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Seasonal Variation of Nutrients that Influence the Growth of Cyanobacteria in Surface Water Reservoirs of River Tapi

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Abstract Water reservoirs as one of the sources for the supply of domestic water to Surat city are having extensive algal blooms that may contain species of toxic cyanobacteria. Current water treatment procedures do not detect or try to remove toxins possibly produced by these toxic cyanobacteria. These toxins are potent health hazard if present in drinking water. The aim of the present study was to study the presence of any such toxic cyanobacteria along with its diversity and their toxins in surface water of river Tapi. Detail analysis of nutrient pollution in surface water along with seasonal variation in cyanobacterial population was studied. Detecting genes responsible for toxin production in cyanobacteria that identify the possible health hazard of cyanobacterial toxins in drinking water reservoirs of Surat city.

Keywords: *cyanobacteria, seasonal variation, health hazards, water reserviour, toxin*

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1. Introduction

The hydrological cycle involves complex network of many water bodies each having different characteristics and distinct uses. Because of its easy availability and relatively less pollution, groundwater remains major source of domestic water supply in India like most other parts of world.

However in certain regions, geological structure do not allow the use of groundwater or the supply of groundwater is not enough. Surface water from rivers can be an alternative in such cases. Some municipal corporations also use surface water because of its perennial availability as drinking water supply. Present study is focused on one such case where Tapi river surface water is used as the source of drinking water supply for Surat city.

Perennial rivers usually have good flushing rate. It is wrongfully believed that this process removes undesirable substances from water. Flushing cannot actually eliminate the contaminants due to adsorption of contaminants to the sediments. Substances bound to riverbed may accumulate, be released back into the water, and may be carried downstream. Riverbed therefore acts as sink for important nutrients such as phosphates, but the same sediments can also serve as source, liberating the phosphates back into the water where it can encourage the growth of cyanobacteria and algae. Further, construction of weirs and barrages may hinder the flow of river water and may aggravate situation.

Cyanobacteria are frequent constituent of many freshwater and marine ecosystems. Cyanobacteria that live dispersed in the water constitute phytoplanktons while those that grow on sediments make the phytobenthos. Under certain conditions, cyanobacteria may reproduce to an alarming level - a condition referred as bloom, especially where waters are rich in nutrients, temperature is favourable and exposure to sunlight is long enough [1].

1.1. Surat City at a Glance

The city of Surat is located on the banks of river Tapi in the western state of Gujarat in India.

The city has a population of 4.66 million as per the Census, 2011 [2]. The Surat municipal corporation operates four different water works with the cumulative capacity of 1300 M.L.D. to fulfill the demand of clean water [3]. Most of this water is provided through four water works of Surat city viz. Rander, Katargam, Varachha and Sarthana. All these water works use surface water from river Tapi. Construction of a weir created a reservoir augmenting this source of surface water.

Figure 1. Location of Surat City

It is observed that eutrophication is a severe problem in river Tapi. It is hypothesized that one of the major contributors are cyanobacteria.

1.2. Eutrophication, Cyanobacteria and Toxicity

The contamination of water resources and drinking water supplies by human excreta has always been a major human health concern since more than a century. Although eutrophication has been recognised as a growing concern since the 1950s, only recently have cyanobacterial toxins become widely recognised as a human health problem. Problem of eutrophication is also aggravated by urbanization.

The general trend has been an increase in concentrations of pollutants in surface waters together with increase in urbanisation. Construction of sewerage first enhanced this trend by concentrating pollutants from latrines. After some decades, construction of sewage treatment systems began extensively in the 1950s. Originally these systems comprised only a biological step which degraded the organic material in the receiving water bodies. However, phosphate remained untreated and led to the nutrient contamination. Wherever conditions like temperature, light and phosphate are conducive, surface waters may host increased growth of algae and/or cyanobacteria. Where such proliferation is dominated by a single or a few species, the phenomenon is referred to as an algal or cyanobacterial bloom. Problems associated with cyanobacteria are likely to increase in areas experiencing population growth with a lack of concomitant sewage treatment and in regions with agricultural practices causing nutrient losses to water bodies through over-fertilisation and erosion. Thus, eutrophication becomes a common nuisance in tropical water bodies due to nutrient contamination [4].

Cyanobacteria producing hepatotoxins are a major apprehension related to river eutrophication [5]. Persistent presence of toxic species of cyanobacteria may pose a health hazard even in treated drinking water if it is acquired from such river water.

According to various literature, toxic freshwater cyanobacteria are reported in as many as 65 countries [6]. They produce a range of hepatotoxins and neurotoxins. Many episodes of acute cyanobacterial toxicity are reported in countries like U.K., U.S.A., Australia, China and Brazil [6]. In one of the worst cases 76 people were killed in Brazil [7]. While acute toxicity is the most obvious problem in cyanobacterial poisoning, a long-term risk may also be present. Short exposures to toxins may result in long-term injury and chronic low-level exposure may cause adverse health effects. Animal experiments have shown chronic liver injury from continuing oral exposure to microcystins [8].

Table 1. Nature of Cyanobacterial Toxins

Cynobacterial toxins and their acute toxicity [a]			
Cynotoxins	**LD (i.p.mouse)[b] of pure toxin (ug/kg)**	**Taxa known to produce the toxin (s)**	**Mechanism of toxicity**
Protein phosphatase blockers (Cyclic peptides with the amino acid ADDA)			
Microcystins in general	45->1000	*Microcystis, planktothrix, Oscillatoria, Nostoc, Anabaena, Anabenopsis, Hapalosiphon*	All block protein phosphatases by covalent binding and cause haemorrhaging of the livers; cumulative demage may occur
(-60 known cogneres)			
Microcystin-LR	60-(25-125)		
Microcystin-YR	70		
Microcystin-RR	300-600		
Nodularin	30-50	*Nodularia spumigena*	
Neurotoxins			
Anatoxin-a (alkaloid)	250	*Anabaena, Oscillatoria, Aphanizomenon, Cylindrospermum*	Block post-synaptic depolarization
Anatoxin-a(s) (unique organophosphaete)	40	*Known only from two species of Anabaena Aphamizomenon*	Blocks acetylcholinesterase block sodium channels
Saxitoxins (carbamate alkaloids)	10-30	*Aphanizomenon, Anabaena, Lyngbya, Cylindrospermopsis racibroskii*	
Cytotoxin			
Cylindrospermopsin (alkaloid)	2100 in 1 day 200 in 5-6 days	*Cylindrospermopsis raciborskii*	Blocks protein sysnthesis; substantial cumulative toxicity
[a] derivied from Turner et al., 1990; Kuiper-Goodman et al., 1999; Sivonten & Jones, 1999			
[b] LD50= lethal dose 50 (the dose of a chemical that will on average, kill 50% of a group of experimental animals); i.p.= intraperitoneal			

The possibility of carcinogenesis and tumor growth promotion need careful evaluation because both have been detected in animal experimentation. Cyanotoxins are not proved to be carcinogenic, however some epidemiological and animal studies have suggested close association between toxin production and elevated frequency of primary liver tumor occurrence [9].

The World Health Organization hase proposed a *provisional* guideline value of Microcystin-LR to be tolerated in drinking water [10]. However in many countries like India there is no such local guideline.

2. Methodology

2.1. Sampling:

Four specimen sites were selected near the intake wells of four major water works namely Rander (A), Katargam (B), Varachha (C) and Sarthana (D) in river Tapi of Surat city (Figure 3). Surface water samples were collected by WHO guidelines in prescribed containers by standard grab method [5]. Samples were analyzed within 2 hours for microscopic identification. For scum, standard sampling method using plankton net was use [11].

Considering specific nature of sampling of cyanobacteria from river, Standard Operation Procedures developed by Klamath Blue Green Algae Working Group was also considered [11].

2.2. Preliminary Identification of Cyanobacteria by Microscopy

Most cyanobacteria can be readily distinguished from other phytoplankton and particles under the microscope by their morphological features at a magnification of 200-1000×. Identification up to genus level was done by wet mount observations in bright field microscope. Identification was carried out with the help of Anagnostidis guidelines [12]. In case of doubt, for establishing cyanobacterial identification in laboratory, occasional consultation with experts was done.

2.3. Physicochemical Analysis of Surface Water

Parameters like temperature, pH and dissolved oxygen concentration were checked [13].

2.4. Indirect Estimation of Cyanobacterial Biomass

Cyanobacterial enumeration was originally carried out using inverted microscope by Utermöhl's counting technique (Utermöhl, 1958). Spectrophotometric estimation of chlorophyll-a was carried out after organic solvent extraction as per ISO method [14].

