Nutrient Enrichment Experiment to Establish Relationship between Chlorophyll and Phosphate

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Abstract In-situ nutrient enrichment experiment was conducted to know the relationship between chlorophyll and phosphate so as to assess the significant role of phosphate in the phytoplankton growth. During the experiment period temperature (27.9-33.7°C), salinity (31-35‰) and pH (7.74-8.07) values were not shown dramatic changes as the experimental studies are conducted for the short period only. DO was found to show an oscillating trend (2.77-6.68 mg/l⁻¹) with phytoplankton population variations. Nutrient concentrations (NO₃: 1.05-12.63 µM, NO₂: 0.29-1.67 µM, PO₄: 0.07-28.32 µM and SiO₃: 4.19-23.89 µM) showed maximum concentration at the first and second day of the experiment in enrichment tanks and it gradually decreased (PO₄ and SiO₃) on last day of the experiment period. The pronounced maximum chlorophyll concentration in tank 5 on 5th day corresponding with addition of highest concentration of phosphorus, clearly pointed out that phosphorus addition had influenced the plankton growth. Increased utilization of PO₄ and support of SiO₃ indicates diatoms prefer silicate and phosphate for growth than the nitrates. Nitrate enrichment in the tank in later part of the experiment indicates nitrogen cycling processes. Increased phytoplankton uptake and growth rate at tank 5 when compared to control, substantially proved the uptake of phosphate by phytoplankton under culture system.

Keywords Chlorophyll; Nitrate; N:P Ratio; Phosphate; Reactive Silicate

1. Introduction

Phytoplankton are the most important primary producers of the aquatic environment and contributes up to 50% of the global primary production. Phytoplankton growth in estuaries and coastal seas is dependent on a number of parameter, especially nutrient availability, light penetration and intensity and mixing with in the water column. Nutrient availability is frequently referred as key factor regulating phytoplankton growth, biomass and species composition (Roelke et al., 1999). Historically, nitrogen and phosphorus (Schindler, 1977; Wyne and Berman, 1980; Birch et al., 1981; Lean and Pick, 1981)
are believed to be the nutrients most commonly limiting phytoplankton production in marine and fresh water ecosystems (Hecky and Kilham, 1988; Elser et al., 1990; Dodds et al., 1993).

Phosphorus plays a crucial role in energy transformation during algal photosynthesis. It is vital to algal metabolic processing including the synthesis of nucleotides, phospholipids and sugar phosphates (Wetzel, 1983). The diatom had reached its limits of growth with the available P. The P-enriched aquatic system utilized atmospheric nitrogen (N) and carbon (C) for algal production and this resulted in significant increase in ecosystem primary production. Phosphorus addition triggers undesirable cyanobacterial blooms unless N is added, however, addition of C or N in the absence of P enrichment the effects are minor (Schindler, 1974). According to Liebig’s law of the minimum single nutrient should limit algal growth at any given time.

A common technique for determining nutrient limitation in phytoplankton is the nutrient enrichment bioassay (Hecky and Kilham, 1998) by measuring the phytoplankton growth in a sample containing a particular nutrient of interest and by keeping all other parameters constant. The effect of altered nutrient regimes on phytoplankton biomass can be quantified by measuring the community growth response to a controlled nutrient environment over short time intervals (Pael, 1982). In this regard, short term (1 week) nutrient manipulation bioassays provide a management tool for addressing the issue of immediate phytoplankton response to enhanced nutrient concentrations (Conley, 2000; Conely et al., 2009; Pearl, 2009). The present study was carried out to assess the significant role of phosphate in the phytoplankton growth for future remote sensing applications.

2. Materials and Methods

An enrichment experiment was carried out to determine the nutrient limitation status of phytoplankton. The experiment was commenced for 10 days with six 100 L open culture tanks containing 50 litres of filtered sea water each and kept in natural environmental condition. The seawater in all tanks was agitated with aerator. The natural phytoplankton concentrate dominated by diatoms (50 ml) was inoculated in all the tanks. The initial nutrient concentrations of experimental seawater are recorded (Table 1) and phytoplankton bloom was triggered with the addition of ammonium sulphate and urea in the ratio 10:2. Super phosphate (NaH$_2$PO$_4$) (5, 10, 15, 20 and 25 µM) was added at different concentrations in order to obtain a situation which concentration of phosphate enhance phytoplankton biomass in tanks. Water samples at every 24 h was analysed for DO, pH, salinity, nutrients and chlorophyll a and temperature was measured. The same experiment was repeated two times for confirmation of the accuracy.

