**Int. J. Aquat. Biol.** (2013) 1(2): 68-75
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com


# Relationships between nutrients and chlorophyll-a concentration in the international Alma Gol Wetland, Iran

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**Abstract:** This study investigated the relationships between nutrients and chlorophyll-a concentration in the International Alma Gol Wetland. Chlorophyll-a is the major photosynthetic pigment in lots of phytoplanktons and has been used as a trophy index in aquatic ecosystems. Water samples were collected fortnightly from five stations in the wetland during summer and autumn. Chlorophyll-a ranged between 4.38 to 156.55 mg/m$^3$, sulfate ranged between 138 to 190 mg/l, total alkalinity ranged between 80 to 280 mg/l, silica ranged between 3.80 to 35.00 mg/l, phosphate ranged between 0.02 to 3.70 mg/l, ammonia ranged between 0.10 to 11.90 mg/l, nitrate ranged between 0.01 to 2.75 mg/l and nitrite ranged between 0.01 to 0.39 mg/l. There was a significant correlation between chlorophyll a and nitrate, nitrite and ammonia but there was no significant correlation between chlorophyll-a and silica, total alkalinity, sulfate and phosphorus.

*Article history:*
Received 15 April 2013
Accepted 1 May 2013
Available online 5 May 2013

*Keywords:*
Nutrients
Chlorophyll-a
Alma Gol
Wetland

## Introduction

Wetlands are providing numerous useful services for people, fish and wildlife (Environmental Protection Agency, 2001). They are one of the most important ecosystems on the earth and is considered unique because of their hydrology and their function as ecotones between terrestrial and aquatic ecosystems (Mitsch and Gosselink, 2007). Also, wetlands are biodiversity shelters (Junk et al., 2006).

Chlorophyll-a is the major photosynthetic pigment of phytoplanktons and a trophy index in aquatic ecosystems (Vollenweider, 1969; Dillon, 1975). Chlorophyll-a (Chl-a) is often used as an estimate of algal biomass, and in blooms concentration chl-a goes above 40 $\mu gL^{-1}$ (Stanley et al., 2003). So a method for the estimation of the growth and development of the phytoplankton community is the analysis of photosynthetic pigments, although the chlorophyll content in the cells changes with the availability of light (Wetzel, 2001), depth and trophic status (Kasprzak et al., 2008).

Eutrophication is defined as the response of aquatic ecosystems to nutrient loading (Edmondson, 1991). Hence, identification of important factors and prediction of subsequent algal blooms using a chl-a equation can be a key in the management of lakes. Both chemical and physical controls can be used to prevent or remove algae or algal byproducts from water (Stanley et al., 2003). In particular, information about the form of nutrient-chlorophyll relationships has allowed lake managers to establish nutrient concentration (e.g. Dillon and Rigler, 1974; Prairie et al., 1989). Nitrogen and phosphorus are often identified as limiting nutrients for algal biomass and silicon is necessary for diatom growth (Hecky and Kilham, 1988). Nitrogen occurs in fresh water in numerous forms: dissolved nitrogen, amino

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acids, amines, urea, ammonium ($NH4^+$), nitrite ($NO2^-$), and nitrate ($NO3^-$) (Wetzel, 2001). In aquatic ecosystems, phosphorus (P) can be found either in particulate form or soluble inorganic phosphorus, orthophosphate ($PO43^-$) (Knud-Hansen, 1997).

A review of the biological abstract published from 1995 to 1997, indicates that, of 596 articles on estuaries and nutrients, 52% consider only nitrogen, 32% refer to both nitrogen and phosphorus, and 16% consider only phosphorus as significant factor for algal bloom, despite the preponderance of studies on N, the evidence for general N limitation of coastal systems is feeble compared to the data for general P limitation of freshwater systems (Meeuwig et al., 1998).

There is a small number of comprehensive analyses on the form of phosphorus-chlorophyll relationships. The phosphorus-chlorophyll relationship most probably originates from the dependence of algae on phosphorus availability (McCauley et al., 1989).

