering method were done between physico-chemical and biological variables and among sites and sampling seasons to determine similarities and dissimilarities among these factors.

Tow- way Clustering Analysis of data were done using UPGMA method based on environmental variables. Before analysis, all data were standardized (Mean= 0, Variance = 1). Euclidian distance among studied samples were determined based on standardized obtained data. Matrix of obtained distance was used to make clustering UPGMA tree. PCA and Tow-Way Clustering were analyzed by Paleontological Statistics (PAST) version 3.04 (Hammer et al., 2001).

Results

Table 1 shows the complete list of phytoplankton taxa identified in Shahrchai dam reservoir. Totally, in this study, 34 taxa were identified in Shahrchai dam reservoir of which Bacillariophyta, Chlorophyta, Cyanobacteria and Euglenophyta with 16, 14, 3 and 1 taxa had the highest number of taxa respectively.

Some physicochemical parameters of shahrchai dam reservoir water in the study period are indicated in Table 2. Total nitrogen (TN) as an indicator of water trophy state ranges between 0.83 to 1.45mg/l.

In spring as well as autumn, the mean of phytoplankton density in depth samples were less than surface ones (Figs 2, 3). Cyanobacteria only occurred in summer and early autumn with 7.3×10^6 cell/l and 8.9×10^6 cell/l respectively (Fig .4). The density of chlorophyta in spring, summer and autumn were 64.3, 5.3 and 4.3×10^6 cell/l respectively. In early spring, the highest algal density was nearly 8.5×10^6 cell/l was related to site 2 – surface and the lowest density 1.462×10^6 cell/l was related to site 6 (Fig. 3).

The analysis of basic components revealed that

first and second axis were responsible for more than 99% of changes (Table 3). PCA showed three distinct groups which were completely met the sampling periods (Figs 6, 7 and 8).

Table 1. List of phytoplankton taxa identified in Shahrchai dam reservoir

Season	Spring	Summer	Autumn
Species			
Cyanobacteria			
Merismopedia glauca (Ehrenberg) Kützing	-	+	+
Gloecapsa punctuate Nägeli	-	+	-
Oscillatoria sp.	+	+	+
Chlorophyta			
Chlamydomonas sp.	+	+	-
Ankistrodesmus falcatus (Corda) Ralfs.	-	+	-
Chlorella sp.	+	+	+
Tetraedran minimum (A.Braun) Hansgirg.	I	-	I
Scenedesmus quadricauda var. *quadrispina* (Chod.) G.M. Smith.	-	-	-
Scenedesmus dimorphus(Turp.) Kuetzing.	+	+	-
Selenastrum gracile Reinsch	+	+	-
Oocystis crassa Wittrock	-	+	-
Volvox aureus Ehrenberg	+	+	+
*Pediastrum duplex*var.gracilimum West.	-	-	+
Pediastrum simplex (Meyen.) Lemmer.	+	-	-
Microspora stagnorum (Kützing) Lagerheim	-	-	+
Dictyocepharium pulchellum H.C.Wood	+	-	-
Cosmarium formosulum Hoff	+	+	-
Bacillariophyta			
Synedra ulna (Nitz.) Her.	-	+	+
Navicula sp.	-	+	-
Cymbella prostrate (Berkley) Cleve.	+	+	-
Diatoma vulgar Bory.	-	-	+
Cyclotella sp.	+	-	+
Amphora ovalis Kuetzing.	+	+	-
Eunotia pectinulia	+	+	-
Nitzschia sp.	-	-	+
Pinnularia hemiptera(Kützing) Rabenhorst	-	+	+
Meridion circular (Greve.) Aghard.	+	+	-
*Amphiprora senestr*Greville	-	+	-
Cocconies pediculus Her.	-	+	-
Gomphonema acuminatum Ehrenberg	-	-	+
Melosira granulate (Ehrenberg) Ralfs	+	+	-
Epithemia turgida (Ehrenberg) Kützing	-	+	+
Tabellaria senestrate (Lyngbye) Kützing	+	+	-
Euglenophyta			
Euglena proxima Dang.	-	+	-

Table 2. Some physicochemical and biological factors of Shahrchai dam Lake, 2008-9.

Season Factor	Autumn 2008	Spring 2009	Summer 2009	Average
Water temperature (°C)	3.93	7.46	15.38	8.93
DO (mgL^{-1})	8.8	10.39	9.59	9.59
EC ($\mu mosc.cm^{-1}$)	251.9	370.75	211.58	244.74
PH	8.07	7.62	8.17	7.95
Transparency (cm)	75	35	57	55.7
PO_4 ($mg.l^{-1}$)	3.56	2.38	2.27	2.74
NO_3($mg.l^{-1}$)	0.65	0.35	0.0068	0.65
TN ($mg.l^{-1}$)	1.45	0.83	1.14	1.14

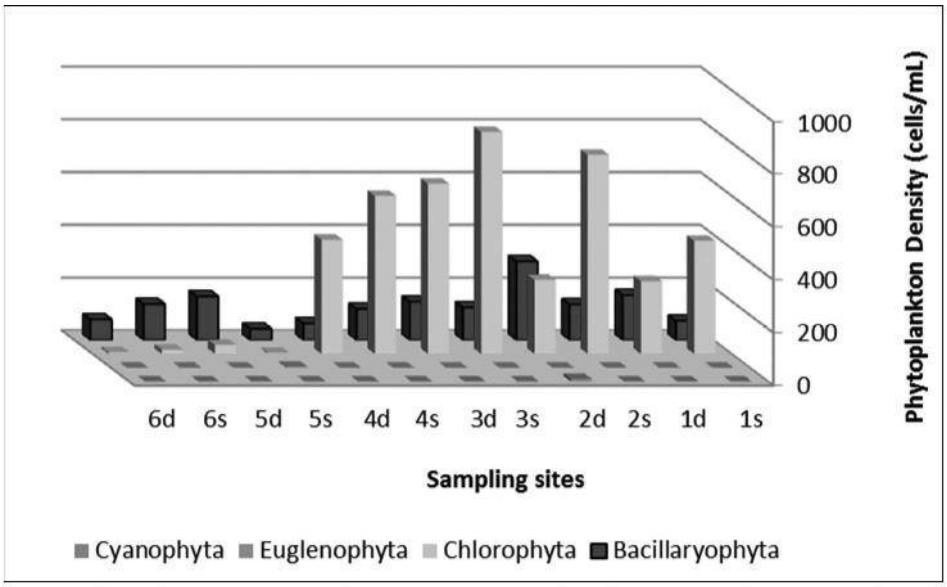

Fig. 2. The density of phytoplankton groups in Shahrchai reservoir in autumn 2008

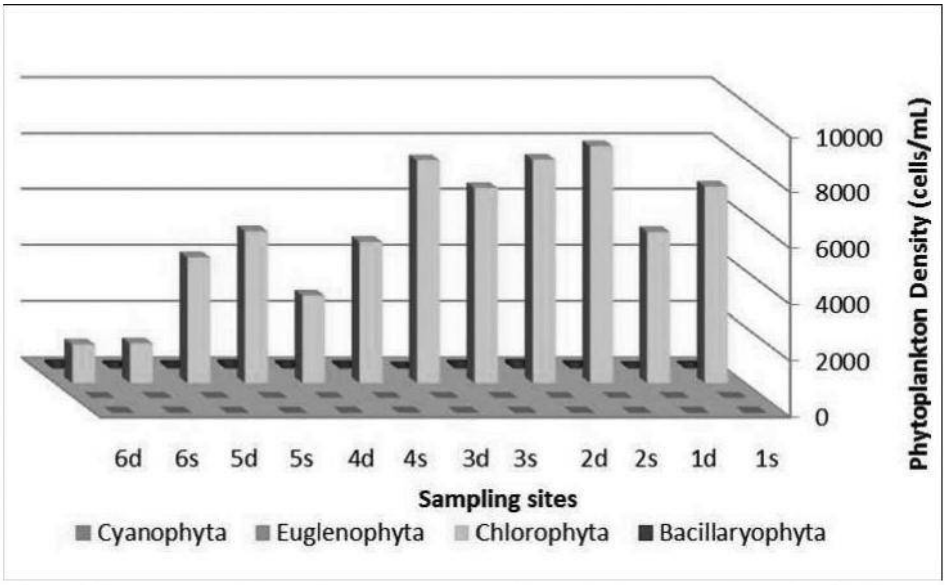

Fig. 3 The density of phytoplankton groups in Shahrchai reservoir in spring 2009

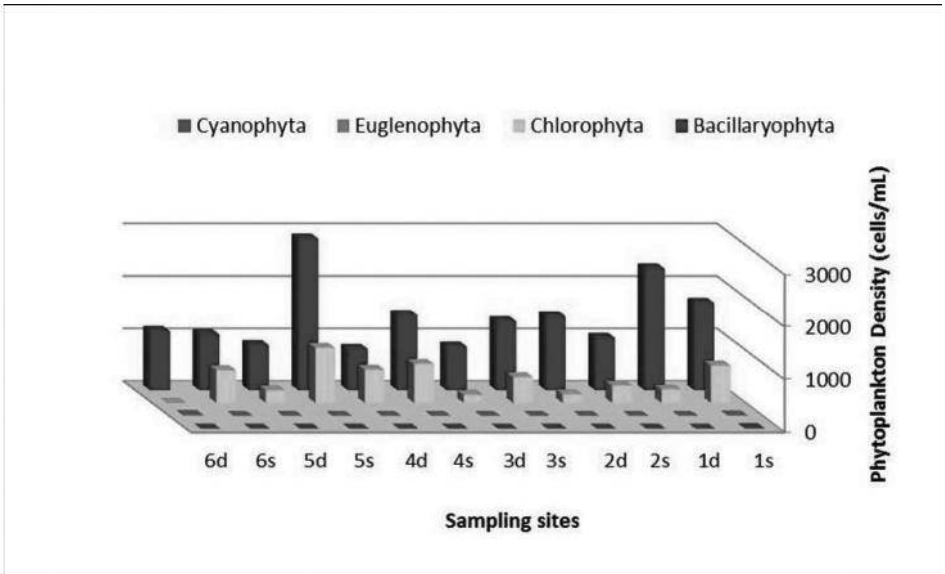

Fig. 4. The density of phytoplankton groups in Shahrchai reservoir in summer 2009