### Table 1: Background Value of Experimental Seawater in the Tanks

<table>
<thead>
<tr>
<th>Experiment</th>
<th>NO$_2$ (µM)</th>
<th>NO$_3$ (µM)</th>
<th>PO$_4$ (µM)</th>
<th>SiO$_3$ (µM)</th>
<th>Chl a (µgl$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.48</td>
<td>5.46</td>
<td>0.12</td>
<td>23.24</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>0.39</td>
<td>5.28</td>
<td>0.18</td>
<td>22.34</td>
<td>2.01</td>
</tr>
<tr>
<td>3</td>
<td>0.51</td>
<td>4.98</td>
<td>0.16</td>
<td>23.02</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Surface water temperature was measured using digital multi-stem thermometer of 0.1°C accuracy. Salinity was estimated using a hand held refractometer (Atago hand refractometer, Japan) and the pH was measured using a pH meter (Cyberscan pH 1100) with the accuracy of ± 0.05. Dissolved oxygen was estimated by following the modified Winkler’s method (Strickland and Parsons, 1972). Filtered water samples (0.45µm GF/C filter paper) were used to analyse dissolved micronutrients such as nitrite, nitrate, reactive silicate and inorganic phosphate by following the methods described by Strickland and Parsons (1972) and measurements were made by using a PC based double beam spectrophotometer (Shimadzu UV-2450).
Chlorophyll a concentration determined by a pre-calibrated fluorometer (Turner Designs, Trilogy) after extraction with 90% acetone (UNESCO, 1994). The growth rate (R) of phytoplankton in each tank was estimated on a daily basis (Reynolds, 1984). Phosphate uptake was determined by measuring the disappearance of nutrients from tank each day. The uptake was calculated as follows:

\[ V = C_i - C_f / t, \]

Where \( V \) is the daily uptake rate (µM), \( C_i \) and \( C_f \) are the initial and final nutrient concentration respectively and \( t \) is the time period of uptake (d) (Domingues et al., 2010). All the experiments were conducted for three times so as to have a check on change in environmental variables as the study was carried out in outdoor system.

3. Results

During the experiment, water temperature varied between 27.9 and 33.7°С in all the experimental tanks and registered maximum temperature on 7th day and minimum on 1st day of the experiment (Figure 1). Salinity values not showed drastic changes (31 to 35 ‰), and the maximum salinity recorded at last 3 days and minimum was recorded on 1st and 2nd day of the experiment in all tanks (Figure 2). pH values were not shown dramatic changes throughout the experiment period and it ranged between 7.74 (3rd days Tanks-2) and 8.07 (10th day tank-5) (Figure 3). Dissolved oxygen concentration varied between 2.77 and 6.68 mg/l, the maximum DO content was observed in tank 3 on 6th day and minimum was observed in tank 3 on 10th day of the experiment (Figure 4).

![Figure 1: Variation of Water Temperature Recorded During the Experiment Period in Different Tanks](image-url)
**Figure 2:** Variation of Salinity Recorded During the Experiment Period in Different Tanks

**Figure 3:** Variation of pH Recorded During the Experiment Period in Different Tanks
The inorganic nutrient concentrations were varied between the experimental tanks based on the nutrient addition with respect to culture period. The highest nitrate concentration (12.63 µM) was recorded in tank-5 on 1st day and lowest (1.05 µM) nitrate content was registered in tank-1 on 4th day (Figure 5). Nitrite concentration varied between 0.29 µM in tank-5 on 6th day and 1.67 µM in tank-2 on 1st day (Figure 7). Highest inorganic phosphate values (28.32 µM) were registered at tank-5 on 1st day of experiment and the minimum 0.79 µM was recorded in tank-1 on 10th day. All the tanks have shown their higher phosphate concentration on the 1st day and lowest concentration on last day of the experiment (Figure 7).
Figure 6: Variation of Nitrite (NO$_2$) Concentration Recorded During the Experiment Period in Different Tanks

Figure 7: Variation of Inorganic Phosphate (PO$_4$) Concentration Recorded During the Experiment Period in Different Tanks