Nitrogen limitation of algal biomass seems to be usually prevalent in subtropical and tropical lakes (Henry et al., 1985; Davalos et al., 1989; Hecky et al., 1993), while phosphorus appears to be the primary limiting nutrient in temperate lakes (reviewed by Smith, 1990). Other nutrients, for example iron and silicate, have been reported to be limiting in other regions (Johnson et al., 1999; Yolanda et al., 1997). The limiting nutrient is determined mainly by the mass equilibrium between elements such as C, N, P, and Si, and their relationship to the growth requirements of the phytoplankton (Wu and Chou, 2003).

This research was conducted to determine the relationships between chl-a and nutrients concentrations in the Alma Gol wetland to identify the important and effective nutrients on chlorophyll a concentration.

## Materials and methods

***Study area:*** This study was conducted on Alma Gol international wetland, which is situated on the Turkmen steppes near the border with Turkmenistan in the Golestan Province, in north of Iran (Fig.1). Area of the wetland was 207 ha.

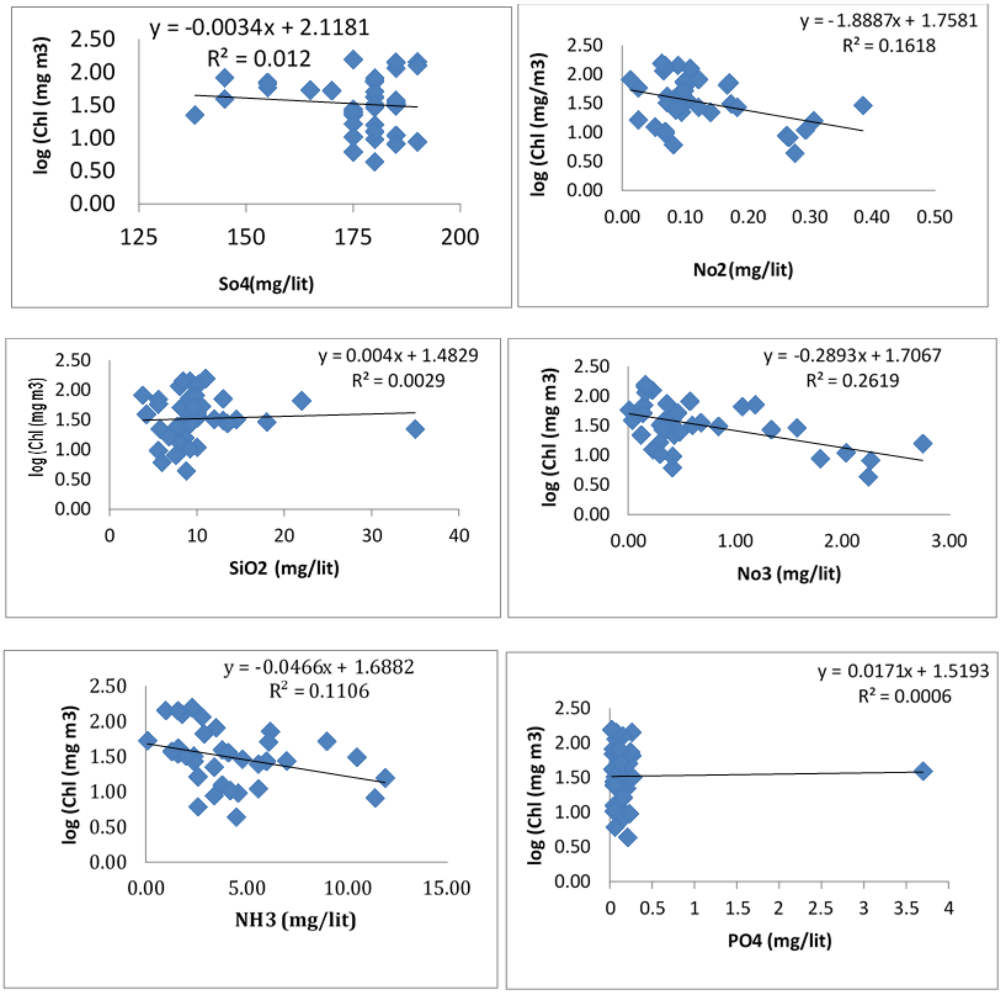

Figure 1. Location of Alma Gol wetland and situation of sampling stations.