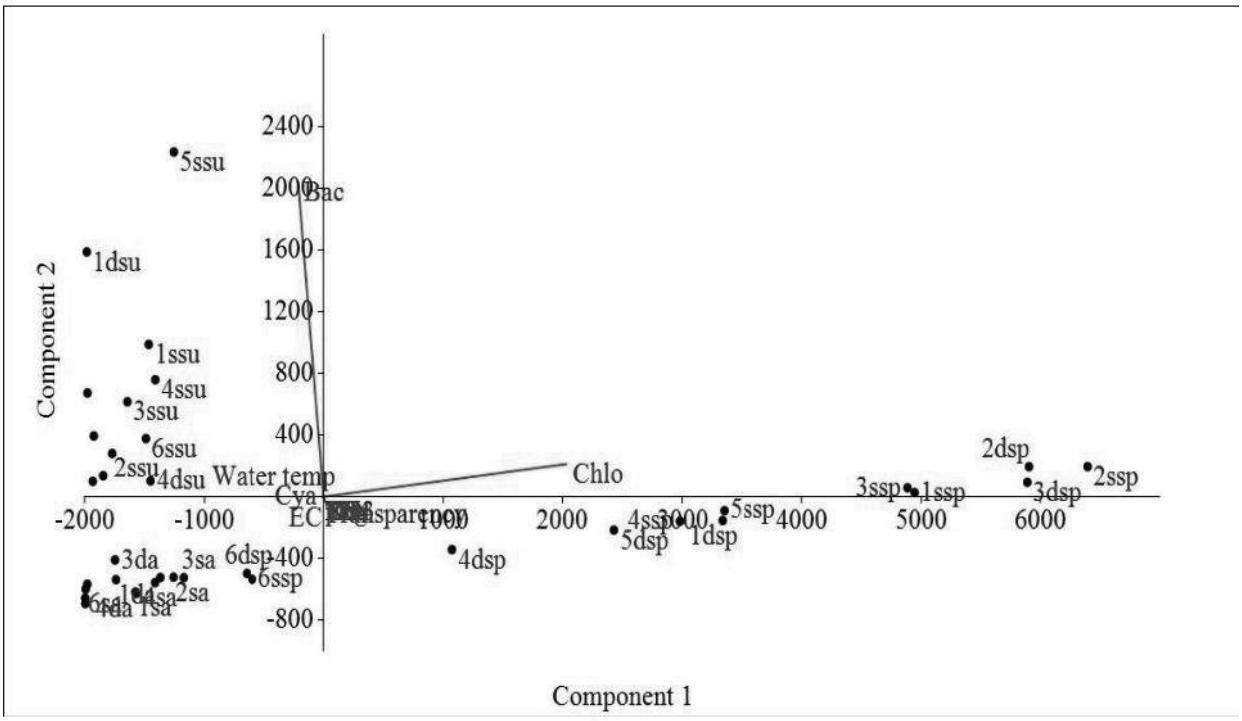

Fig. 5. Principal Component Analysis (PCA) of phytoplankton groups, physicochemical factors, sampling sites and seasons in Shahrchai reservoir

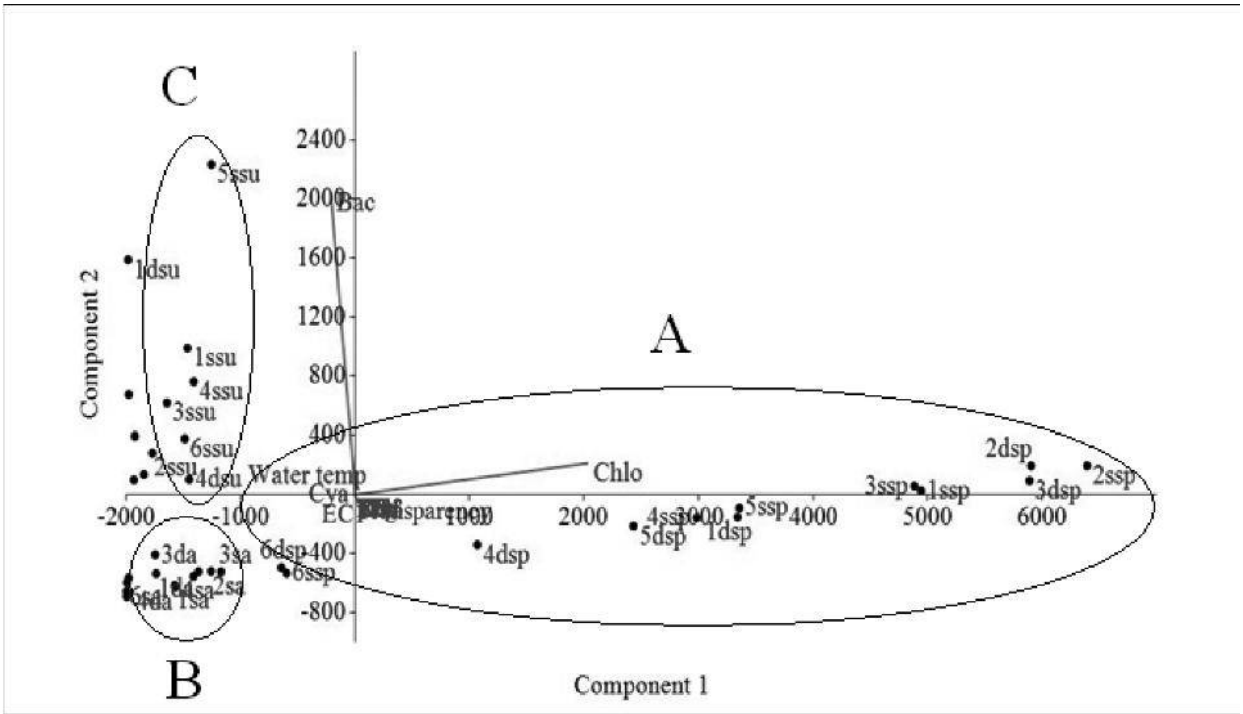

Fig. 6. Principal Component Analysis (PCA) of phytoplankton groups, physicochemical factors, sampling sites and seasons in Shahrchai reservoir (note to the grouping based on the seasons: A- spring, B- autumn, C- summer)

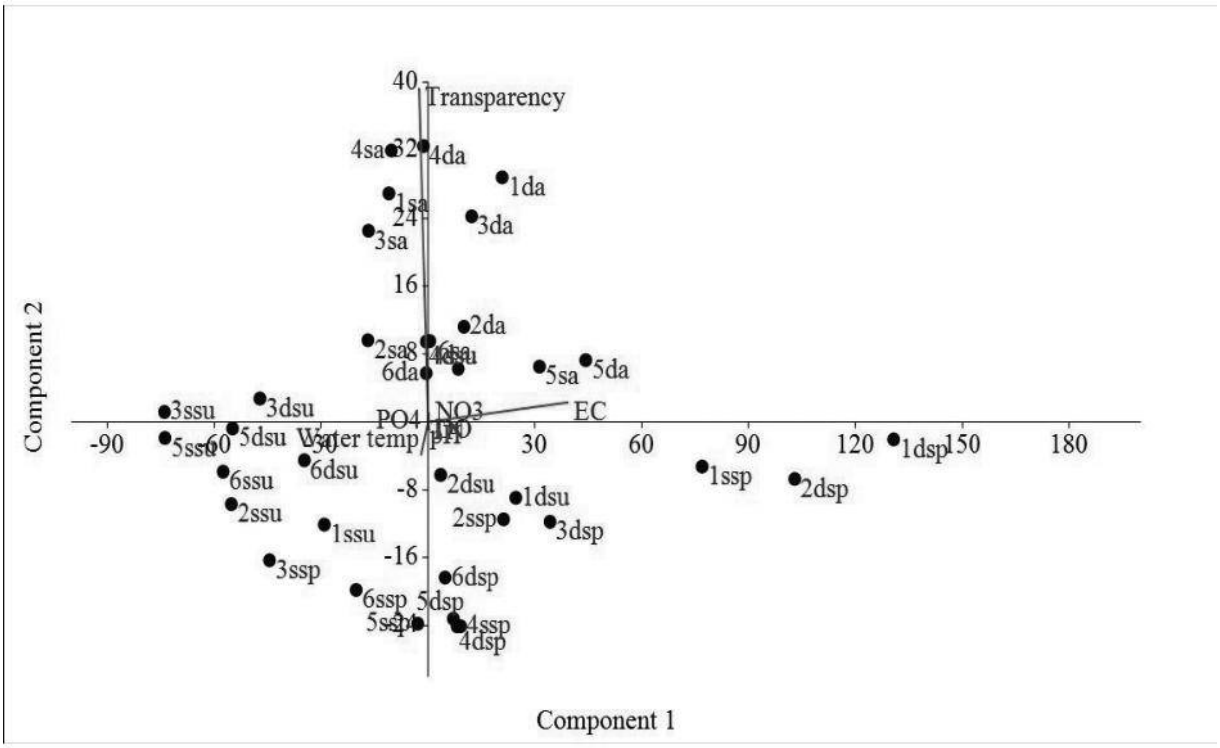

Fig. 7. Principal Component Analysis (PCA) of physicochemical factors, sampling sites and seasons in Shahrchai reservoir