2.5. Cultivation, Isolation and Maintenance of Cyanobacterial Species

Enrichment of cyanobacteria was initially carried out in four different media out of which BG11 Broth gave good results and was used in all later studies [15]. However, axenic growth of cyanobacteria on solid media is difficult due to high sensitivity of cyanobacteria to acidity and towards contaminants present in agar agar powder. To overcome this, isolation was carried out using BG11 agar prepared in Mili-Q water with agar agar been replaced with molecular biology grade agarose [16]. Isolates were preserved in soft agar stabs of oligotrophic version of BG11 medium at 4°C.

2.6. DNA Extraction from Isolates

A modified method using non-ionic detergents was used for the extraction of genomic DNA from cyanobacterial isolates [17].

2.7. Confirming the Presence of Toxic Cyanobacteria

In order to identify capacity to produce all these molecules, primer set used in this study was designed to target the aminotransferase domain of microcystin synthetases and nodularin synthetases from all hepatotoxic cyanobacteria [18].

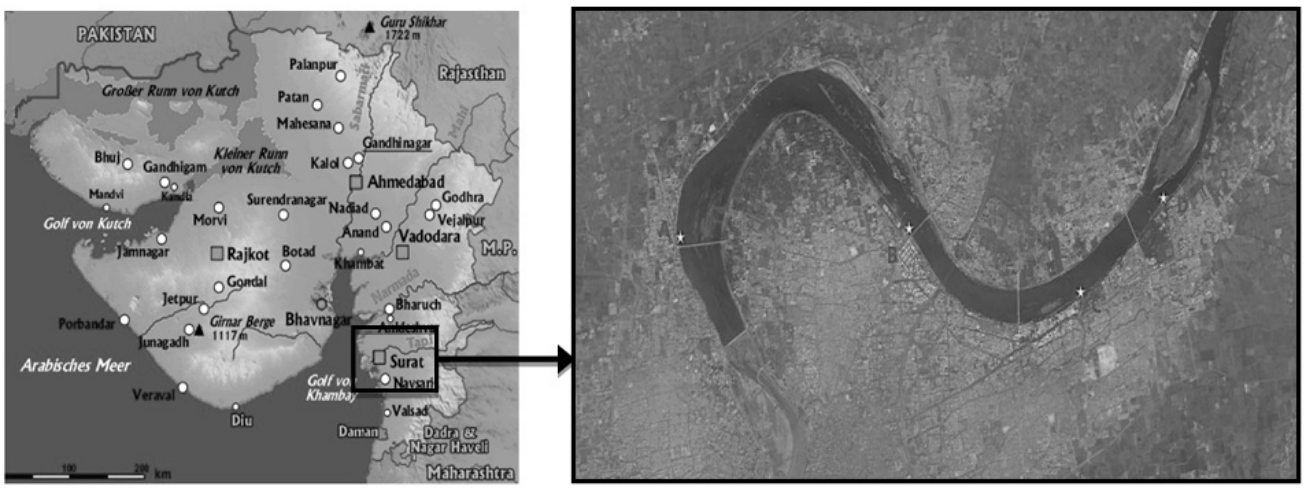

Figure 2. Location of sampling sites across River Tapi

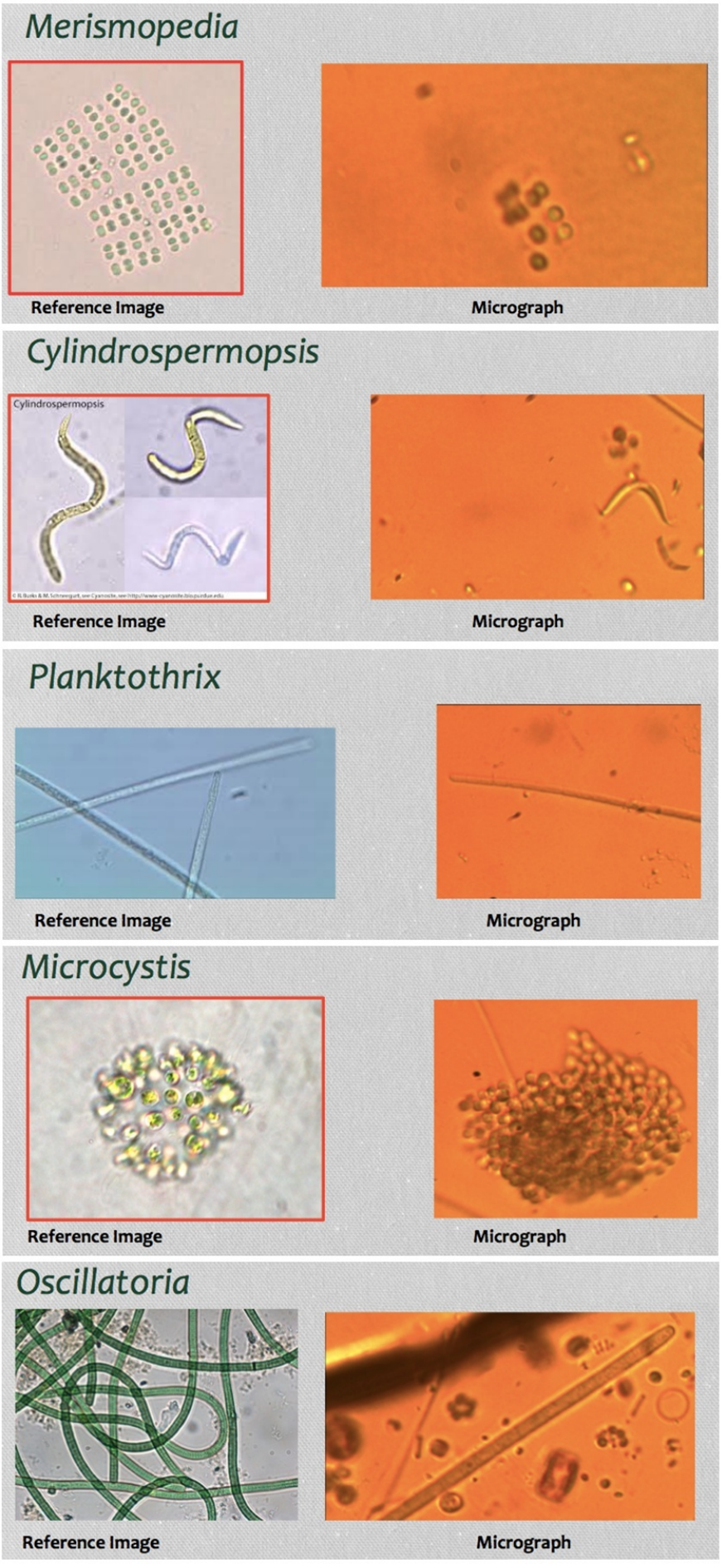

Figure 3. Identification of common genera of cyanobacteria (All reference images are from http://www-cyanosite.bio.purdue.edu)

3. Results

3.1. Preliminary Microscopic Identification of Cyanobacteria

Microscopy identified many genera of cyanobacteria. However, dominant genera in most samples belonged to the *Oscillatoria*, *Microcystis*, *Planktothrix*, *Cylindrospermopsis* and *Merismopedia*. (Figure 4)

Besides common cyanobacteria, certain genera of algae were also observed. These commonly included *Agmenellum*, *Scenedesmus*, *Actinastrum*, *Stauroneis* and *Navicula*.

3.2. Physicochemical Analysis of Surface Water

Physicochemical analysis of surface water samples from each sampling site for two consecutive years gave following results.

