 DOI: 10.22102/JAEHR.2019.140617.1092

ORIGINAL PAPER

Effects of nutrients on the primary production and determination of the restricting factors in primary production in the international wetland of Choghakhor (Iran)

Mohammad Hadi Abolhasani[1,✉], Niloofar Pirestani[2], Saeed Ghasemi[2]

1. Waste and Wastewater Research Center, Department of Environmental Science, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran
2. Department of Environmental Science, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran

Date of submission: 13 Jul 2018, ***Date of acceptance:*** 27 Apr 2019

ABSTRACT

Limitations in nutrients and physicochemical parameters play a key role in aquatic ecosystems. The present study aimed to determine the influential physicochemical factors in the chlorophyll-a content for wetland management by identifying the restricting factors in primary production. Sampling was conducted during March 2017-February 2018. Factors such as water salinity, temperature, pH, nitrate and phosphate concentrations, biochemical oxygen demand, total dissolved solids, electrical conductivity, total suspended solids, and dissolved oxygen were measured in triplicate at each station. In addition, the trophic state index (TSI) was used to determine the trophic state of the wetland. No significant difference was observed in chlorophyll-a contents in different seasons ($P>0.05$), with the highest values reported in spring and autumn, and the lowest values reported in summer. The maximum and minimum chlorophyll-a content were observed in stations A and C, respectively. Station A had a significantly higher value compared to the other stations ($P<0.05$). In addition, no significant differences were observed in the water physicochemical parameters in different seasons ($P>0.05$). The highest (5.9 mg/l) and lowest water nitrate levels (4.1 mg/l) were observed in spring and autumn, respectively (mean nitrate level: 4.84 mg/l). The highest (2.1 mg/l) and lowest water phosphate levels (0.47 mg/l) were observed in spring and autumn, respectively (mean nitrate level: 1.04 mg/l). Moreover, TSI indicated that the wetland is oligotrophic in spring and winter, while it is mesotrophic in summer and autumn. Overall, the nitrate level in water was the main restricting factor in the management of Choghakhor wetland.

Keywords: TSI, Primary production, Choghakhor wetland, Nutrient effects

Introduction

The beneficial functions of wetlands include water reservation and transportation, production of flora and fauna, decomposition of organic materials, and serving as valuable habitats for various organisms.[1] Moreover, wetlands are considered to be important conservation habitats for wildlife such as birds, mammals, fish, amphibians, insects, and plants.[2] Wetlands are classified as natural and artificial, and water is the key factor in both categories. With an area of 1,360 hectares, Choghakhor wetland is the only artificial wetland registered in the international wetlands of Iran and an important wintering habitat for native and migrating birds. Choghakhor wetland is located at 2,270 meters above the sea level with the geographical coordinates of 31°54′17″N-31°56′31″N and 50°52′40″E-50°56′14″E (Document p120, 1996).[3]

Phytoplanktons are at the base of the food chain, serving as primary producers due to the capacity to convert inorganic materials into organic forms.[4] The primary production of phytoplanktons is defined based on the amount of organic materials per unit area and time.[5] Primary producers absorb sunlight by

✉ *Mohammad Hadi Abolhasani*
Hadi.Mha2001@yahoo.com

Citation: Abolhasani MH, Pirestani N, Ghasemi S. Effects of nutrients on the primary production and determination of the restricting factors in primary production in the international wetland of Choghakhor (Iran). J Adv Environ Health Res 2019; 7(2): 94-100

chlorophyll-a and produce organic compounds by combining water and carbon dioxide during photosynthesis. In this process, chlorophyll-a is the main production pigment, as well as an indicator of phytoplankton biomass.[6] In addition, chlorophyll-a concentration and distribution are the indicators of nutrient concentrations and primary production.[7] Considering the direct correlation between nutrient concentrations and primary production, investigation of nutrient limitation could contribute to recognizing the controlling factors of production in aquatic ecosystems.[8]

Phosphate, nitrate, and silicate are the major nutrients for the growth and distribution of phytoplanktons. In general, phosphate is the restricting factor for primary production in freshwater, while nitrate is considered to be the restricting factor in oceans.[1] Moreover, increased nutrient concentrations have adverse effects on aquatic organisms, and accumulation of phosphate in water and sediments causes eutrophication.[9]

Eutrophication increases phytoplankton bloom and decreases water oxygen, thereby leading to cyanobacteria bloom. Consequently, the population of macrophyte, algae, invertebrate, and fish decline, causing fundamental changes in biodiversity. Nutrient monitoring is essential to avoiding eutrophication. The physicochemical and biological factors in aquatic ecosystems affect the composition of species and biomass of phytoplanktons.[10]

Currently, data is scarce regarding the eutrophication of Choghakhor wetland in Iran. The present study aimed to measure water physicochemical factors to determine the influential factors in primary production and contribute to wetland management by clarifying the restricting factors in primary production.