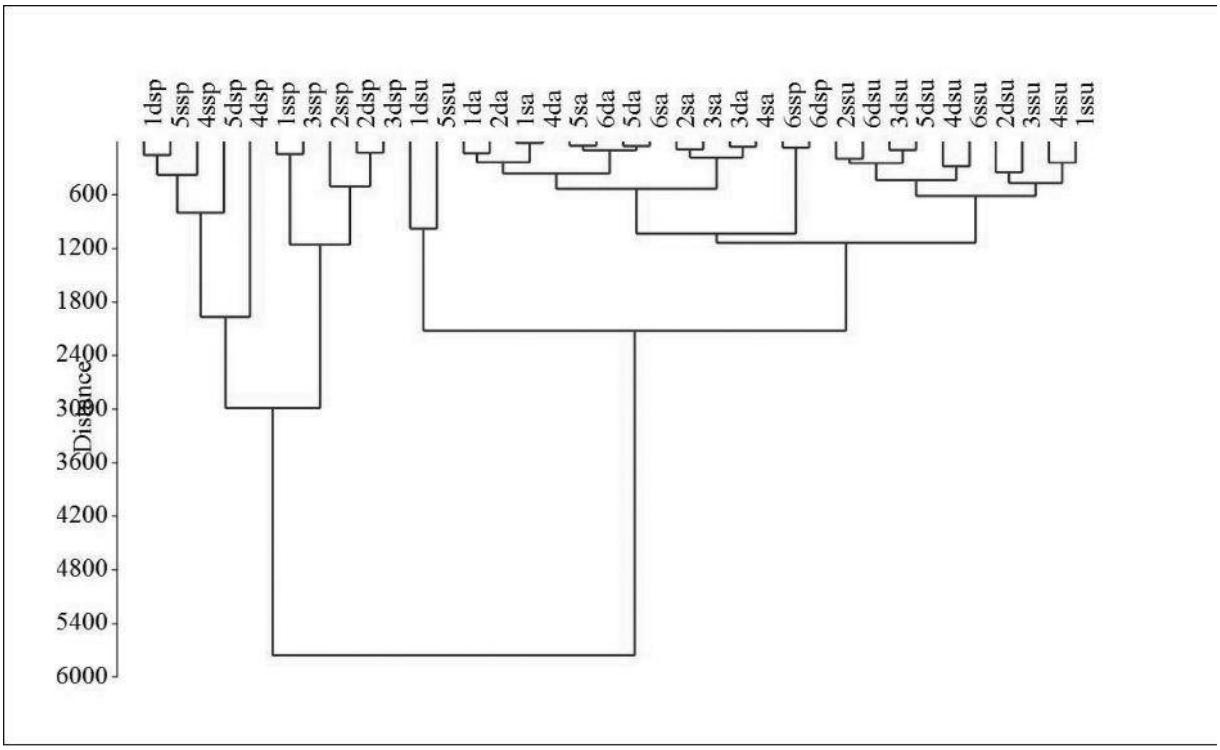

Fig. 8. Clustering analysis of sampling sites and seasons in Shahrchai reservoir by UPGMA method

Table 3. Total components factor loadings in PCA of Shahrchai reservoir limnological parameters

Component	Eigenvalue	Variance (%)
1	7.6×10^6	94.6
2	432031	5.375
3	1316.15	0.016375
4	163.785	0.0020277
5	7.62473	9.4862×10^{-5}
6	7.50029	9.3314×10^{-5}
7	4.27768	5.322×10^{-5}
8	0.389737	4.8488×10^{-6}
9	0.169695	2.1112×10^{-6}
10	0.14498	0.14498×10^{-6}
11	0.0912185	0.0912185×10^{-6}
12	0.065562	8.1568×10^{-7}

Discussion

The phytoplankton are the base of food chain and constitutes the bulk of primary producers in all water bodies. The ecology of phytoplankton has a principal importance because strongly depics the water quality and also reflects the average ecological condition (Tyagi and Nalik, 2017). In our study, Chlorophyta was the most abundant algal group followed by Bacillariophyta and Cyanobacteria. Similar findings were reported by Tyagi and Malik (2017) and Mishra et al. (2010). Martinel et al. (2016) studied the phytoplankton and trophic state of a nearly impounded reservoir. They concluded that phytoplankton structure may be a suitable indicator to follow the trophic status of a young reservoir. Shahrchai reservoir is a relatively new reservoir (impounded in 2005). So, our study shows an appropriate approach that might be recommended to follow ecological condition of the reservoir in the future studies.

In spring as well as autumn, the mean of phytoplankton density in depth samples were less than surface ones. That shows phytoplankton density is largely related to sun light and its depth of penetration in the water. Cyanobacteria only occurred in summer and early autumn with 7.3×10^6 cell/l and 8.9×10^6 cell/l respectively, which indicates oligotrophic status of Shahrchai reservoir. Low level of cyanobacteria indicated the non- contamination or low contamination in all sampling sites. Year round high density of chlorophyta indicated low levels of contamination in the reservoir. In this study *Chlorella* as a green alga showed a high abundance in all samples in both spring and autumn. Also, in early spring the algae bloom in the Shahrchai Lake was related to *Chlorella*. In early spring, the highest algal density was nearly 8.5×10^6 cell/l was related to site 2 surface and the lowest density 1.462×10^6 cell/l was related to site 6. The low density of phytoplankton at site 6 can be attributed to increase in water depth at this site due to rainfalls that caused to increase water level at that time. The analysis of basic components revealed that first and second axis were responsible for more than 99% of changes (Table 2). The results showed 3 completely distinct groups related to sampling seasons (Fig. 7). The most important physicochemical variables affecting this classification were water transparency, EC and water temperature (Fig. 7).

Wassie and Melese (2017) studied the effects of physicochemical factors on phytoplankton structure in Selameko reservoir in Ethiopia. Their results confirmed that anthropogenic activities can influence the phytoplankton population by changing the phosphorus concentration. Mohebbi et al. (2015) showed that agricultural activities and urbanization were the most important factors in eutrophication of Aras reservoir. Water quality and trophic structure of the Shahrchai reservoir may be negatively af-

fected by agricultural and livestock activities (Mutlu and Kutlu, 2017) performed around the dam in the future. Therefore, to avoid such disturbances in the Shahrchai reservoir, basin and reservoir management plans are strongly recommended. In conclusion, Shahrchai reservoir is an important fresh water source fed by Shahrchai river and snow waters and contains noticeable freshwater potential. Besides, its eutrophication status was not exceeded the limits recommended for Lakes, ponds and dams. Therefore, the reservoir management should be streamed toward integrated approaches to let the condition goes well.

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Reproductive Characteristics of Four *Artemia* Populations with Different Geographical Origin Fed on a Halophylic Unicellular Algae: *Dunaliella tertiolecta*

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Abstract

This study was conducted to investigate reproductive characteristics of four *Artemia* populations (*A. urmiana*, *A. franciscana*, Pakistan and Turkmenistan strains) cultured under the identical laboratory condition and fed on a unicellular alga. Cysts were hatched by standard methods and the nauplii from the populations were cultured in laboratory condition using 80 g/l salinity, 25±1ºC with photoperiod (12L: 12D). *Dunalliella tertiolecta* fed to all *Artemia* populations. To determine the reproductive characteristics, 30 pairs of adult *Artemia* of each population were randomly placed in 50ml conical falcons so that each conical falcon contains a pair of *Artemia*. The number of cysts and nauplii in each falcon were counted daily. All data were analysed using SPSS, one way ANOVA. The results showed that in all *Artemia* populations, the daily nauplii production were higher than cyst production. Also, during reproductive period, A. *franciscana* had the highest cyst production (639±105) and *A. urmiana* had the highest nauplii production (78.5±7). The highest and the lowest birth rate were related to A. *franciscana* (1225±193) and Turkmenistan strain (362±29), respectively. Therefore, it is suggested that *A. urmiana* and A. *franciscana* were perefered species for cysts production. Turkmenistan strain was not recommended for production plans, due to low cysts and nauplii production.

Key words: *Artemia*, Reproduction, *Dunaliella tertiolecta*, Geographical origin.

Introduction

Artemia as a live food has a high nutritional value for larval rearing of the most marine fishes (Sorgeloos et al., 2001) and shelfish species (Leger et al., 1986; Bengetson et al., 1991; Sorgeloos et al., 1998). *Artemia* is used to feed aquatic animals in different types as decapsulated cysts, early hatched nauplii, juvenile, adult, dry and frozen (Bengtson et al.,1991). *Artemia* genus consists of bisexual and parthenogenetic strains. *Artemia* has two types of reproduction, namely ovi-viviparous and oviparous depending on environmental conditions (Liang and Macrae, 1999; Jackson and Clegg, 1996). Nauplii production occurs in suitable culture conditions, while cyst production occurs in improper condition. Factors such as age of mother, photoperiod, salinity and oxygen and water temperature interfere in controlling reproductive

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mode. In *Artemia* strains the differences in genetic tendencies of females, determines the time of viviparous or cyst production pattern of reproduction. Bisexual reproduction is effective to maintain genetic diversities between individuals of a population that cause live potentials and dispersal in different habitats and enhance development rate in environment change. Parthenogenesis has the advantage of rapid production (Hafezieh, 2003). Lavens and Sorgeloos (1996) mentioned the most effective factor on *Artemia* cyst production as selection of suitable culture strain based on their growth, reproduction and tolerance level into temperature and salinity. In fact, native strain of the region that enjoys maximum growth and reproductive potential should be selected. Usually, strains with smaller size cyst and nauplii production is preferred to cyst production and strains with rapid growth and nauplii producers would be selected for biomass production (Tackaert and Sorgeloos, 1992). Ahmadi (2003) mentioned that population density influenced the cyst production of earthen pond cultured *Artemia* and regarding that parthenogenetic strain has a higher level of heterozygotic level compared to bisexual one, suggesting that parthenogenetic strain should be used to cyst production and *Artemia urmiana* bisexual strain used for biomass production (Ahmadi, 2003). The aim of this study was to compare the growth and cyst production and live birth rate in various geographical *Artemia* strains and determining the best species for production plans.