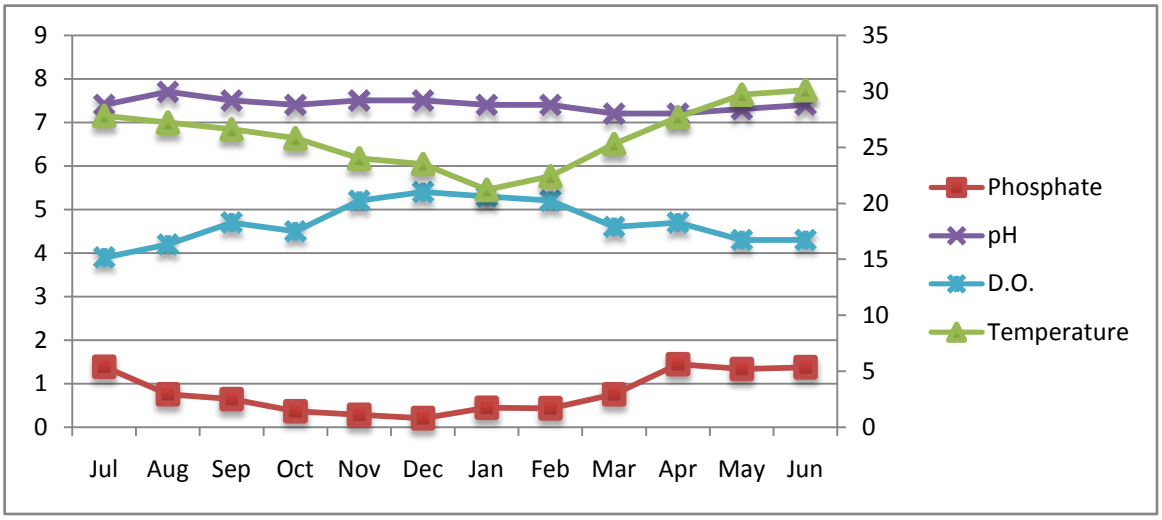

Figure 4. Physiochemical analysis of water from sampling site A (Rander) during first year of study

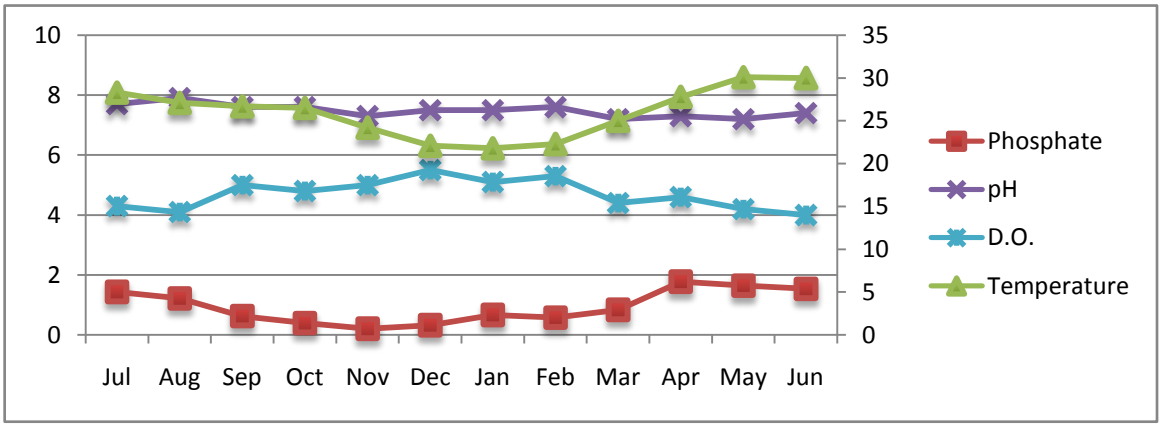

Figure 5. Physiochemical analysis of water from sampling site B (Katargam) during first year of study

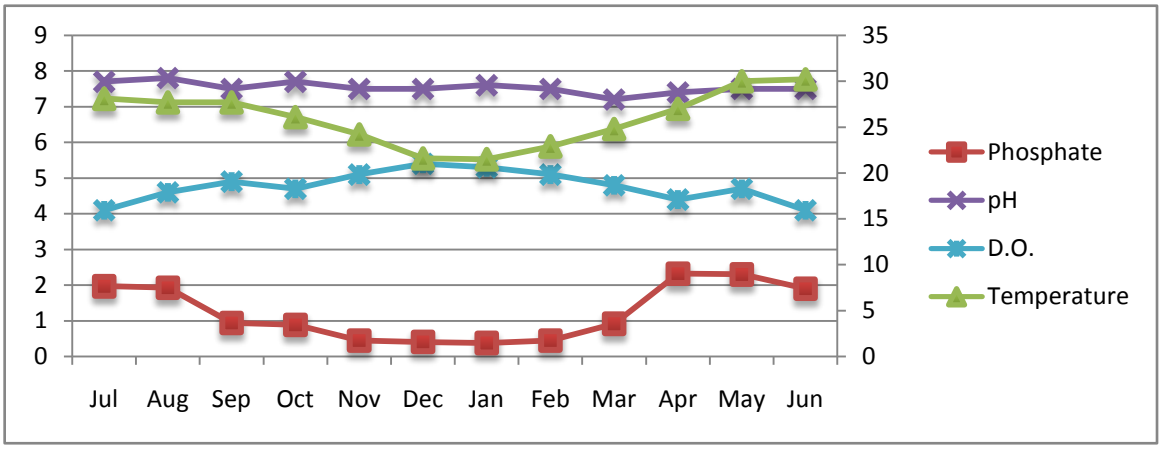

Figure 6. Physiochemical analysis of water from sampling site C (Varachha) during first year of study

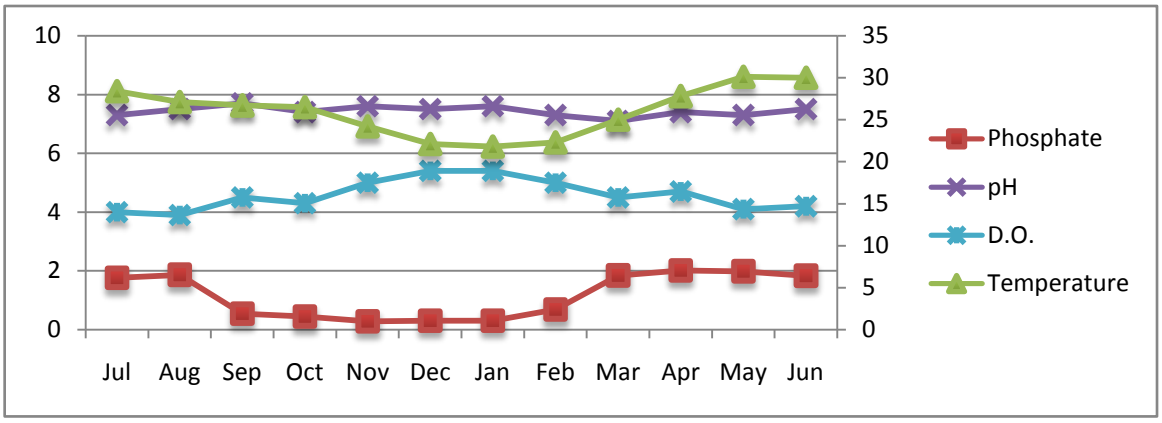

Figure 7. Physiochemical analysis of water from sampling site D (Sarthana) during first year of study

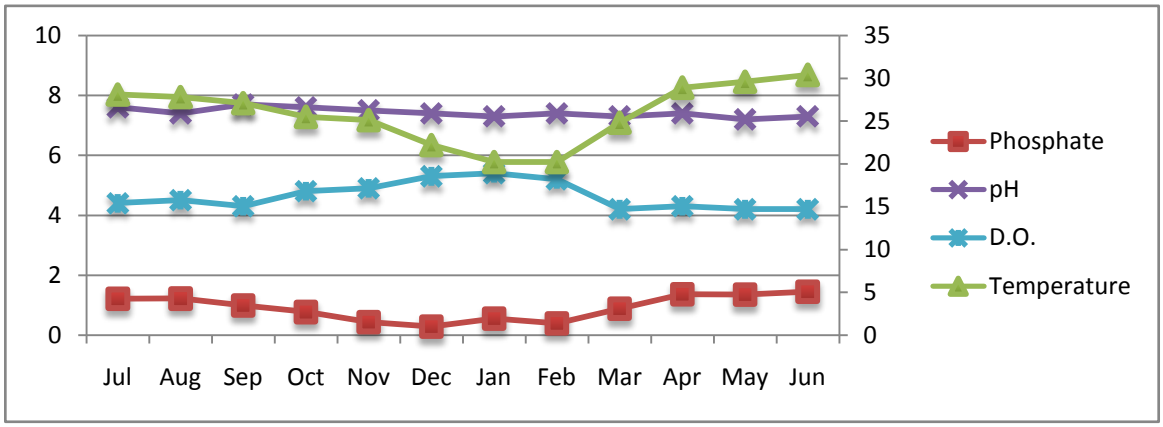

Figure 8. Physiochemical analysis of water from sampling site A (Rander) during second year of study