The silicate concentrations in the tanks were ranged from 4.19 to 23.89 µM. The minimum silicate concentration was registered on 10$^{th}$ day and the maximum concentration was observed on 1$^{st}$ day in tank-3 (Figure 8). Chlorophyll a concentration varied between 1.5 and 10.77 µgL$^{-1}$ with the highest and lowest value in tank-5 on 5$^{th}$ and 1$^{st}$ day respectively (Figure 9). The N:P was found to be low (<1) up to 9$^{th}$ day of the experiment and it became higher (>1) on 10$^{th}$ day only (Figure 10). While, Si:P ratio shows higher values >1 from 7$^{th}$ day to end of the experiment and lower values (<1) were observed up to 7$^{th}$ day of the experiment (Figure 11).
Figure 8: Variation of Reactive Silicate ($SiO_3$) Concentration Recorded During the Experiment Period in Different Tanks

Figure 9: Variation of Chlorophyll Concentration Recorded During the Experiment Period in Different Tanks
Figure 10: Variation of N:P Ratio During Experiment in Six Tanks

Figure 11: Variation of Si:P Ratio Recorded During the Experiment Period in Different Tanks

Significant phosphate consumption occurred in all tanks during the experiment, with no significant phosphate consumption in the control were observed in (Figure 12). The phosphate uptake rate was ranged between 6.728 d⁻¹ and 36.8365 d⁻¹ and it was found to be high on 2nd day of the experiment in tank-5.
The highest growth rate (0.451 d\(^{-1}\)) of the phytoplankton also observed on 2\(^{nd}\) day of the experiment in tank-5 and other tanks were also shown their highest growth rate on 3\(^{rd}\) day (Figure 13). While the lowest growth rate (0.083d\(^{-1}\)) was observed in tank-3 on 10\(^{th}\) day of experiment. The pronounced increase in phytoplankton growth rate was significantly coincide with the maximum uptake of phosphate, suggest that phosphorus uptake enhanced the phytoplankton growth in enrichment tanks and confirmed that it serve as one of the limiting nutrient in phytoplankton production.

**Figure 12: Trends of Phosphate Uptake Rate (R) Recorded During the Experiment Period in Different Tanks**

**Figure 13: Trends of Phytoplankton Growth Rate (R) Recorded During the Experiment Period in Different Tanks**
4. Discussion

Understanding the role of essential nutrients to the phytoplankton growth is crucial for the successful control of eutrophication in coastal areas. The limiting nutrients can be detected by using different methods, e.g. by inorganic nitrogen to phosphorous ratios (Neill, 2005), enrichment experiments (Ryther and Dunston, 1971; Granéli, 1987) or measuring intracellular concentrations of nutrients (Hecky and Kilham, 1988). Despite providing significant information on the effects of nutrient availability on phytoplankton growth and community structure, nutrient enrichments do not constitute a straightforward methodology when it comes to interpreting and extrapolating results to natural systems.

However nutrient limitation of phytoplankton has been reported extensively in different seas. Among those nutrients, nitrogen and phosphorus play an important role in limiting biological productivity (Gruber, 2004). Nitrate addition was strongly promoted phytoplankton bloom, which was also proved by the nutrient enrichment experiment in the northwestern Indian Ocean (Takeda et al., 1995) nitrate and phosphate in central Indian Ocean (Tang et al., 2009) and nitrate, phosphate and silicate (Zou et al., 2001) in Yellow sea.

As the experiment was carried out at a common place, this physical environment was kept as common for all the experimental tanks and not registered any drastic variation. Salinity increased from the first day up to the end of the experiment which might be due to the evaporation of the water with increase in temperature in the culture tanks and there is no water compensation was made for this purpose. In the present study, the pH not showed significant variation (7.74 - 8.07) and was well within the optimal pH range (6.5 to 8.2) for sustainable aquatic life (Adeyemo et al., 2008) and it favors the phytoplankton growth. Generally, a fluctuation in pH value is attributed to factors like removal of CO$_2$ by photosynthesis through bicarbonate degradation, low primary productivity, reduction of salinity and temperature and decomposition of organic materials (Rajasegar, 2003) among others.

The DO level varied between 2.77 mg/l (10$^{th}$ day) and 6.68 mg/l (6$^{th}$ day). The decreased DO content at the end of the experiment could be attributed to decrease in oxygen release, increased respiration of higher population and increase in salinity and temperature reducing the oxygen dissolution. The increased DO content is coincided with the increased phytoplankton biomass up to 6$^{th}$ day of the experiment after that phytoplankton biomass was started to decrease with decrease in DO content. DO is the measure of photosynthetic activity of phytoplankton biomass and after reaching saturation in growth competition for nutrients space and utilization of DO by the cells itself during night hours led to the reduction in DO after 6$^{th}$ day.