Materials and Methods

Bisexual strains of *A. urmiana* and *A. franciscana* and parthenogenetic *Artemia* of Pakistan and Turkmenistan were cultured under standard condition in 4 treatments, each consisted of 4 replicates, feeding *Dunaliella tertiolecta*. *Artemia* were cultured in 120× 53× 31 cm aquariums. After a 20 days of culture period in bisexual species when males began to catch females and in parthenogenetic ones when the signs of ovarian development were observed, 30 pairs of each species were isolated and added to 50 ml conical falcons where each conical falcon bore a pair of *Artemia* of the studied species. Females with oocystes migration into uterus considered as adult (Triantaphyllidis et al., 1995). Culture period continued as they live. During reproduction period, *Artemia* fed with *D. tertiolecta* according to Coutteau et al., (1984). During the test period in bisexual species, active swimming males replaced with dead males (Browne et al., 1988). All cysts and nauplii of each falcon were counted daily. Reproductive characteristics including number of birth rate, cyst production, nauplii production , spawners , birth rate per spawner were determined daily for each *Artemia* population until day 28 (Browne et al., 1998). All data were analyzed using ANOVA – SPSS software (Triantaphyllidis et al., 1995; Sokal and Rohlf, 1981) and means were compared by Tukey test.

Results

The results showed that there were no difference in nauplii production in 4 *Artemia* strains ($p>0.05$). There was a significant difference in *A. urmiana* and *A. franciscana* compared to Turkmenistan strain ($p< 0.05$) and no significant difference among other species ($p> 0.05$) in cyst production and total fecundity. There was a significant difference in Turkmenistan strain and other strains in daily nauplii produc-

tion ($p< 0.05$). *A. urmiana* and *A. franciscana* showed a significant difference compared to Pakistan and Turkmenistan *Artemia* in daily cyst production and daily cyst production of each spawner ($p< 0.05$). Also, *A. urmiana* and *A. franciscana* showed a significant difference compared to Turkamanestan *Artemia* in daily fecundity of each spawner ($p< 0.05$).

The mean of reproductive parameters in different *Artemia* populations were shown in Table 1 and Figures 1-4.

Table 1. Mean of reproductive parameters of *A. urmiana*, *A. franciscana*, Pakistan and Turkmenistan populations

Artemia population Parameter	*A. urmiana*	*A. franciscana*	Pakistan	Turkmenistan
Total birth rate	1208±182^{a*}	1225±193^{a}	715±74^{ab}	362±29^{b}
Total cyst production	619±91^{a}	639±105^{a}	342±40^{b}	220±16^{b}
Total nauplii production	589±134^{a}	587±141^{a}	373±48^{ab}	141±18^{b}
Mean daily cyst production	59.6±5.7^{a}	61±6^{a}	28.2±2.3^{b}	24.2±1.5^{b}
Mean daily nauplii production	78.5±7^{a}	66±8^{a}	64±6.6^{a}	29±2.4^{b}
Number of daily cyst producer spawners	11.1±1.4^{a}	10.9±1.5^{a}	13.4±1.5^{a}	9.3±0.5^{a}
Number of daily nauplii producer spawners	6.8±1.6^{a}	7.8±1.6^{a}	5.6±0.6^{a}	4.6±0.4^{a}
Daily fecundity of each spawner	25.1±3.7^{a}	25.5±4^{a}	14.9±1.5^{ab}	7.5±1^{b}
Total cyst production of each spawner	361±36^{a}	372±26^{a}	199±23^{b}	129±9^{b}
Total nauplii production of each spawner	343±35^{a}	342±28^{a}	217±32^{b}	82±9^{c}
Total birth of each spawner		715±21^{a}	417±28^{b}	211±9^{c}

Significant differences were determined by ANOVA ($p<0.05$).
*Different superscript letters in a row show significance and similar letters in a row show non-significance.

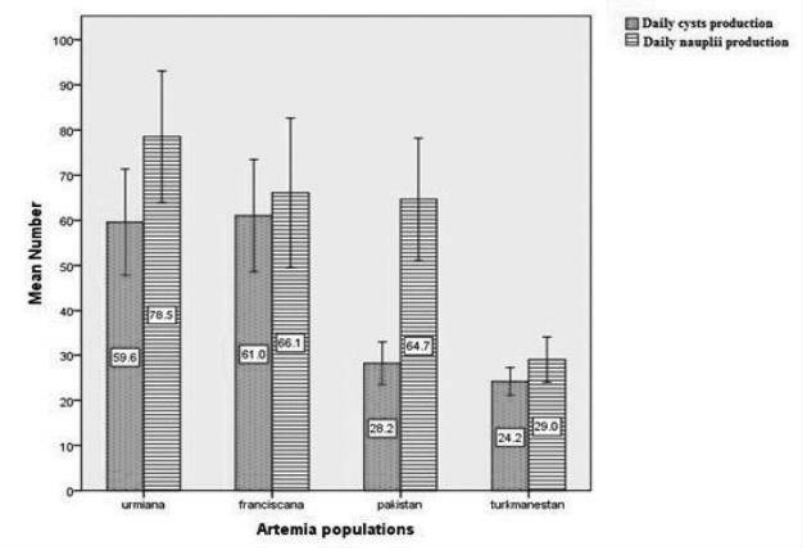

Fig. 1. Comparing the daily cyst and nauplii production rate among different *Artemia* populations

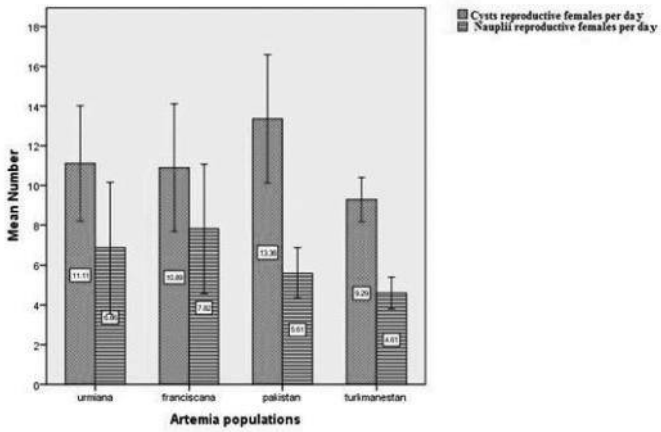

Fig. 2. Comparing daily cyst and nauplii producer spawners among different *Artemia* populations

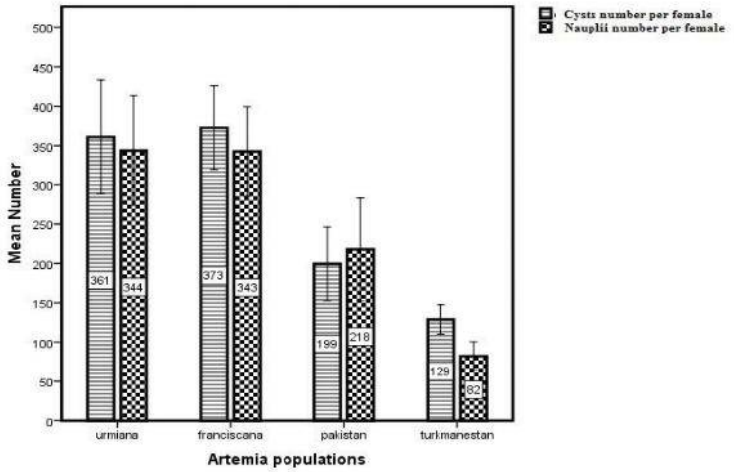

Fig. 3. comparing the total cyst and nauplii production of spawners from different *Artemia* populations

Artemia populations were shown in Table 1 and Figures 1-4.

In all *Artemia* populations, the mean daily nauplii production were higher than the mean daily cyst production. That *A. franciscana* had the highest daily cyst production and *A. urmiana* had the highest daily nauplii production. Also, the lowest daily cyst and nauplii production was observed in Torkamanestan population (Fig. 1).

In all *Artemia* daily spawner populations, the number of nauplii producers was higher than cyst producers and the Pakistan *Artemia* had the highest daily cyst producers and *A. franciscana* had the highest nauplii producers.