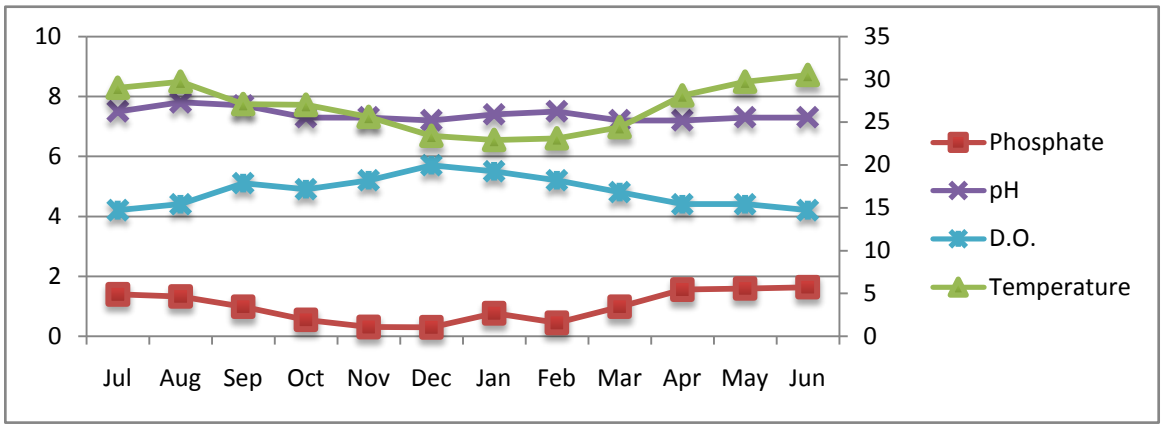

Figure 9. Physiochemical analysis of water from sampling site B (Katargam) during second year of study

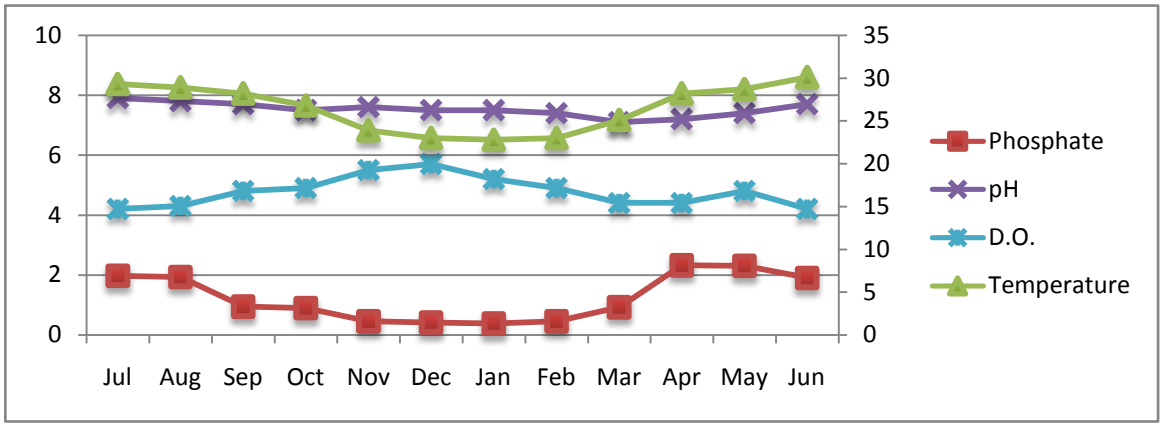

Figure 10. Physiochemical analysis of water from sampling site C (Varachha) during second year of study

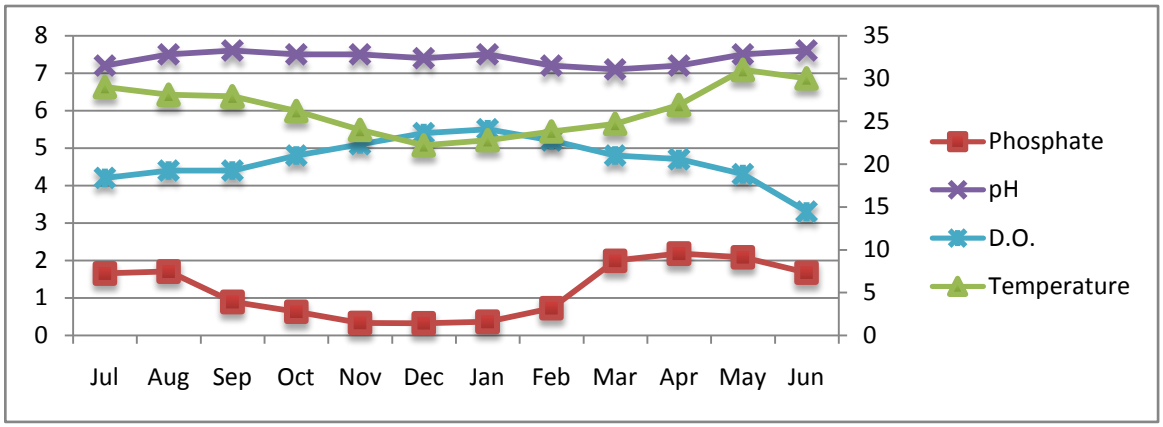

Figure 11. Physiochemical analysis of water from sampling site D (Sarthana) during second year of study

3.3. Indirect Estimation of Cyanobacterial Biomass

Seasonal variation in the Cholorophyll-a concentration in surface water from each sampling site was found as per following.

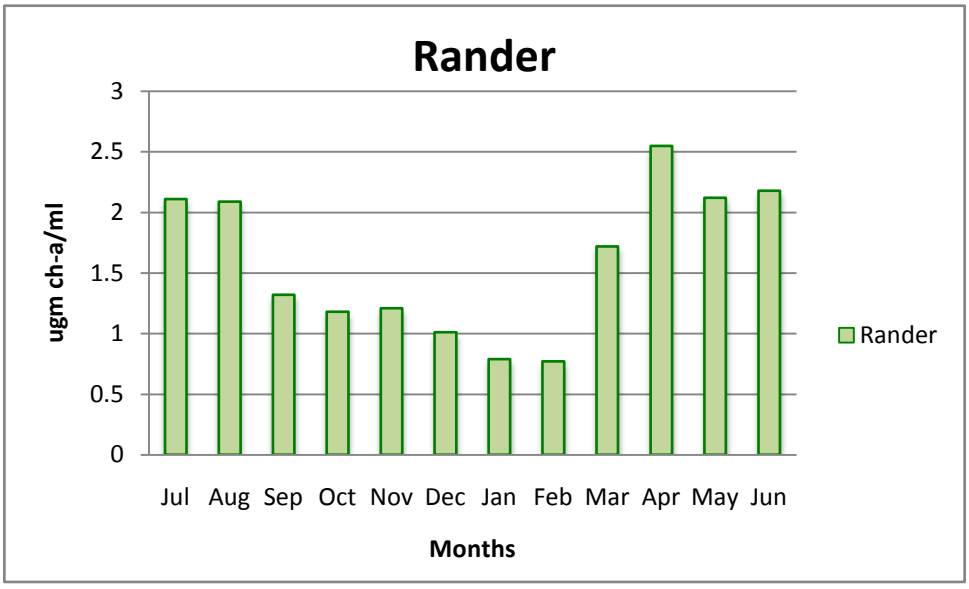

Figure 12. Chlorophyll-a concentration in the water from sampling site A (Rander)

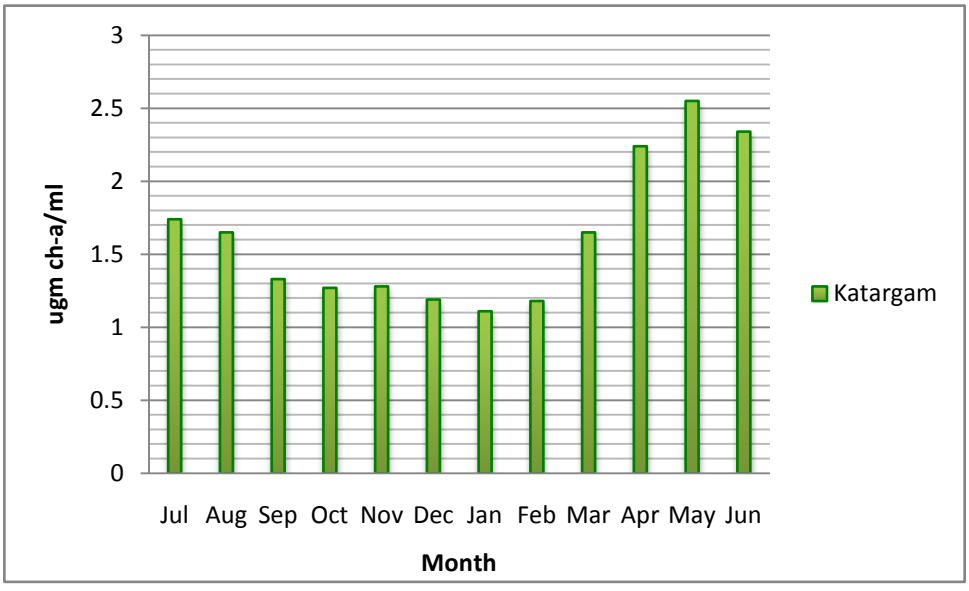

Figure 13. Chlorophyll-a concentration in the water from sampling site B (Katargam)