Materials and Methods

Choghakhor wetland is an artificial freshwater wetland located in Boldaji town, which is in Boroujen county of Charmahal-and-Bakhtiyari province, Iran.[11] The wetland is located at the distance of 12 kilometers from Boldaji town, which is the nearest region to the wetland,[11] and 65 kilometers from Shahrekord. Surface waters are slightly involved in the water supply of the wetland. However, the main suppliers are natural springs, such as Sibak, Tong-E-Siah, Cheshme Zardegan, Cheshme Avardegan, Cheshme Eskiabadi, and Cheshme Galoogard. The region is mesothermal in summer and cold in winter, with the maximum and minimum temperature of 21 ºC (August) and 6 ºC (December), respectively.[12]

Sampling

The stations in the north, south, east, and west of the studied region were selected based of availability and the wetland shape. The coordinates of the stations were as follows: station A (31o54'39"N 50o54'36"), station B (31o54'27"N 50o54'40"), station C (31o55'02"N 50o55'23"), and station D (31o54'26"N 50o53'59"). Sampling was performed at the depth of 10-15 centimeters from the surface of water.

Water analysis

Triplicate samples were obtained from the stations at 10 AM-2 PM. To determine the physicochemical parameters of water, one liter of water was collected from each station and transferred to the laboratory, avoiding light and heat. Water salinity, temperature, and pH were determined at the stations using a digital apparatus. In addition, water nitrate and phosphate levels were measured using standard methods and a spectrophotometer.[13] Water biochemical oxygen demand (BOD_5), total dissolved solids (TDS), electrical conductivity (EC), and total suspended solids (TSS) were also measured using standard methods.[14]

The trophic state index (TSI) was used to determine the trophic state of the wetland.[15] TSI classifies the water body into three categories of oligotrophic, mesotrophic, and eutrophic based on a digit scale (0-100). Near-zero TSI shows ultra-oligotrophic water, and the values of near 100 represent hyper-eutrophic water.

$$TSI\ (CHL\ a) = 9.81 \ln (CHLa) + 30.6$$

CHLa=chlorophyll-a concentration (mg/m^3)

Various methods are used to determine the primary production of aquatic ecosystems, the most common of which involves the measurement of chlorophyll-a content based on the following formula:[16]

$$P = \frac{R}{K} \times C \times 3.7$$

where P represents the phytoplankton photosynthesis (g carbon/m^2/day), R is the relative amount of the light radiated to water, K shows the reflection coefficient (m), and C is the chlorophyll-a concentration (mg/m^3).

In addition, the value of 3.7 in the formula is based on the gram on fixed carbon per gram of chlorophyll within one hour of photosynthesis, and the K coefficient is 0.3 for shallow wetlands.

In this study, chlorophyll was extracted using acetone for 48 hours in dark and cold conditions (temperature: 3-5 ºC). The transmittance rate of the extracts was read at the wavelengths of 645 and 665 nanometers using a UV-Vis spectrophotometer in accordance with the method proposed by Vollenweider (1974) in order to calculate the concentration of chlorophyll-a.[16] All the experiments were performed in triplicate.

Statistical analysis

Comparison of the primary production between the seasons and stations was performed using completely randomized blocks (sampling of the stations). Data analysis was carried out using two-way analysis of variance (ANOVA) and Duncan's test at the significance level of 0.05. In addition, the principal component analysis (PCA) was applied to determine the main influential physicochemical factors. Multivariate stepwise regression was also employed to verify the correlation between chlorophyll-a content and environmental factors. All the analyses were performed in SAS software.

Results and Discussion

In the present study, no significant differences were observed in the physicochemical parameters of water in different seasons (P>0.05) (Table 1). The highest (5.9 mg/l) and lowest water nitrate levels (4.1 mg/l) were observed in spring and autumn, respectively, with the mean nitrate value of 4.84 mg/l. The highest (2.1 mg/l) and lowest water phosphate levels (0.47 mg/l) were observed in spring and autumn, respectively, with the mean phosphate value of 1.04 mg/l.