***Field sampling:*** From summer to autumn of 2011, water samples were collected fortnightly from five sampling stations to determine nutrients and chl-a concentration. Water samples were light-protected and transferred to laboratory at 4 ºC.

***Chlorophyll a determination:*** To measure chl-a concentration, samples were shaken and a certain volume of water (based on water color) was filtered using a vacuum pump and GF/F filter. Thereafter, filter was pulverized with 90% acetone in a mortar. The resulting mixture was centrifuged for 10 min. (3000 rpm) and supernatant was poured into a glass cuvette. The optical density was read at 630, 647, 664 and 750 nm. Chlorophyll-a concentrations were calculated according to Jeffrey and Humphrey (Jeffrey and Humphrey, 1975).

***Nutrient determination:*** Ammonia, nitrite, nitrate, silica, sulfate, total alkalinity and dissolved phosphorus were measured using especial tablets and a photometer set (Wagtech, Berkshire, UK) with specific recipe.

***Statistical analyses:*** Data were analyzed by statistical software SPSS v. 18 and Microsoft office Excel 2007. Data were examined using pearson correlation test to find significant relationship between chl-a and nutrient concentration.



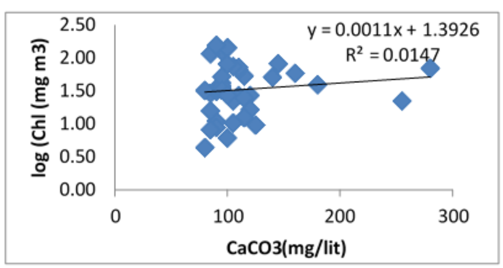




Figure 2. Log Chl-a:NO2, Log Chl-a:NO3, Log Chl-a:NH3, Log Chl-a:SO4, Log Chl-a:SiO2, Log Chl-a:PO4 and Log Chl-a:CaCO3 relations for Alma Gol wetland.

Table 1. Nutrients and chl-a relationship in Almagol wetland.

| | Chl -a | Log Chl-a | $NO_2$ | $NO_3$ | $NH_3$ | $PO_4$ | $SiO_2$ | $CaCO_3$ | $SO_4$ |
|---|---|---|---|---|---|---|---|---|---|
| Chl-a | 1 | | | | | | | | |
| Log Chl-a | 0.889$^{**}$ | 1 | | | | | | | |
| $NO_2$ | -0.486$^{**}$ | -0.654$^{**}$ | 1 | | | | | | |
| $NO_3$ | -0.489$^{**}$ | -0.701$^{**}$ | 0.895$^{**}$ | 1 | | | | | |
| $NH_3$ | -0.444$^{*}$ | -0.457$^{*}$ | 0.482$^{**}$ | 0.535$^{**}$ | 1 | | | | |
| $PO_4$ | -0.038 | 0.005 | -0.005 | -0.120 | -0.013 | 1 | | | |
| $SiO_2$ | 0.061 | 0.124 | 0.186 | 0.092 | -0.043 | -0.160 | 1 | | |
| $CaCO_3$ | 0.043 | 0.158 | -0.220 | -0.365$^{*}$ | -0.117 | 0.249 | -0.312 | 1 | |
| $SO_4$ | 0.055 | -0.105 | 0.227 | 0.283 | 0.027 | -0.373$^{*}$ | 0.159 | -0.763$^{**}$ | 1 |

** Correlation is significant at the 0.01 level (2-tailed) and * Correlation is significant at the 0.05 level (2-tailed).