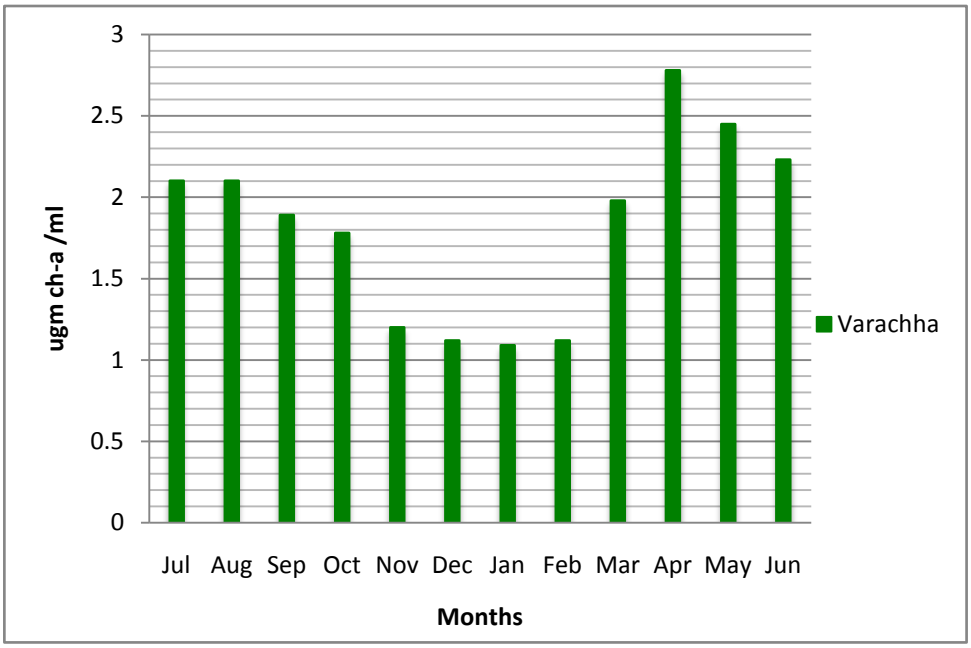

Figure 14. Chlorophyll-a concentration in the water from sampling site C (Varachha)

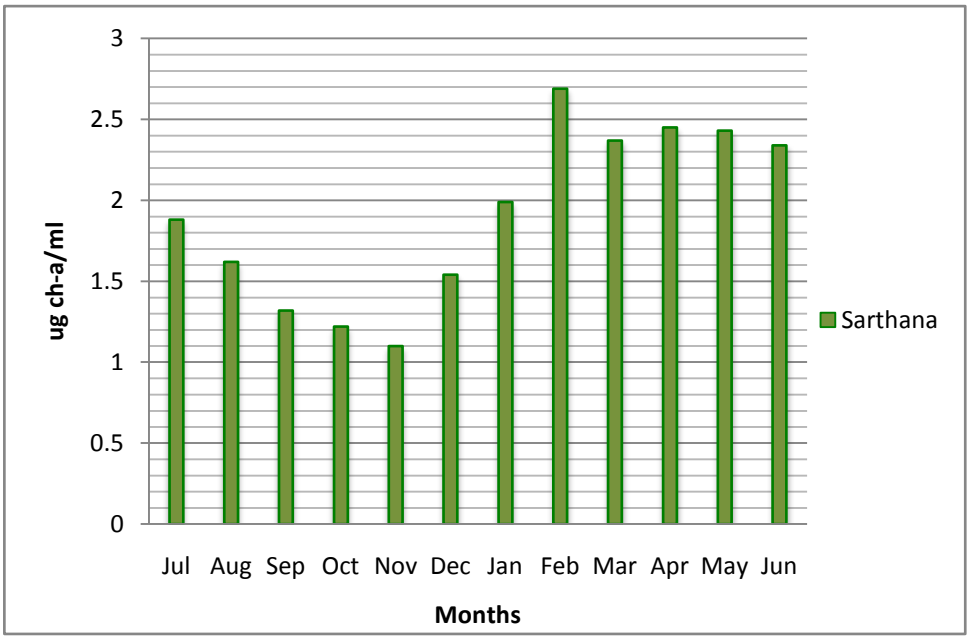

Figure 15. Chlorophyll-a concentration in the water from sampling site D (Sarthana)

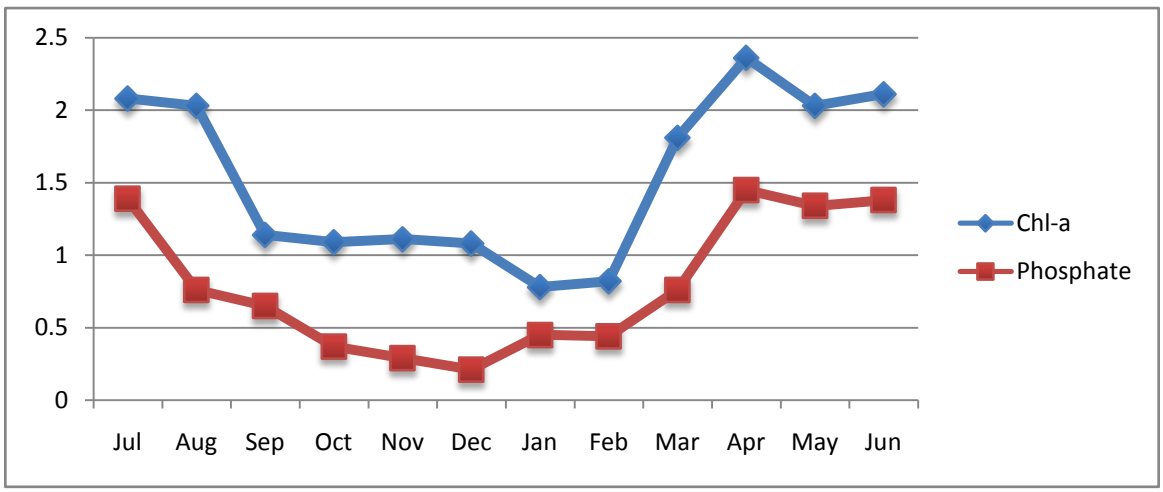

Figure 16. Correlation between Phosphate and Chlorophyll-a (ug/ml) concentrations at sampling site A (Rander)

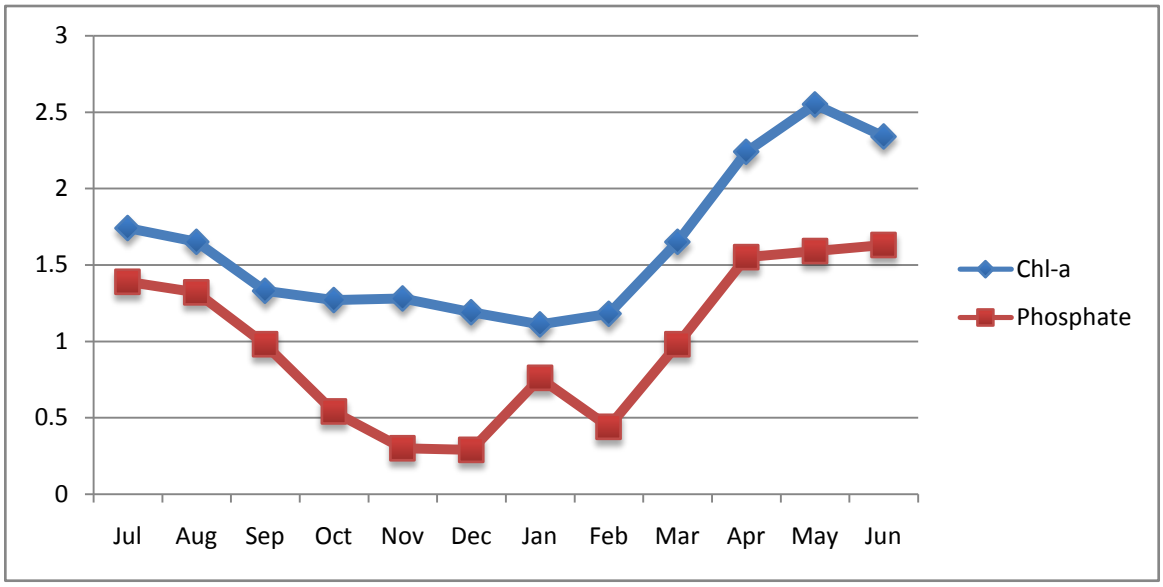

Figure 17. Correlation between Phosphate and Chlorophyll-a (ug/ml) concentrations at sampling site B (Katargam)