The mean water salinity was 14.37 ppt, and the maximum and minimum levels were observed in summer (25.75 ppt) and winter (8 ppt), respectively. The mean dissolved oxygen level during the study period was 5.26 mg/l, with the highest value observed in winter (6.77 mg/l), and the lowest level observed in summer (2.55 mg/l).

Table 1. Physicochemical parameters of water (trophic index and TSI) in different seasons in Choghakhor wetland, Iran (2017-2018)

Parameter	Spring	Summer	Fall	Winter
DO (mg/L)	1.16±5.16	0.93±2.55	1.1±6.57	1.22±6.77
BOD	0.42±2.77	0.26±1.6	0.45±3.17	0.78±3.27
Primary production (g/m^{2}day C)	1.13±12.5	1.06±11	1.44±12.5	1.76±11.75
pH	0.5±7.23	0.76±7.75	0.8±8.45	0.76±7.95
Temperature (°C)	1.6±19.75	1.91±24.75	1.23±12.12	0.78±38.87
Salinity (ppt)	1.17±10.5	1.75±25.75	1.43± 13.25	1.1±8
Nitrate (ppm)	0.43±4.85	0.34±4.1	0.56±5.9	0.34±4.52
Phosphate (ppm)	0.12±0.79	0.09±0.47	0.56±2.1	0.12±0.81
Ec	3.33±45.2	2.67±39.75	3.2±40.25	3.45±52.75
TDS	1.16±16.5	1.09±10.25	1.1±10.8	1.23±18.5
Deep (m)	0.49±2.17	0.33±1.6	0.67±2.04	0.45±1.95
Trophy Index (TSI)	34.3 (Oligotroph)	41.8 (Mesotroph)	40.2 (Mesotroph)	38.1 (Oligotroph)

Based on the TSI, the wetland was considered to be oligotrophic in spring and winter, while it was considered to be mesotrophic in summer and autumn. PCA was applied to determine the influential physicochemical parameters of water in chlorophyll-a content (Table 2). The identified factors encompassed 59.54% of the total variance and considered to be the major influential factors.

Table 2. Results of PCA regarding reduction of physicochemical parameters of water in Choghakhor wetland, Iran (2017-2018)

Cumulative variance	Component	Total	Percentage of variance
20.8	1	2.78	20.56
42.6	2	1.57	19.23
59.54	3	1.13	14.3

To date, no integrated studies have been focused on biological and ecological parameters and their fluctuations in Choghakhor wetland, and research in this regard is quite outdated. The present study aimed to complete the current data regarding the effects of nutrients on primary production and contribute to wetland management by determining the restricting factors in primary production.

According to the findings of Abolhassani *et al.* regarding the assessment of the primary production status of the international Gavkhooni wetland, nitrate is the restricting factor in primary production in Gavkhooni wetland. In the mentioned research, TSI indicated that the wetland is oligotrophic in spring, autumn, and winter, while it is mesotrophic in summer.[17]

In another study by Ardila *et al.* on ecological assessment based on the analysis of physico-chemical and biological interactions in the trophic state of Libelula wetland in Bogotá (Colombia), the results indicated that the eutrophic state has led to the formation of specific phytoplankton, zooplankton, and macrophyte species, which have been associated with nutrient- and organic matter-enriched water ecosystems. Furthermore, the changes in the bathymetry of Libelula wetland was studied over time using the geographic information system (GIS) and unmanned aerial vehicles (UAV). The findings showed a significant negative impact principally due to the residential activities that lead to the loss of important ecosystem services.[18]

In the current research, the influential factors determined by PCA were used in stepwise regression in order to identify the optimally fitted model for the main factors. The stepwise test revealed that among these parameters, nitrate and phosphate had the highest cumulative variance compared to the other factors. Table 3 show output of cumulative rate of physicochemical parameters of water in Choghakhor wetland.

Table 3. Output of cumulative rate of physicochemical parameters of water in Choghakhor wetland

Factor	Cumulative percentage
DO	0.67
BOD	0.65
pH	0.6
Temp	0.61
Salinity	0.52
NO_3	0.72
PO_4	0.73
EC	0.56
TDS	0.63
Deep	0.52
TSS	0.4

As is observed in Fig. 1, there was no significant difference in the chlorophyll-a concentration in different seasons (P>0.05). The highest chlorophyll-a content was observed in spring and autumn, whereas the lowest content was observed in winter.