Figure 3 shows that during reproduction period, each spawner of *A. urmiana*, *A. franciscana* and Turkmenistan had the highest cysts production and each spawner of Pakistan *Artemia* had the highest nauplii production among their populations. Totally, each spawner of *A. franciscana* had the highest and each Turkmenistan *Artemia* had the lowest birth and fecundity. During reproductive period, *A. urmiana*, *A. franciscana* and Turkmenistan had the highest cysts number and Pakistan strain had the highest nauplii production in their populations. *A. franciscana* and *A. urmiana* had the highest cyst and nau-

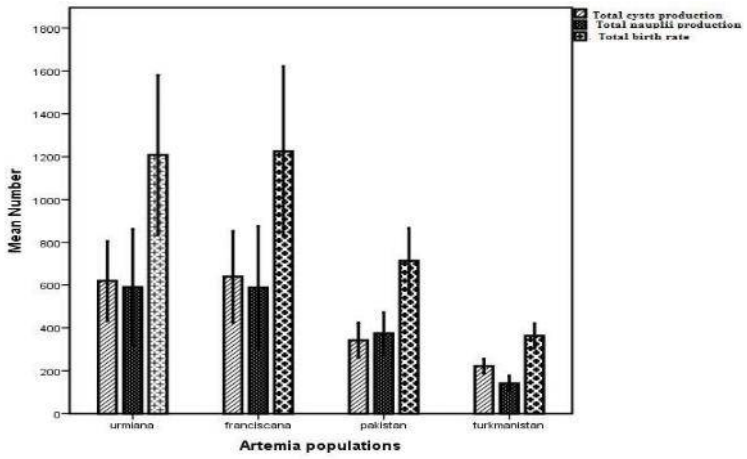

Fig. 4. Total cysts and nauplii production and total birth rate of four *Artemia* population

plii production compared to other strains. The highest and the lowest birth rate were belonged to *A. franciscana* and Turkmenistan *Artemia*, respectively (Fig. 4). Most of researchers have studied the reproductive characteristics and life cycle of bisexual and parthenogenetic *Artemia* populations of different geographical regions (e.g. Triantaphyllidis et al.,1995; Browne and Wanigasekera, 2000; Abatzopoulos et al., 2003; El- Bermawi et al., 2004; Baxevanis et al., 2004).

Triantaphyllidis et al., (1995) reported that the number of total offspring (cyst and nauplii), number of offspring at each birth (cyst and nauplii production) and the number of birth rate from each *Artemia* spawner was significantly higher in *A. franciscana* than Tanggu partenogenetic *Artemia*. But comparing bisexual *Artemia* from Egypt with parthenogenetic strains had a converse result (Baxevanis et al., 2004). Also, the percentage of nauplii production in bisexual Eygept *Artemia* and *A. franciscana* were significantly higher than parthenogenetic *Artemia*. Gajardo et al. (2002) suggested that *Artemia* strains with higher heterogygotic level was more suitable for cyst production. Also, Ahmadi (2003) mentioned that *A. urmiana* cyst production such as *A. franciscana* is under the genetic control and depends on heterozygotic level of studied species. Sorgeloos and Lavens (1996) mentioned that the most important factor affecting the cyst production of *A. urmiana* was the selection of appropriate cultured species, which should be based on scientific data of any kind regarding growth, especially reproduction. In fact, for breeding, we choose the species of each region that has the maximum growth and the maximum reproductive capacity in the heat and salinity regime of that area. In examining the results, there was no correlation between the number of breeding times and the reproductive model (cyst or nauplii production) of *Artemia* species with each other during the reproductive days, and each *Artemia* had specific features of that strain for birth, that depends on the genetic diversity of the *Artemia* population that was the same as Triantaphyllidis et al. (1995), Baxevanis et al. (2004), Guajardo et al. (2002). Regarding that total nauplii production has not significant difference in *Artemia* strains, and bisexual *A. urmiana* and *A. franciscana* have a significant difference than

Turkmenistan parthenogenetic *Artemia* in cyst production and number of offspring, that *A. franciscana* enjoys the highest birth rate of offspring and Turkmenistan parthenogenetic *Artemia* had the least birth rate that corresponds to Triantaphyllidis et al. (1995), Guajardo et al. (2002) and Ahmadi (2003).

A. urmiana, *A. franciscana* and Torkamanestan have enjoyed the highest cyst production within their own populations, while Pakistan parthenogenetic *Artemia* had enjoyed the most nauplii production within its own population the same as Triantaphyllidis et al. (1995) and Baxevanis et al. (2004) mentioned heterogenetic characteristics and intra population differences of each species. The results from each *Artemia* spawner, revealed that each *A. franciscana* and Turkmenistan has the most and the least birth rate and the most intra population cyst production were due to *A. urmiana*, *A. franciscana* and Torkamanestan population. Pakistan *Artemia* spawner had the highest nauplii production that was the same as Triantaphyllidis et al., (1995), Guajardo et al. (2002) and Ahmadi (2003). They mentioned the cyst production of bisexual *A. urmiana* and *A. franciscana* were higher and under genetic control but this results are incontrast with Baxevanis et al. (2004).

As bisexual *A. urmiana* and *A. franciscana* have a significant difference in total number of offspring, number of birth rate, and number of offspring from each birth than other species ($p>0.05$), therefore, bisexual *Artemia* has a different reproductive characteristics than parthenogenetic *Artemia* that was the same as El-Bermawi et al. (2004) and Baxevanis et al., (2004) that distinguished bisexual and parthenogenetic *Artemia* species of Egypt according to reproductive characteristics. As Turkmenistan *Artemia* has a significant difference in total number of offspring, number of birth rate, and number of offspring in each birth, than other species ($p< 0.05$), it can be mentioned that this *Artemia* has a different reproduction characteristics as Browne et al. (2002), reported that reproductive characteristics of *Artemia* have greatly influenced by environmental factors. *Artemia* populations situated far apart each other biotopes, live in different environmental conditions is according to Triantaphyllid et al. (1995), Browne and Wanigaseker (2000), Abatzopoulos et al. (2003), El-Bermawi et al. (2004) , Baxevanis et al. (2004). They proved that most *Artemia* populations show different responses based on their survival, growth and reproductive characteristics. Therefore, each strain based on high heterogenetic characteristics and population differences, may reflect the best accommodation at the resident strain of the area. On the other hand, cyst production of bisexual *Artemia* is under genetic control and depends on its heterozygoty level and the culture of each species has adapts with growth, reproductive pattern, reproductive characteristics and tolerable level of organism regarding to salinity and temperature of culture environment. Therefore, in order to select a species, the growth adaptation, breeding model, reproductive characteristics and living standards should be considered in relation to the salinity and temperature of the breeding place in order to select the best priority. Also, in choosing a species, the region's aquaculture should be directly linked with the use of *Artemia*. If, due to the diet of aquatic animals, the need for small naples, it is necessary to choose a species of *Ar-*

temia, which produces small nauplii and cysts, than other species, or if it requires biomass of *Artemia* in the aquaculture of the area, There should be a species of *Artemia* that has the power to grow, and grow better with the predominance of nauplii production, to be selected for *Artemia*. Therefore, if the goal of *Artemia* cultivation is to produce daily live mass, Pakistani parthognagmatic *Artemia* is more suitable than other species, because it has the most nauplii production within its population, although in the breeding season, *A. urmiana* and *A. franciscana* have more in breeding and reproduction. If the goal of the *Artemia* breeding is the production of cysts, it is recommended to breed *A. urmiana* and *A. franciscana* species. Turkmenistan's *Artemia* is not recommended for reproduction because of poor nauplii and cyst production and poor growth.

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Some New Records of Chromosome Numbers in Iranian Charophytes

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Abstract

Chara is an interesting genus from cytological view has been extensively worked out in Europe and North America, but there is a few reports on Asian Charophytes. Chromosome number were determined for 10 species of *Chara* from 33 populations. Chromosome counts were as follows: *C. connivens*, n=14, *C. contraria*, n= 28; *C. crassicaulis*, n=21; *Chara gymnophylla* var. *gymnophylla*, n=14; *Chara gymnophylla* var. *rohlenae*, n=14; *C. kirghisorum*, n=14; *C. kohrangiana*, n=21, *C. socotrensioides*, n=14; *C. tomentosa*, n=14; *Chara vulgaris* var. *longibracteata*, n=28 and *Chara vulgaris* var. *vulgaris*, n=28. Authors made cytological studies of Iranian charophytes in twelve taxa which 10 taxa are new for Iran and five taxa are new for science.

Keywords: Chromosome number, *Chara,* Charophytes, Iran.

Introduction

Cytological investigation of chromosome number in Characeae have been made in the last several decades and reported distinct chromosome number within different genus of Characeae (Hotchkiss, 1958 and 1963; Gillet, 1959; Corillion and Guerlesquin, 1959 and 1961; Imahori and Kato, 1964). Nagl and Furening (1979) have been reported that the chromosome number of algae are like to the higher plants, and posses localized centromers. Gillet (1956) suggested 7 base chromosome number in *Chara* and 6 base chromosome number for *Nitella* and 14 or possibility 7 base chromosome number for *Chara.* Ramjee and Sarma (1971) showed that there is 34% polypoloidy within different species of *Chara*. Polypoloidy is common mechanism for speciation and intraspecific barrier to gene flow in the genus *Chara* (Williams and Tindal, 1975). Several studies on *Chara* species showed that polypoloidy in dioecious species is more common than monoecious species (Grant and Proctor, 1972; Corillion and Guerlesquin, 1972; Guerlsquin 1967).

Study of the chromosome number in Characeae is useful tool for taxonomic problems (John et al., 1975). Several authors have been reported chromosome number of Characeae from India and Pakistan (Noor, 1969; Noor and Mukherjee, 1977; Khan and Sarma, 1967; Sinha and Verma, 1970), but there are a few works that have been done on Iranian Characeae (Sheidai et al., 1995). In the present study chromosome numbers have been reported in ten taxa belonging to the genus *Chara* L. which is new for Characeae.