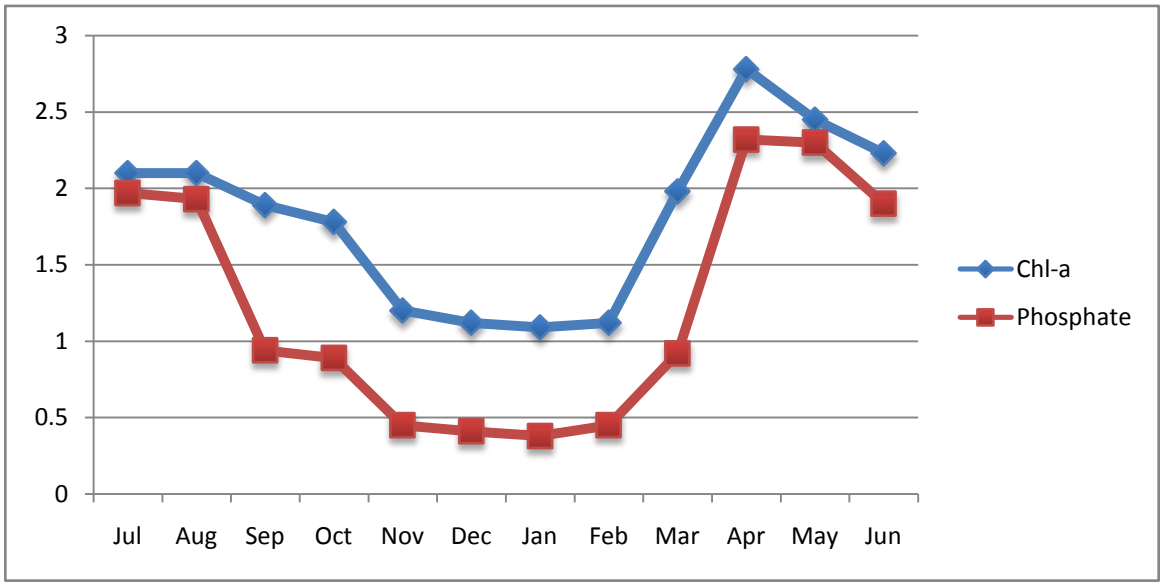

Figure 18. Correlation between Phosphate and Chlorophyll-a (ug/ml) concentrations at sampling site C (Varachha)

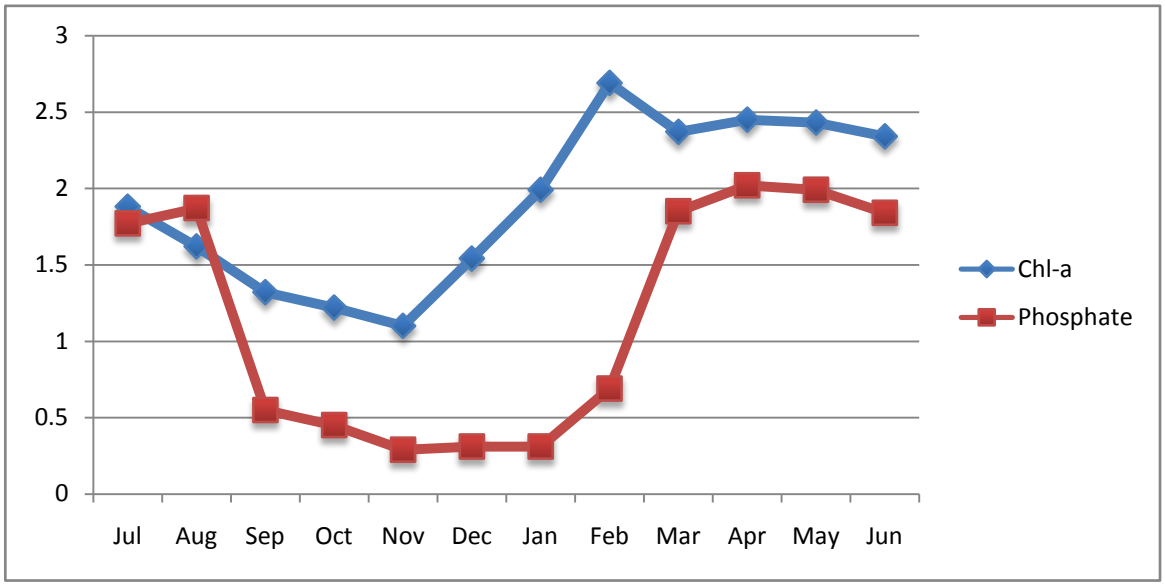

Figure 19. Correlation between Phosphate and Chlorophyll-a (ug/ml) concentrations at sampling site D (Sarthana)