Table 2. Mean ± SD of nutrients during sampling in Almagol wetland.

| Variables | August | September | October | November | December |
|---|---|---|---|---|---|
| $NO_2$ | 0.14±0.1[ab] | 0.18±0.1[a] | 0.08±0.02[b] | 0.11±0.03[ab] | 0.06±0.02[b] |
| $NO_3$ | 0.60±0.58[b] | 1.34±0.95[a] | 0.18±0.03[b] | 0.47±0.32[b] | 0.33±0.08[b] |
| $NH_3$ | 4.72±3.50[a] | 4.78±3.88[a] | 1.90±0.69[a] | 5.00±2.32[a] | 3.56±0.92[a] |
| $PO_4$ | 0.50±1.13[a] | 0.14±0.06[a] | 0.12±0.09[a] | 0.11±0.07[a] | 0.11±0.08[a] |
| $SiO_2$ | 10.15±6.28[a] | 9.67±1.94[a] | 9.36±1.24[a] | 12.27±8.18[a] | 7.16±1.51[a] |
| $CaCO_3$ | 153.00±67.59[a] | 88.50±5.80[b] | 94.00±6.52[b] | 108.50±13.75[b] | 113.00±10.37[b] |
| $SO_4$ | 160.30±16.53[b] | 184.00±3.16[a] | 185.00±6.12[a] | 176.50±3.37[a] | 177.00±2.74[a] |
| Chl a | 52.46±20.79[b] | 23.03±14.59[cd] | 136.83±15.80[a] | 42.08±21.67[bc] | 11.03±3.82[d] |

Different letters show significant difference between columns ($P$ <0.05).

## Results

Results illustrated that there was negative and significant relationships between chl-a and logarithm of chl-a with nitrate, nitrite ($P$<0.01) and ammonia ($P$<0.05) but there was no significant correlation between chl-a and logarithm of chl-a with silica, total alkalinity, sulfate and resolve phosphorus ($P$>0.05) (Table 1). The average of various factors and significant differences between months are presented in table 2.

Regression lines for Log Chl-a:NO2, Log Chl-a:NO3, Log Chl-a:NH3, Log Chl-a:SO4, Log Chl-a:SiO2, Log Chl-a:PO4 and Log Chl-a:CaCO3 relations were shown in figure 2 and also presented at below.

y=-0.003x+2.118, $R^2$=0.012, r=-0.105, Log Chl-a:SO4

y=-1.888x+1.758, $R^2$=0.161, r=-0.654, Log Chl-a:NO2

y=0.004x+1.482, $R^2$=0.002, r=0.124, Log Chl-a:SiO2

y=-0.289x+1.706, $R^2$=0.261, r=-0.701, Log Chl-a:NO3

y=-0.046x+1.688, $R^2$=0.110, r=-0.457, Log Chl-a:NH3

y=0.017x+1.519, $R^2$=0.000, r=0.005, Log Chl-a:PO4

y=0.001x+1.392, $R^2$=0.014, r=0.158, Log Chl-a:CaCO3

## Discussion

A significant correlation was found between chl-a and nitrite, nitrate and ammonia. When there was a high concentration of chl-a, a low concentration of nitrite, nitrate and ammonia were found. Chl-a was not affected by silica, total alkalinity, sulfate and dissolved phosphorus.

Power relationships between phosphorus, chlorophyll, and water clarity have been observed for freshwater systems in the world (e.g., Sakamoto 1966; Brown et al., 2000). The strong relationship between chlorophyll and phosphorus established by Sakamoto (1966) in numerous Japanese lakes forms an appropriate testable hypothesis: chlorophyll is both a useful and an easy estimator of phytoplankton standing crop and is now more generally used than cell number or cell volume.

Smith (1982) achieved the most comprehensive analysis of phosphorus-chlorophyll relationships to date, and was the first to illustrate that information of both total phosphorus (TP) and total N (TN) concentrations could improve predictions of algal biomass. A hypothesis that fits more closely with the classic Liebigian paradigm (von Liebig, 1840; Hutchinson, 1973; Droop, 1974; Tilman, 1982) would consider other nutrients that might limit algal growth at high levels of ambient phosphorus. It would predict that chlorophyll should rise linearly until some other factor (such as nitrogen, silicon, molybdenum and light) becomes limiting. Despite the fact that nonlinear relationships between TP and chlorophyll are possible in lakes (Forsberg and Ryding 1980; Canfield, 1983), there is few quantitative examinations on their shape or form.