Materials and Methods

Living speciemens were collected in June,

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July and August 2009 from different localities, then plants with Antheridia were kept in glass beakers in soil water medium. The specimens were identified with the matrix keys for *Chara* and *Nitella* (Van Raam and Stewart, 2009) and with the figures in Han and Li (1994), Krause (1997), Pal et al. (1962), Wood and Imahori (1964). Taxon names are in accordance with the names in Algaebase web site (www.algaebase.org) and the Synopsis of the Characeae (Van Raam and Stewart, 2009). Voucher mounted specimens were deposited in the Herbarium of Shahid Beheshti University (HSBU) and there is a list of localities in appendix table. *Chara connivens* Salzmann ex A. Braun, *Chara contraria* A. Braun ex Kützing, *Chara crassicaulis* Schleicher, *Chara gymnophylla* (A. Braun) A. Braun var. *gymnophylla*, *Chara gymnophylla* var. *rohlenae* (Vilhelm) Filarszky, *Chara kirghisorum* Lessing, *Chara pedunculata* Kützing, *Chara socotrensioides* R. D. Wood and *Chara kohrangia*., *Chara vulgaris* var. *longibracteata* (Kützing) J. Groves and Bullock-Webster, *Chara vulgaris* Linnaeus var. *vulgaris* and *C. tomentosa* Linnaeus. Branchlets with developing antheridia were removed and fixed in absolute ethanol and glacial acetic acid and aceto-carmine or aceto-orcein squashing method was used for chromosome preparations. Five to ten methaphase cells have been counted for chromosome number.

Results

In most of prophase cells there was clumping between chromosomes and produce a compact mass of chromosome or in some cells there is number of chromosome masses. Chromosome number of investigated species are included in Table 1 and Figures 1-12.

Table 1. Species of Characeae and their chromosome numbers.

Name of the taxa	Sexuality	Chromosome number (n=)	Section	Subsection	Figure
C. connivens	dioecious	n=2x=14	*Grovesia*		1
C. contraria	monoecious	n=4x=28	*Chara*		2
C. crassicaulis	monoecious	n=3x=21	*Chara*		3
Chara gymnophylla	monoecious	n=2x=14	*Chara*		4
Chara gymnophylla var. *rohlen* ((Vilhelm) Ahmadi in Ahmadi et al. 201	monoecious	n=2x=14	*Chara*		5
C. kirghisorum	dioecious	n=2x=14	*Chara*		6
C. kohrangiana Ahmadi 2012	monoecious	n=3x=21	*Chara*	Charopsis	7
C. pedunculata Kützing	monoecious	n=5x=35	*Chara*	Hartmania	8
C. socotrensioides	monoecious	n=2x=14	*Chara*		9
C. tomentosa	dioecious	n=2x=14	*Chara*		10
Chara vulgaris var. *longibracteata*	monoecious	n=4x=28	*Chara*		11
Chara vulgaris var. *vulgaris*	monoecious	n=4x=28	*Chara*		12

In most of the metaphase cells in *C. connivens* chromosome number was n=14 (Fig. 1). In *C. contraria* it was n=28 (Fig. 2). Mitotic cells in *C. crassicaulis* showed chromosome number n=21 (Fig. 3).

In *C. gymnophylla* two populations have been observed which were separated as *C. gymnophylla* var. *gymnophylla* and *C. gymnophylla* var. *rohlenae*, A chromosome number of 14 was observed for two varieties but there was some variations in chromosome number n=7, 21 for these two varieties (Figs 4, 5). In *C. kirghisorum* chromosome counts was obtained n=14 (Fig. 6). Our counts on chromosomes in antheridial cells for *C. kohrangiana* were n=21 (Fig. 7) . *C. pedunculata* chromosome number counts was n=35 (Fig. 8). *C. socotrensioides* chromosome counts was n=4 (Fig. 9). Chromosome number in *C. tomentosa* were obtained as n=14 (Fig. 10). Chromosome number in two varieties of *C. vulgaris* var. *longibracteata* and *C. vulgaris* var. *vulgaris* were n=28 (. 11, 12).

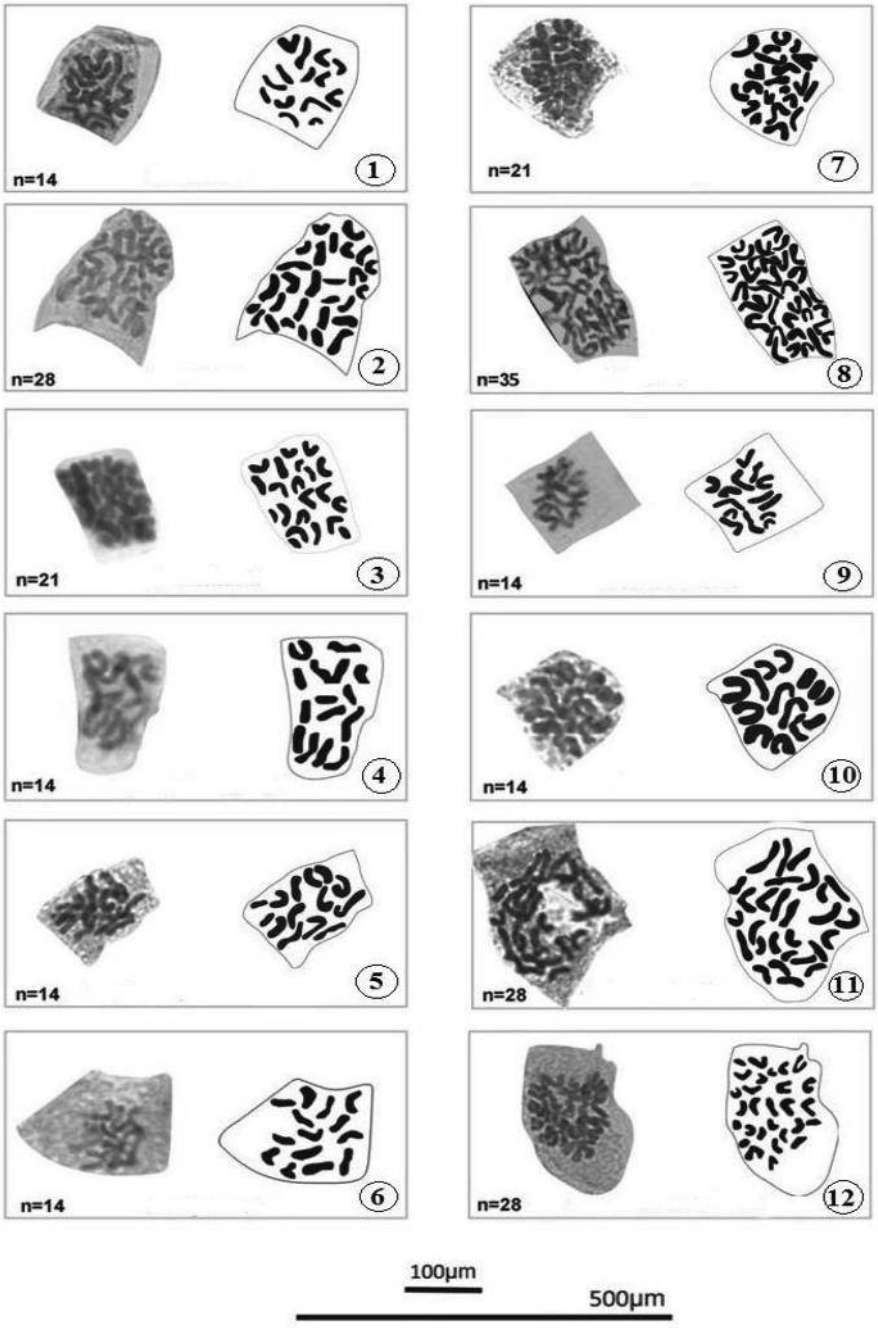
Discussion

The present finding support base chromosome number n=7 as ancestral basic chromosome number for the genus *Chara*. Kasaki (1964) proposed reduction theory of evolution in base chromosome number. Sarma et al. (1970), Sawa (1974) and Bhatnagar (1983) discussed on evolutionary sequences and interrelationships of Charophytes based on cytological findings. Sawa (1974) realised that ancestral forms of Charophyta possess x=3.

Antheridial spermatogenesis cells in Characeae have synchronous nucleous division, it helps to see easily metaphase chromosomes. There was chromosomes mass because of clumping in prophase cells and it may be happen because of homologues between repeated chromosomes. Grant and Proctor (1972) showed Chromosome number interspecific variation. They reported different chromosome number in *C. contraria* n=14, 28, 42. Grant and proctor (1970) have been studied cytotypes in *C. contraria* and found three different chromosome number (n= 14, 28, 42). Williams and Tindall (1975) reported n=42 for *C. contraria* and we found n=28. Our counts of *C. vulgaris* n=28 but Grant and Proctor (1972) reported n=14, 28, 42, John et al. (1975) determined n=42 for this species and Maszewski and Kolodziejzyk (1991) determined n=28. Noor and Mukherjee (1977) reported chromosome number in *C. vulgaris* var. *vulgaris* f. *artrovirens* n=35. *C. crassicaulisis* morphologically similar to *C. vulgaris* and we found n=21 in this species. Subrahmanyam and Chowdary (1992) reported chromosome counts in *Chara vulgaris* var. *gymnophylla* f. *grovesi* in=35 but our counts for two varieties of *C. gymnophylla* var. *gymnophylla* and *C. gymnophylla* var. rohlenaewere n=14,It was the first report for twovarieties of *C. gymnophylla*.

C. kirghisorum is also first report of chromosome numbern=14, in the point of taxonomic view it is similar to *C. vulgaris* but this species is dioecious. *C. kohrangiana* is a new species belongs to the genus *Chara* subgenus *Charopsis* section *Agardhia* subsection *Agardhia* (Ahmadi et al., 2012. under publication) and chromosome number was n=21. Chromosome counts in *C. pedunculata* is n=35. *C. socotrensioides* chromosome number is n=14 and it has not been reported before for this species. We counted chromosome number for *C. tomentosa* n=14, Kunachowicz et al. (2001) reported

n=14. Bhatnagar (1988) suggested that haplostephanous forms of *Chara* (subgenus: Charopsis) are primitive than the diplostephanous forms of *Chara* (subgenus: *Chara*). In this study most of dioecious species are diploid but monoecious species have different polypoloidy level (n=2x, 3x, 4x, 5x).