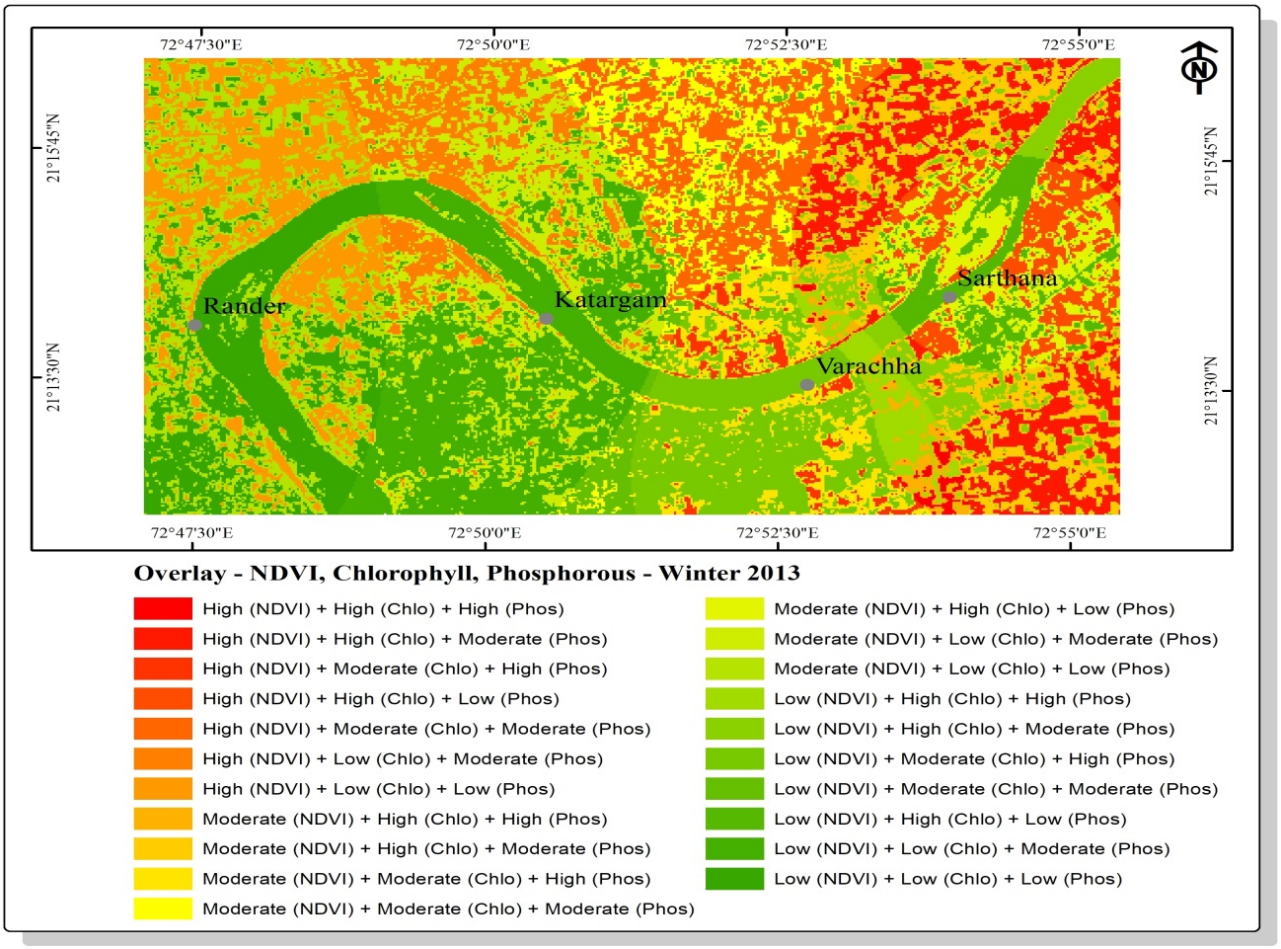

Figure 20. Overlay of NDVI, Chlorophyll, Phosphorous during winter season-1

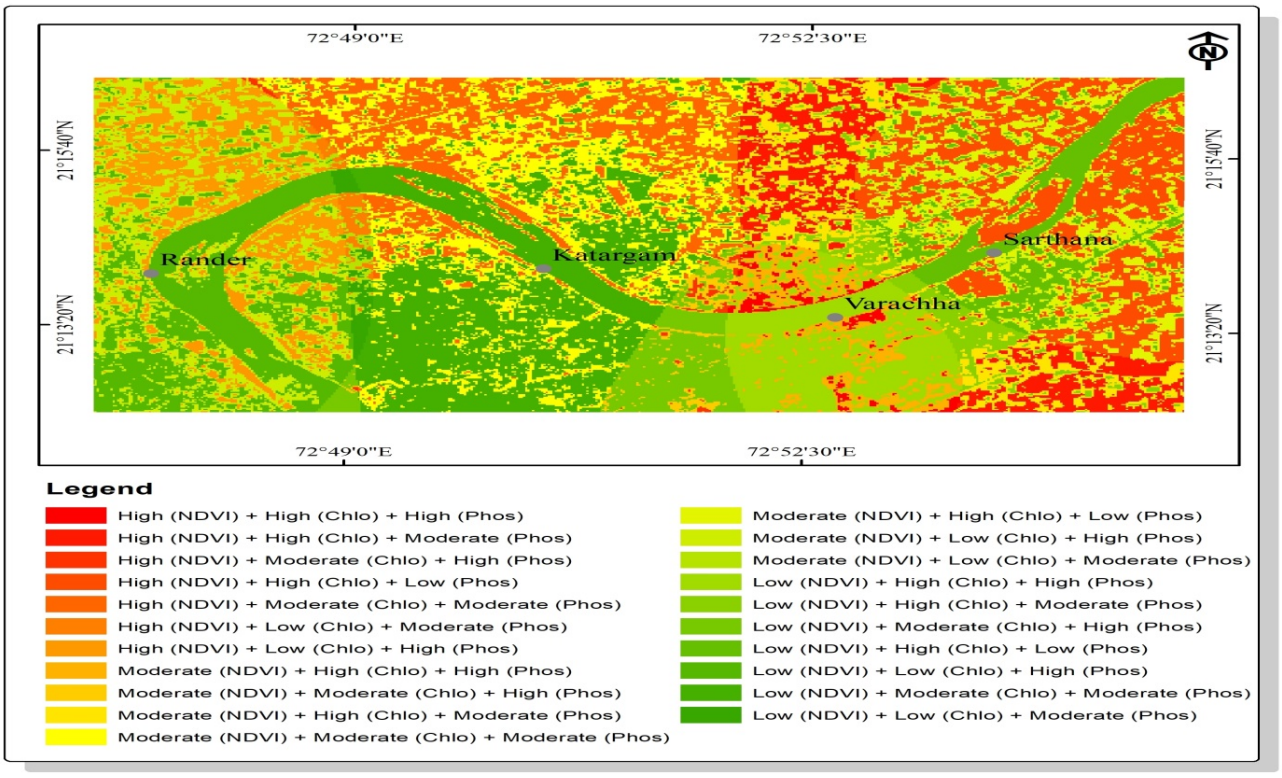

Figure 21. Overlay of NDVI, Chlorophyll, Phosphorous during winter season-2

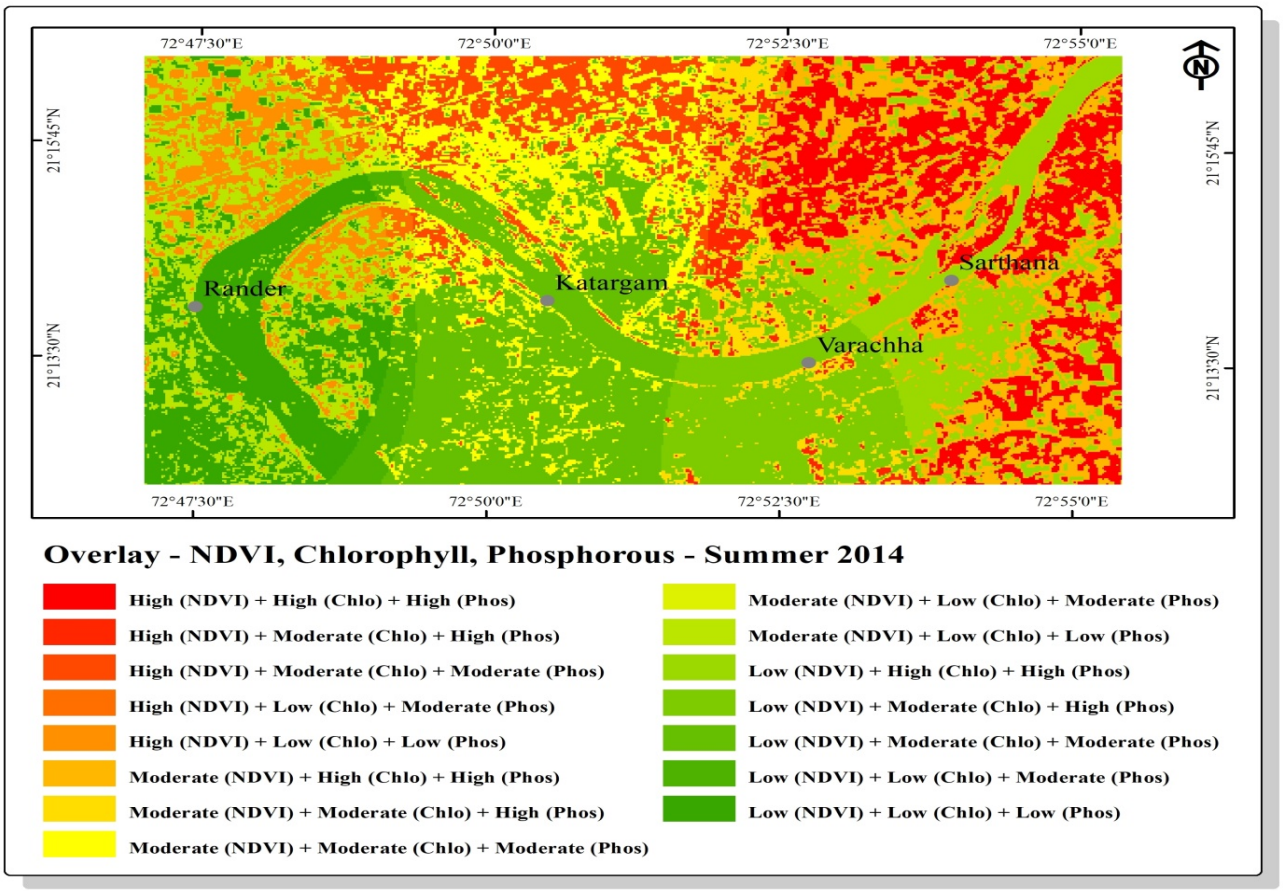

Figure 22. Overlay of NDVI, Chlorophyll, Phosphorous during summer season-1

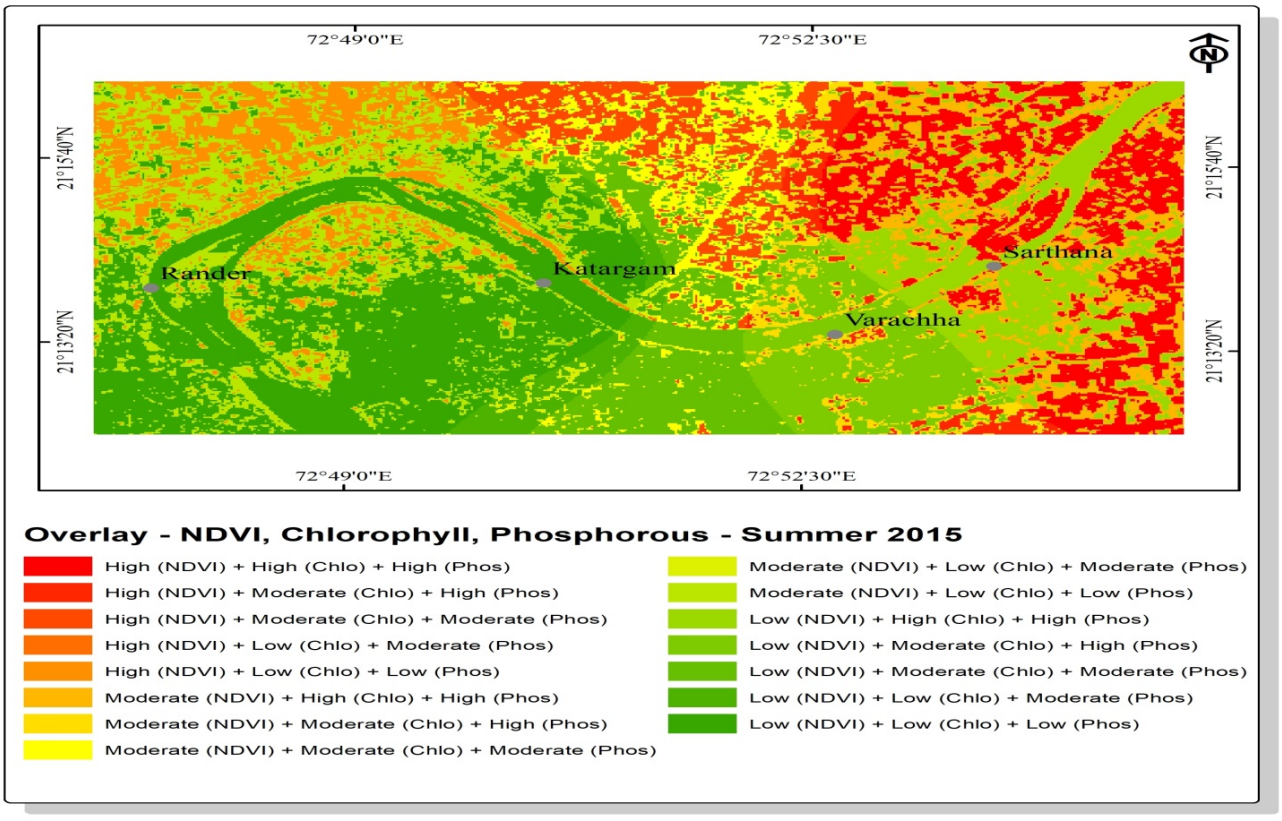

Figure 23. Overlay of NDVI, Chlorophyll, Phosphorous during summer season-2

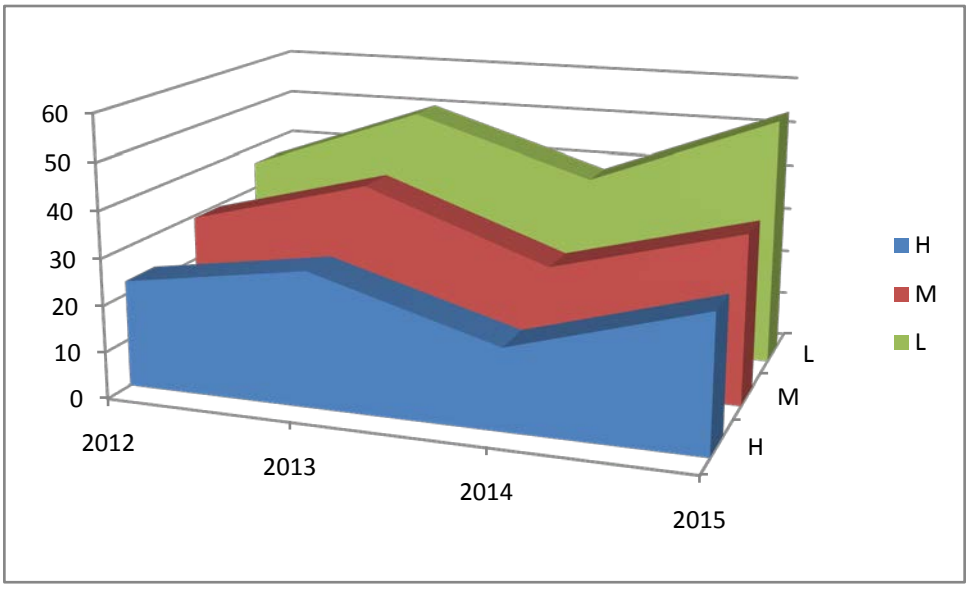

Figure 24. Time series of coverage in km of chlorophyll and NDVI area in Tapi River

3.4. Confirming the Presence of Toxic Cyanobacteria

PCR reactions resulted in detecting the presence of toxin producing aminotransferase (AMT) domain of *mcyE* gene or other equivalent genes in 20 distinct isolates.

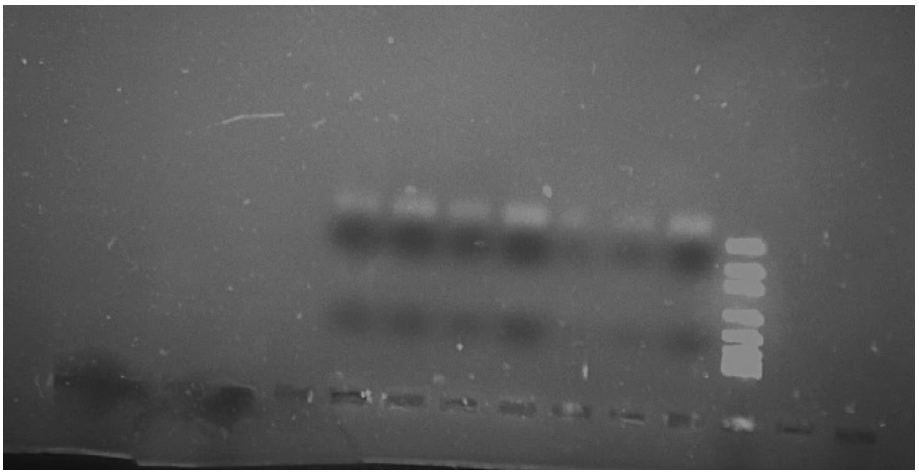

Figure 25. Detection of AMT domain of *mcyE* gene using PCR

4. Discussion

Monthly samples were compared for their phosphate and cyanobacterial biomass content to assess seasonal variation in Tapi water. Results suggested that chlorophyll-a levels peak up in late summer and before monsoon season (Figure 12, Figure 13, Figure 14, Figure 15), especially in April. Further, out of the collected four water works samples, Varachha-the northeast part of the city (upstream river area) was found to have maximum values of chlorophyll-a (2.67 mg/m^3) (Figure 15).

High cyanobacterial population in terms of high chlorophyll-a concentration can also be correlated with higher temperatures as seen in physicochemical analysis. Due to less fluctuations, the values of pH and dissolved oxygen does not seem to have larger impact on cyanobacterial growth.