Rapid uptake of nutrients (NO$_3$, NO$_2$ and SiO$_3$) were observed beyond 2$^{nd}$ day of the experiment in all the tanks with increase in phytoplankton biomass which is inferred from chlorophyll a concentration and it started to increase with decrease in chlorophyll concentration on 7$^{th}$ day onwards up to the end of the experiment. It clearly showed that addition of nitrogen and phosphorus greatly stimulated algal growth as measured by chlorophyll a production. Zaret et al. (1981) reported an increase in chlorophyll concentrations after 48 hour of incubation in the presence of added nitrogen and phosphorus. This increase is an excellent augment with the results of our experiment.

Nutrient concentrations in enrichment tanks will tend to decrease over time as result of cellular uptake. Therefore, a certain nutrient that was not limiting at the beginning of the experiment may become limiting after a few days of incubation. If a nutrient is not limiting at the time of beginning of the experiment, nutrient consumption in enriched tanks after nutrient addition will not be different from consumption in the controls (Domingues et al., 2011). Ault et al. (2000) argued that increases in
growth rate in response to nutrient enrichment over the course of an experiment do not necessarily mean that phytoplankton growth was nutrient limited.

The inorganic phosphate (IP) concentration in all the experimental tanks were found to be higher at the first day of the experiment and started to decrease on 2nd day with enhanced phytoplankton growth, suggested the uptake of phosphate by the phytoplankton. This is also confirmed with the increasing uptake rate of phosphorous on 2nd day of experiment along with pronounced increase in phytoplankton growth rate. But the phytoplankton growth rate and nutrient uptake rate was started to decrease at the end of the experiment due to the decay of phytoplankton cells after its proliferation. Increased levels of phosphorous led to a massive proliferation in phytoplankton, indicating phosphorous initially as the limiting nutrient in the tanks (Higgins et al., 2006). The relationship between phytoplankton biomass and phosphorous availability in marine systems has been examined by several researchers (Riley, 1965; Smith, 1984; Downing, 1997; Guildford and Hecky, 2000 and Hoyer et al., 2002). However, a strong correlation between instantaneous measurement of chlorophyll a and dissolved inorganic phosphorous was reported 30 years ago by Ketchum (1969).

Phytoplankton growth responded significantly to nutrient enrichment in most tanks. However, increased nutrient consumption rates without simultaneous increase in phytoplankton net growth were also observed on several occasions. Considering the specific phytoplankton groups, diatoms were the main component of phytoplankton biomass.

The N:P and Si:P ratios were found to be low (<1) up to the 9th day of the experiment after that it become higher (>1) at the end of the experiment. For most marine coastal waters and inland seas nitrogen is thought to be the most limiting nutrient for phytoplankton production (Ryther and Dunstan, 1971, Howarth, 1988, Granell et al., 1990). However, a few cases have been reported where phosphorous limits production (Berland et al., 1980, Smith, 1984), and phosphorous has been suggested to be limiting in marine coastal waters only when large nutrient loads with high N:P ratios reach coastal waters (Howarth, 1988). Rapid phytoplankton growth in tanks depleted nitrogen supplies and declined N:P ratio. However, nutrient (NO₃) enrichment in culture tanks was noted at the end of the experiments indicating liberation of nitrogen through ammonia liberated by the culture organisms. Axler et al. (1994) determined that N:P ratio >1 indicated phosphorus limitation, while nitrogen limitation was associated with ratios <1. Thus, in the present study phosphorous limitation is evident in the end of the experiment and nitrogen limits the phytoplankton growth at the initial stages of experiment.

Overall, the net growth of phytoplankton in enrichment tanks seemed to be phosphorous limited at the end of the experiment. Increased phosphate net consumption rates in all phosphate enriched tanks were associated to significant increase in community biomass (from 1.5 to 10.77 µg l⁻¹).

Phosphorus is an essential nutrient utilized by all organisms for energy transport and growth, yet little is known about the role of phosphorus plays in the production and distribution of plankton in the world’s ocean.

5. Conclusion

To conclude the present study, it was found that the phosphate plays vital role in the phytoplankton production at higher nutrient concentrations and it extensively used to determine the phytoplankton community structure of the aquatic environment. Hence, establishing a clear relationship between the target nutrients and chlorophyll would help assess the nutrients at spatial scale using satellite datasets.
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References


Wyne, D., and Berman, T. *Hot Water Extractable Phosphorus - An Indicator of Nutritional Status of Peridinium Cinctum (Dinophyceae) from Lake Kinneret (Israel)*. Biology. 1980. 16; 40-44.