Figs. 1-12. Chromosome morphology in species of *Chara*: 1. *C. connivens*,2. *C. contraria*, 3. *C. crassicaulis*,4. *C.gymnophylla*var. *gymnophylla*, 5. *C. gymnophylla*var. *rohlenae*, 6. *C. kirghisorum*, 7. *C. kohrangiana*, 8. *C. pedunculata*, 9. *C. socotrensioides*, 10. *C. tomentosa*, 11. *C. vulgaris* var. *longibracteata*, 12. *C. vulgaris* var. *vulgaris*

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Table 2: Details of the localities.

C. connivens
Isfahan Province
31°12’36” N 51°40’12” E 2360 HSBU-8800320; 10.06.2009
C. contraria
Markazi province
39°48′40″N, 37°06′08″E 2275 HSBU-8800863; 3.04.2009
35°30′40″N, 37°55′25″E 1995 HSBU-8808610; 20.04.2009
35°36′25″N, 37°40′37″E 2200 HSBU-8808613; 29.03.2009
Fars province
30°25′05″N, 53°25′46″E 2375 HSBU-8800715; 12.05.2009
East Azarbaijan
36°36′13″N, 47°14′06″E 2202 HSBU-8800448; 26.06.2009
C. crassicaulis
Qom Province:
34°23′22″N, 50°51′41″E 1468 HSBU-8800251; 6.06.2009
Chara gymnophylla var**. *gymnophylla***
Fras province
30°27’37” N 51°47’22” E 2143 HSBU-8808719; 25.08.2009
Markazi province
39°13’84” N 38°98’15” E 2100 HSBU-8808627; 10.06.2009
34°87’96” N 37°81’28” E 1800 HSBU-8808611; 29.05.2009
34°80’96” N 37°71’76” E 1900 HSBU-8808614; 29.05.2009
Ardebil Province
38°32’07” N 47°53’55” E 1076 HSBU-8800451; 28.08.2009
East Azarbaijan
37°21’13” N 45°09’18” E 1334 HSBU-8800444; 26.08.2009
Chara gymnophylla var**. *rohlenae***
Fras province
30°37’36” N 53°10’41” E 2313 HSBU-8800711; 25.08.2009
Markazi province
34°80’96” N 37°71’76” E 1900 HSBU-8808614; 29.05.2009
Chaharmahal and Bakhtiari Province
32°17’24” N 50°38’52” E 2059 HSBU-8800381; 15.08.2009
Kohkiliyo and BoyerahmadProvice
30°51’56” N 51°20’06” E 1542 HSBU-8800741; 14.08.2009
Isfahan Province
32°47’07” N 51°01’50” E 1979 HSBU-8800313; 9.08.2009
C. kirghisorum
Markazi province
33°73’68” N 37°38’98” E 2034 HSBU-8808616; 31.07.2009
Chara kohrangiana
Chaharmahal and Bakhtiari Province
32°22’15” N 50°26’03” E 2324 HSBU-8800383; 15.06.2009
C. socotrensioides
Chaharmahal and Bakhtiari Province
31°51′35″ N 51°19′59″ E 1552 HSBU-8800742; 14.06.2009
C. pedunculata
IsfahanProvince
31°12’36” N 51°45’12” E 2360 HSBU-8800321; 10.06.2009
C. tomentosa
Isfahan Province
32°47’07” N 51°01’50” E 1979 HSBU-8800312; 06.08.2009
31°12’36” N 51°45’12” E 2360 HSBU-8800319; 10.06.2009
Chara vulgaris **var. *longibracteata***
Markazi province
39°87’23” N 38°28’31” E 2105 HSBU-8800864; 10.06.2009
33°84’34” N 37°99’36” E 1800 HSBU-8808615; 31.07.2009
34°36’20” N 50°21’21” E 1446 HSBU-8808619; 10.06.2009
Isfahan Province
33°57’56” N 51°15’03” E 1994 HSBU-8800311; 07.06.2009
Fars province
30°20’35” N 53°53’40” E 1891 HSBU-8800714;12.06.2009
Chara vulgaris **var. *vulgaris***
Markazi province
39°05’52” N 38°24’71” E 1943 HSBU-8800865; 10.06.2009
35°30’40” N 37°55’25” E 1995 HSBU-8800869; 20.07.2009
East Azarbaijan Province
37°27’45” N 44°55’32” E 1521 HSBU-8800445; 26.08.2009
Lorestan province
36°30’91” N 37°49’09” E 2000 HSBU-8800661; 20.08.2009
Qom Province
34°21’10” N 50°54’19” E 1600 HSBU-8800254; 06.08.2009

Study of Algal Flora of the 15th Khordad Dam

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Abstract

Algae have been described as more than 1800 genera and 2100 species which are different in terms of the biology, size, structure, and physiology. In this study, the phytoplankton populations of 15 khordad dam reservoir in Qom Province were determined. Four sampling sites were selected for this study. Sampling was carried out from January 2014 to June 2015 to assess algal flora and frequency of phytoplanktons. The results showed that Phytoplankton assemblage comprised several taxa from Diatomaceae (41.1%), Dinoflagellaceae (32.5%), Chlorophyceae (23.2%) and Cyanophyceae (3.2%). Among identified taxa, *Cyclotella* from Diatomaceae, *Peridinium* from Dinoflagellaceae, *Chlorella* from Chlorophyceae and *Oscillatoria* from Cyanophyceae being the predominant genera in each phylum. The maximum and minimum frequencies were recorded at one meter depth in west side edge during June and at five meter depth in the east side edge of the reservoire during December, respectively.

Keywords: Frequency, Algal flora, Phytoplankton, 15thKhordad dam

Introduction

Pyhtoplanktons are unicellualr or multicellular organisms that grow and propogate using sunlight, minerals and organic matter suspended in water which in turn are foodstock for other organisms. Algae are aquatic and photoautotrophic protists able to turn non organic to organic matter through photosynthesis. Algae have a special position in the biosphere and because of their presence in various habitats from air to underground waters have occupied an expansive area in comparison with other plants. Algae are prominent in bodies of several water reservoirs which occupy more than 70% of the Earth's surface (Gholami and Jamaloo et al., 2007; Mohammadi and Heidari, 1992; Mohammadi, 1999). Some species of algae cause problems with taste or smell in drinking water (Hedieh Loo, 2011). In fact, 80% of the disease worldwide are attributed to unhealthy water (Afsharzadeh et al., 2003). So far, many studies have been carried out to identify phytoplanktons and investigate their variability, propagation, and population in Iran and arround the world. 15th Khordad dam is located in 34°7' 47"72" and 50°61'30"50" N in the west of Sang Siahkooh village, Delijan, Markazi Province of Iran, 80 km for from Qom to Isfahan Road on Labshoor Qomrood river. The lake behind this dam which started to reserve water in 1997 contains 220 milion m^3. Average annual percipitation is 160 mm suggesting a dry region

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in the country. 15th Khordad dam is the first storage dam in Iran built on a saline river to procure urban drinking water. The aim of the present study was investigating algal flora of 15th Khordad dam and identifying and determining disperssion of various algal taxa in different regions of the dam and at different times of the year.

Materials an Methods

Sampling

In order to determine the concentration of algal flora, samples were collected from different depths and at different distances and location in the dam resrvoire. Water contents of the sampling bottles were kept in sampling containers which were transported in ice during the hot days for analysis within almost 24 hours. Equal volume of formalin solution 8% was used to stabilize the 1 litter samples. A total of 32 samples at two different depths were obtained from water near dam crest, coaslines, and lake center (Tashiee, 2013).

$$\text{No/ml} \ \frac{C \times 1000mm^3}{A \times D \times F}$$

Biological Assays

The type and concentration of algae were determined through biological assays. Samples were filtered through 0.8μm Whatman paper and then the paper filters were washed with 1 ml distilled water in a beaker. The washed content was then put on a simple slide or Sedjwick rafter slide and the algal flora were analyzed and counted using a microscope with monitor (Model: NLCD-120) with 10x or 40x magnification based on a counting protocole [13]. Sedjwick rafter slides which are usually used for counting planktons provide for $1000mm^3$ or 1mm in volume of the specimen. In samples with a great number of planktons (10 or more in each field) field counting was used instead of linear counting. Planktons were counted randomly in the fields 1 whiple grade. The number of fields counted depends on the density of the planktons. The number of planktons in 1ml volume of the sample was caculeted using the following equation:

Where C is the number of organisms counted, A is the area of the field (area of the whiple) in mm^2, D is the depth of each field (the depth of S-R slides) in mm^2, and F is the number of fields counted. The number of cells in each mm^2 was divided or multiplied by the correction factor and the results were obtained for the diluted or conentrated samples. The algae were determined in samples using the references books and monograph for algae (Yaghmaeeyan and Feiz Bakhsh, 2002; 2004; Madkour et al., 2010; Abed at al., 2014; Shah et al., 2008; Kolayli and Shahin, 2009, WHO). Statistical Package for Social Sciences (SPSS) was used for analysis of the repetitive data.