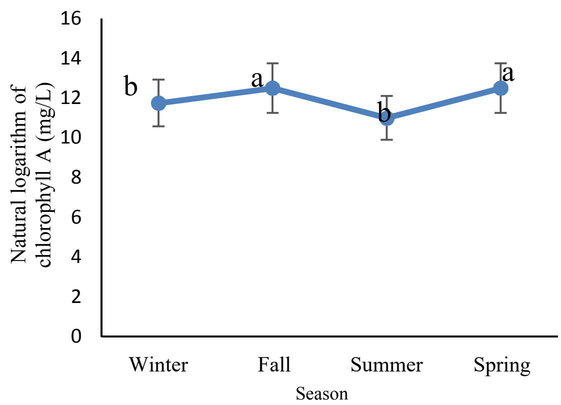

Fig. 1. Mean natural log of chlorophyll-a (mg/m^3) in different seasons in Choghakhor wetland (2017-2018)

Compared to the results obtained by Fathi *et al.*, our findings suggested a decrement in the chlorophyll-a content in the wetland. In the mentioned study, they reported the annual mean chlorophyll-a content to be 18.75 mg/l, with the peak observed in summer and autumn.[19] Moreover, our findings indicated that the wetland was not more eutrophic compared to the study by Ebrahimi *et al.* On the other hand they reported that the wetland is mesotrophic.[3]

According to the results of the present study, there were two peaks in the chlorophyll-a content of the wetland in spring and autumn, which was due to the optimization of phytoplankton bloom conditions. It is notable that the minimum chlorophyll-a content was reported in summer and winter.[20]

In the current research, TSI was estimated at 38.6 in the wetland, suggesting that the wetland was in an oligotrophic state. The highest index value was observed in summer, demonstrating the mesotrophic state of the wetland in this season. However, summer represented the lowest chlorophyll-a content, while the highest content was reported in autumn and spring, denoting an oligotrophic state in these seasons.

According to the results of PCA, water nitrate and phosphate were the most significant influential factors in chlorophyll-a content, which emphasizes on the importance of nutrients in primary production. Furthermore, the stepwise regression analysis showed that water nitrate was a major restricting factor in the wetland as when the wetland contained high phosphate concentrations, and the nitrate to phosphate ratio dropped to certain levels, nitrate-N was the restricting factor.[1] Similarly, water nitrate was reported to be the restricting factor in Karst Lake (Germany), and addition of nitrate to the water increased the phytoplankton population.[21] Phosphate has been reported to be the restricting factor in Bitter Lakes (Egypt), suggesting that the lake is oligotrophic.[22] Overall, nitrogen seems to be more important in primary production compared to other water nutrients.

In another study, Ghorbani *et al.* evaluated the effects of physico-chemical factors on chlorophyll-a in Shadegan international wetland, located in Khouzestan province (Iran), reporting that based on the TSI, Shadegan wetland is mesotrophic in spring and winter, while it is eutrophic in summer and autumn. Overall, the lake was reported to be a suitable environment for warm water species.[23]

Abolhasani *et al.* assessed the primary production status of the international Gavkhooni wetland (Iran), observing that the mean water nitrate level of the sample stations was 4.255 mg/l, with the highest (5.07 mg/l) and lowest levels (3.35 mg/l) recorded in summer and autumn, respectively. In addition, the mean water phosphate level of the sample stations in the mentioned study was estimated at 1.082 mg/l, with the highest (1.75 mg/l) and lowest levels (0.57 mg/l) recorded in winter and summer, respectively.[17]

As is depicted on Fig. 2, the significantly highest chlorophyll-a content was observed in station A ($P<0.05$), while the lowest concentration was observed in station C.