On microscopy of samples, it was observed that five genera *Oscillatoria*, *Microcystis*, *Planktothrix*, *Cylindrospermopsis* and *Merismopedia* predominated the phototrophic diversity of river water. Some of these cyanobacteria are known to produce hepatotoxins. As observed in (Figure 16, Figure 17, Figure 18 and Figure 19); phosphate contamination in water is the major cause for the formation of cyanobacterial blooms. There was a strong correlation between seasonal variation in the concentrations of phosphate and chlorophyll-a. It is clearly evident that season and ambient temperature have stimulatory effect on the cyanobacterial biomass. It was measured in terms of chlorophyll-a and values increased to maximum in the month of April and remained considerably high up to June. With advance of monsoon, it gradually decreases due to mixing with estuarine water and dilution. At the onset of winter, a major reduction in chlorophyll-a concentration is observed. These results

were analogous with other similar studies on the global tropical water bodies [19,20,21].

PCR amplification of toxin producing domain of enzyme microcystin synthetases and nodularin synthetases convincingly proves ability of isolates to produce hepatotoxins.

5. Conclusion

Monthly study of phosphate and chlorophyll-a concentrations establish a strong correlation between nutrient contamination and resulting eutrophication in the river. Both of these values peak late in summer before monsoon. Identification of cyanobacteria using microscopy suggests presence of previously known hepatotoxin-producing genera in river that may pose public health hazard. PCR amplification proves genetic basis of toxin production.

Being heavily reliant on the surface water as a source of domestic water supply, presence of cyanobacterial toxins is a major concern for Surat city. This demands further studies on cyanobacterial toxins in the river water. Continuation of this study in terms of advanced molecular technology along with metagenomic profile of river water and study of peptides can throw more light on this problem.

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