Results

Four taxa of algae, namely, Diatomceae, Cyanophyceae, Chlorophyceae, and Dinoflagellceae were determined in the samples obtained from reservoire near the west and east coastlines, behind the dam crest, and the lake center at one m and five meter depths during January, Feburary, March, April, May, and June. Table 1 shows list of algal groups and their mean number in samples from different regions of the 15th Khordad dam reservoir. Figure 1 shows the graph of the mean algae numbers counted in the samples obtained from different regions of the 15th Khordad dam. As the Figure

suggests, the most frequent algae belonged to Diatomceae, Dinoflagellceae, and Chlorophyceae taxa in that order while the least frequent algae belonged to Cyanophyceae taxa. The largest numbers of Diatomceae were counted near the west coast line water and lake center, respectively. The counts for dam crest water and east coast water were almost the same. Figure 2 represents the mean algae count in 15th Khordad dam in six different months. As the Figure shows, the number of algae increases as we get close the warm months of the year. In fact, the largest number of algae were observed during June, May, April, March, February, and January. The mean of algal counts observed at depths 1m and 5m of the reservoire shows in Figure 3, where the number of algae counted in depth 1m was more than those in depth 5m. Tukey HSD test shows that there is a significant difference between the number of algae in the edge of west side, the edge of east side, behind the dam era of Cyanophyceae, 6 genera of Chlorophyceae, 1 genus of Xanthophyceae, and 1 genus belonged to Dinoflagellceae. The highest frequency of occurance was 282390 per cm^2 in July. These findings confirm the results of the present study. According to our findings, the growth of algae depends on nutrients temperature and flow rate. Mohebbi et al. (2012) studied phytoplangton population and its character-

Table 1. List of algal groups and their mean number in samples from different regions of the 15th Khordad Dam reservoir

Algal group	West side edge		Behind dam crest		Center of the dam reservoir		East side edge	
	Depths 1m	Depths 5m	Depths 1m	Depths 5m	Depths 1m	Depths 5m	Depths 1m	Depths 5m
Chlorophyceae	1653	759	1624	734	1600	714	1577	704
Cyanophyceae	243	103	222	96	211	93	202	87
Diatomceae	3329	1217	3276	1144	3270	1138	3206	1122
Dinoflagellceae	2504	980	2477	963	2446	954	2412	940

Table 2. Tukey SHD test for location

Location (I)	Location (J)	Mean Difference (I-J)	Std. Error	Sig.	%95 Confidence Interval	
					Lower Bound	Upper Bound
Western Side	Behind the Dam Crest	54.490*	1.8464	.000	49.47	59.34
	Center of the Reservoire	3.723*	1.8464	.000	29/980	39.467
	Eastern Side	67.432*	1.8545	.000	62.668	72.196
Behind the Dam Crest	Western Side	-54.490*	1.8464	.000	-59.234	-49.747
	Center of the Reservoire	-19.767*	1.8625	.000	-24.552	-14.982
	Eastern Side	12.942*	1.8705	.000	8.136	17.747
Center of the Reservoire	Western Side	-34.23*	1.8464	.000	-39.467	-29.980
	Behind the Dam Crest	19.67*	1.8625	.000	14.982	24.552
	Eastern Side	32.709*	1.8705	.000	27.903	37.514
Eastern Side	WesternSide	-67.432*	1.8545	.000	-72.196	-62.668
	Behind the Dam Crest	-12.942*	1.8705	.000	-17.747	-8.136
	Center of the Reservoire	-32.709*	1.8705	.000	-37.514	-27.903

istics in Aras dam through sampling from three stations (dam crest, center, and sides) along the dam reservoire to determine and count pyhto-planktons and the relevant fluctuations in their population during different seasons. Similarly, abundance of the taxa during spring time and its difference in the western side station were more because of the wind direction.

As a conclusion it revealed Chlorophyceae, Xanthophyceae, Chrysophyceae, Diatomceae,

Table 3. Tukey HSD test analysis for the effect of various sampling months on species

Month (I)	Month (J)	Mean Difference (I-J)	Std. Error	Sig.	%95 Confidence Interval	
					Lower Bound	Upper Bound
January	February	-37.927*	2.3754	.000	-44.696	-31.158
	March	-102.051*	2.3556	.000	-108.764	-95.338
	April	-217.835*	2.3280	.000	-224.469	-211.201
	May	-343.657*	2.2945	.000	-350.196	-337.119
	June	-481.442*	2.2651	.000	-487.897	-474.987
February	January	37.927*	2.3754	.000	31.158	44.696
	March	-64.124*	2.3462	.000	-70.810	-57.438
	April	-179.908*	2.3185	.000	-186.515	-173.301
	May	-305.730*	2.2848	.000	-312.242	-299.219
	June	-443.515*	2.2553	.000	-449.42	-437.088
March	January	102.051*	2.3556	.000	95.338	108.764
	February	64.124*	2.3462	.000	57.438	70.810
	April	-115.784*	2.2981	.000	-122.333	-109.235
	May	-24.606*	2.2642	.000	-248.059	-235.154
	June	-379/391*	2.2344	.000	-385.759	-373.24
April	January	217.835*	2.280	.000	211.201	224.469
	February	179.908*	2.3185	.000	173.301	186.15
	March	115.784*	2.2981	.000	109.235	122.333
	May	-125.822*	2.2355	.000	-132.193	-119.452
	June	-263.607*	2.2053	.000	-269.891	-257.322
May	January	343/657*	2.2945	.000	337.119	350.196
	February	305.730*	2.2848	.000	299.219	312.242
	March	241.606*	2.2642	.000	235.154	248.059
	April	125.822*	2.2355	.000	119.452	132.193
	June	-137.785*	2.1699	.000	-143.968	-131.601
June	January	481.442*	2.2651	.000	474.987	487.897
	February	443.515*	2.2553	.000	437.088	449.942
	March	379.391*	2.2344	.000	373.024	385.759
	April	263.607*	2.2053	.000	257.322	269.891
	May	137.785*	2.1699	.000	131.601	143.968

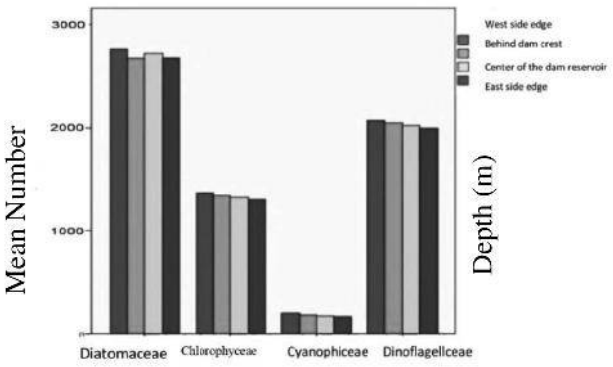

Fig. 1. Mean algae number in samples from different regions of the 15th Khordad dam reservoir

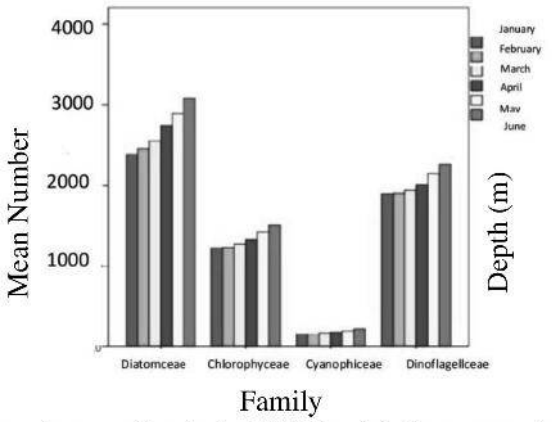

Fig. 2. Mean algae number in the 15th Khordad Dam reservoir in 6 different months

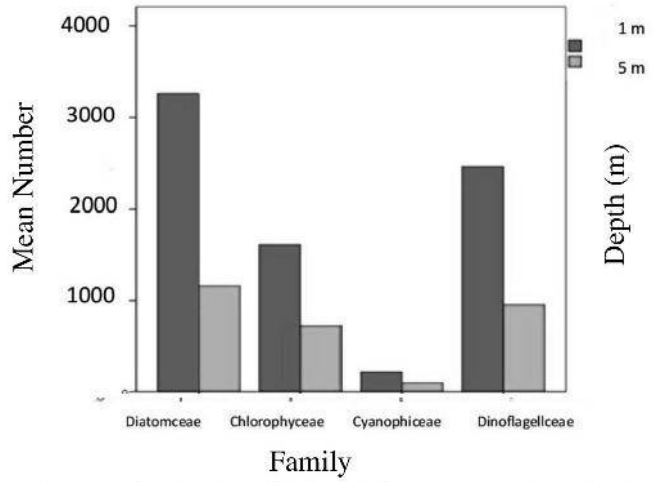

Fig. 3. Mean algae number in the 15th Khordad Dam reservoir at depths 1 and 5 m

Dinoflagellceae, Euglenophyceae, Rhodophyceae, and Cyanophyceae were probable to be present in the 15th Khordad dam reservoire. Analysis of data showed the presence of Chlorophyceae alga (23.2%): *Cosmarium* Corda ex Ralfs, *Tetraspora* Link ex Desvaux, *Oocystis* Nägeli ex A. Braun, *Chlorella* Kützing, *Brachimonas* Bohlin, *Pediastrum* Meyen, *Closterium* Nitzsch ex Ralfs, *Pandorina* Bory and *Ankistrodesmus* Corda, genera; Cyanophyceae (3.2%): *Chroococcus* Nägeli, *Oscillatoria* Vaucher ex Gomont and *Anabaena* Bory ex Bornet and Flahault, genera; Dinoflagellceae (32.5%) belonging to *Peridinium* Ehrenberg and *Ceratium* F. Schrank, genera and Diatomceae (41.1%): *Cymbella C. Agardh, Cyclotella* Kützing, *Synedra* Ehrenberg and *Diatoma* Bory. On the other hand, no instances of Rhodophyceae, Chrysophyceae, Xantophyceae and Euglenophyceae algae were observed in the study. Also the study revealed that the disperssion of algae in the 15th Khordad dam reservoire was not the same under different conditions and there was variability under the factors such as the depth, place, and seasons of sampling.

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