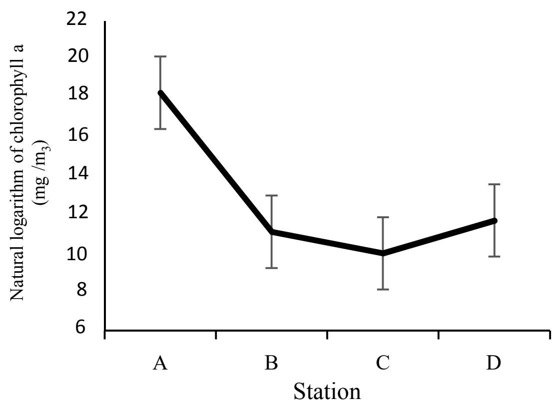

Fig. 2. Mean content of chlorophyll-a (mg/m^3) in various stations in Choghakhor wetland (2017-2018)

Finally, the stepwise analysis demonstrated that among various water parameters, nitrate was the only significant influential factor in the concentration of chlorophyll-a in the wetland.

Chlorophyll-a=11.23 – 2.14 NO_3; R^2=0.83

According to the results of the present study, water nitrate was the main restricting factors in primary production in Choghakhor wetland. The lowest chlorophyll-a content was observed in summer, which might be due to higher temperature as most phytoplanktons die or have limited growth under over-radiation conditions.[20] Moreover, low phytoplankton population and high water temperature increase the activity of organisms, thereby leading to the depletion of the water oxygen content. Low light intensity and shortened daytime might be the causes of the low primary production in the wetland in winter. The highest water TSS could be another negative influential factor in the rate of phytoplankton photosynthesis. Accordingly, the lowest chlorophyll-a was observed in station C, which might be due to the high water TSS during the study period.

In this regard, Ghorbani *et al.* evaluated the effects of physico-chemical factors on chlorophyll-a content in Shadegan international wetland, reporting the annual average chlorophyll-a concentration to be 10.28 mg/m^3. However, no significant seasonal differences were observed (P>0.05). The maximum chlorophyll-a content was observed in autumn (29.63 mg/m^3), whereas the minimum value was observed in spring (4.07 mg/m^3).[23]

Abolhasani *et al.* assessed the primary production status of the international Gavkhooni wetland, reporting no significant difference in the mean chlorophyll-a content in different seasons. Furthermore, the highest and lowest chlorophyll-a contents were observed in spring and winter, respectively; and the significantly highest content was measured in station A (Shakh Kenar).[17]

Nutrient fluctuations may largely influence the structure and composition of phytoplankton populations over time.[1, 24] According to the results of the present study, fluctuations in the nutrients in Choghakhor wetland were parallel as the highest water nitrate and phosphate levels were observed in spring, and the lowest levels were reported in summer. Moreover, a direct correlation was denoted between the water chlorophyll-a content and nutrient levels. Surface water nitrate was found to increase in winter, autumn, and early spring, while it decreased in late autumn and summer.[11]

In the current research, water nitrate showed a specific cycle in different seasons as it decreased from spring to summer and increased in autumn, followed by further reduction in winter.[18] Phosphate had a similar change pattern, which indicated that primary production levels change along with the changes in water nutrients in Choghakhor wetland. In general, nitrogen and phosphorous are considered to be the main restricting factors in aquatic ecosystems, and the growth of phytoplanktons depends on the concentrations of these compounds.[25]

Comparison of the present study with the findings of Fathi et al. demonstrated that the levels of water nutrients increased markedly, while the chlorophyll-a content decreased, thereby leading to the reduction of phytoplankton growth and diversity. Elevations in the water nutrients of wetlands may be an alarm for eutrophication.[19]

Conclusion

According to the results, TSI indicated that Choghakhor wetland was oligotrophic in spring and winter, while it was mesotrophic in summer and autumn. In general, water nitrate was considered to be the main restricting factor in Choghakhor wetland.

Acknowledgements

Hereby, we extend our gratitude to the Research Institute for Waste and Wastewater affiliated to the Islamic Azad University of Isfahan (Khorasgan) for assisting us in this research project.

References

1. Reynolds CS. The ecology of phytoplankton: Cambridge University Press. 2006.
2. Downard R, Frank M, Perkins J, Kettenring K, Larese-Casanova M. Wetland Plants of Great Salt Lake, A Guide to Identification, Communities & Bird Habitat. Utah State University Extension, Logan, Utah. 2017.
3. Ebrahimi S, Moshari M. Evaluation of the Choghakhor wetland status with the emphasis on environmental management problems. Publs Inst Geophys Pol Acad Sc 2006; 6: 390-398.
4. Lichti DA, Rinchard J, Kimmel DG. Changes in zooplankton community, and seston and zooplankton fatty acid profiles at the

freshwater/saltwater interface of the Chowan River, North Carolina. Peer J 2017; 5:e3667.