Hoyer et al. (2002) suggested that phosphorus accounts for more variance in chlorophyll than that of nitrogen in inshore coastal waters and fresh water of Florida. While nitrogen had been shown to limit algal populations in both systems (Elser et al., 1990; Downing, 1997), the data presented suggested that phosphorus was the primary nutrient limiting algal populations amongst the 300 nearshore coastal locations sampled. They recognized, though, that some of those 300 stations may at some times be limited by nitrogen. Canfield (1983) and Brown et al. (2000) have formerly affirmed that phosphorus is the primary limiting nutrient in Florida lakes, and when the total nitrogen to total phosphorus ratio decreased to < 10, nitrogen may become limiting. It is normally assumed, that nitrogen is the primary limiting nutrient for phytoplankton production in most coastal waters (Downing, 1997).

Brown et al. (2000) explained that TP accounted for a significant amount of the variance ($R^2$=0.76) of observed chlorophyll measurements and TN accounted for lower variance related to observed chlorophyll measurements ($R^2$=0.46), but a multivariate model using both TP and TN also accounted for a significant amount of the observed variance ($R^2$=0.78). The coefficient of determination values for TP-chlorophyll and the multivariate nutrient-chlorophyll, were similar ($R^2$ =0.76 against 0.78), suggesting that chlorophyll concentrations can be predicted rationally well using TP alone. The coefficient of correlation for the relationship between TP and chlorophyll for both equations was positive and significant.

Redfield (1958), expressed that phosphorus (P) has been considered a key limiting nutrient in marine systems. Furthermore, P controls phytoplankton biomass in numerous freshwater systems and similarities in phytoplankton physiology and nutrient requirements in coastal and freshwater systems (Hecky and Kilham, 1988). Nevertheless, following Ryther and Dunstan's (1971) work, nitrogen is generally the limiting nutrient in coastal systems and has received the bulk of research interest. Meeuwig et al. (1998) illustrated that the relation between chlorophyll and TN is marginally stronger than that between chlorophyll and TP suggesting that TN, rather than TP, limits estuarine chlorophyll. The average TN:TP ratio of 4.5 also supports the argument for TN as the key limiting nutrient in estuaries in Prince Edward Island in Canada (Meeuwig et al., 1998). However, the relative strength of these patterns and, the TN:TP ratio cannot be used to conclude which nutrient is limiting phytoplankton biomass, the low yield of chlorophyll per unit of nutrient points to the importance of other factors such as herbivory and turbidity, and potentially to indirect control by iron, in determining phytoplankton biomass.

Canfield (1983) developed a chl-a equation using samples from 223 Florida lakes, 27% of which were considered N-limited. Because of the long growing season in Florida, these samples were taken during August 1979 to September 1980. Chl-a showed significant correlations with both TP (r=0.79, $P$<0.01) and TN (r=0.87, $P$<0.01).

It has been shown that there is often a strong correlation between TP and algal biomass (Sakamoto 1966; Dillion and Rigler 1974; Jones and Bachmann 1976; Carlson, 1977). This suggests that P may be the element controlling algal growth. Though, lakes surrounded by rich phosphate deposits and P-containing soils may be N-limited.

Wu and Chou (2003) indicated that both the concentration of chlorophyll a and phytoplankton biomass have shorter Euclidean distances to silicate, nitrate, biochemical oxygen demand, and temperature, than to phosphate, nitrite, ammonium, or physical factors such as conductivity, pH, and dissolved oxygen, suggesting that phytoplankton are associated with silicate, nitrate, biochemical oxygen demand and temperature. These results supported the hypothesis that nutrients such as silicate and nitrate play a more important role in regulating phytoplankton in subtropical eutrophic estuary of Taiwan than do other factors.

In conclusion, results illustrated that there was a negative and significant relationships between chl-a and logarithm chl-a with nitrate, nitrite and ammonia but there was no significant correlation between chl-a and logarithm chl-a with silica, total alkalinity, sulfate and dissolved phosphorus in this research. Some research supported the result of this study and some of them were against. Although we could not find any relationship between chl-a and P, it can be, because of measuring dissolved phosphorus instead of total phosphorus.

**Acknowledgements**

Thanks to A. Jafar Node, critic of Shahid Naser Fazli barabadi Aquaculture Research Station for helpful and constructive comments. This research was supported from grants from the Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources.

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