5. Cloern JE, Foster S, Kleckner A. Phytoplankton primary production in the world's estuarine-coastal ecosystems. Biogeosciences 2014; 11(9): 2477- 2501.
6. Carder K, Steward R. A remote sensing reflectance model of a red-tide dinoflagellate off west Florida. Limnol Oceanogr 1985; 30(2): 286-298.
7. Behrenfeld MJ, Falkowski PG. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnol Oceanogr 1997; 42(1): 1-20.
8. Warren DR, Collins SM, Purvis EM, Kaylor MJ, Bechtold HA. Spatial variability in light yields colimitation of primary production by both light and nutrients in a forested stream ecosystem. Ecosystems 2017; 20(1): 198-210.
9. Rathore S, Chandravanshi P, Chandravanshi A, Jaiswal K. Eutrophication: Impacts of Excess nutrient inputs on aquatic ecosystem. IOSR J Agric Vet Sci 2016;9(10): 2319-2372.
10. Naz M, Türkmen M. Phytoplankton biomass and species composition of Lake Gölbaşı (Hatay-Turkey). Turk J Biol 2005; 29(1): 49-56.
11. Zamani-Ahmadmahmoodi R, Jafari A, Alibeygi-Beni H. Potential ecological risk assessment, enrichment, geoaccumulation, and source identification of metals in the surface sediments of Choghakhor Wetland, Iran. Environ Earth Sci 2017; 76(11): 398-407.
12. Malekmohammadi B, Jahanishakib F. Vulnerability assessment of wetland landscape ecosystem services using driver-pressure-state-impact-response (DPSIR) model. Ecol Indic 2017; 82: 293-303.
13. Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater. APHA, AWWA and WPCF, Washington DC. 1996.
14. W Rice. Eugene & Public Health Association, American. Standard methods for the examination of water and wastewate, 2012.
15. Kulshreshtha A, Shanmugam PD. Development of optical models for assessing the trophic status of coastal waters, OCEANS 2017 - Aberdeen, Aberdeen, 2017, pp. 1-7.
16. Aleem AA, Samaan AA. Productivity of Lake Mariut, Egypt. Part II. Primary production. Int Revue ges Hydrobiol Hydrogr 1969; 54(4): 491-527.
17. Abolhasani MH, Pirestani N, Ghasemi S. Assessment of the primary production statues of the international Gavkhooni Wetland, Iran. Int J Aquat Biol 2018; 6(5): 248-253.
18. Ardila L, Mora S, Flórez R. Ecological assessment by physical chemical and biological interactions analysis in trophic state of Libelulas wetland–Bogotá-Colombia. Int J Environl Sci Dev 2018; 9(9): 236-243.
19. Fathi P, Ebrahimi E, Mirghafari N, Esmaeili A. The study spatial and temporal changes of water quality in Choghakhor wetland using water quality index (WQI). J Aquat Ecol 2016; 5(3): 41-50.
20. González-García C, Forja J, González-Cabrera M, Jiménez M, Lubián L. Annual variations of total and fractionated chlorophyll and phytoplankton groups in the Gulf of Cadiz. Sci Total Environ 2018; 613: 1551-1565.
21. Camacho A, Wurtsbaugh WA, Miracle MR, Armengol X, Vicente E. Nitrogen limitation of phytoplankton in a Spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment bioassay approach. J Plankton Res 2003; 25(4): 397-404.
22. El-Serehy HA, Abdallah HS, Al-Misned FA, Irshad R, Al-Farraj SA, Almalki ES. Aquatic ecosystem health and trophic status classification of the Bitter Lakes along the main connecting link between the Red Sea and the Mediterranean. Saudi J Biol Sci 2018; 25(2): 204-2012.
23. Ghorbani R, Hosseini SA, Hedayati SAA, Hashemi SAR, Abolhasani MH. Evaluation of effects of physico-chemical factors on chlorophyll-a in Shadegan international Wetland-Khouzestan province-Iran. Iran J Fish Sci 2016; 15(1): 360-368.
24. Winder M, Hunter DA. Temporal organization of phytoplankton communities linked to physical forcing. Oecologia 2008; 156(1): 179-192.
25. Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, et al. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol lett 2007; 10(12): 1135-1142.