RESULTS
AND
DISCUSSION
CHAPTER 4

Characterization of sewage wastewater in terms of physicochemical characteristics and microalgal diversity

4.1. Introduction

Water is indispensable for the sustenance of mankind. In developing countries like India; many communities or tribes reside near various water resources and are directly or indirectly dependent on these for their livelihood. The increasing population load and mixing of contaminated wastewater from different sources e.g. industries or agricultural fields, emphasize the need to preserve our precious water resources for future generations. Additionally, the impact of contaminated wastewater release on the overall health of aquatic bodies is extremely important (Oliveira et al., 2007; Yang et al., 2008; Senthil et al., 2012). Most types of untreated wastewater are usually very rich in nutrients (nitrogen, phosphorus) along with other contaminants (heavy metals, pesticides etc.). Discharge of such untreated sewage wastewater into the water bodies leads to increased nutrient load and eutrophication, formation of algal blooms and imbalance in the ecology of such environments (Heisler et al., 2008). Heavy metals released in the environment enter into the food chain, and exert toxic effects on living organisms by bioaccumulation and biomagnification; which may also lead to loss of key species (Atici et al., 2008; Doshi et al., 2008; Ogoyi et al., 2011). A thorough knowledge about the impact of mixing of contaminated wastewater and development of cost effective technologies for treatment represent the major issues related to wastewater management.

The biota of an aquatic ecosystem comprises micro/macrofauna, besides a wide range of organisms including micro/macrophytes. Microalgae present in wastewater systems can be used as indicators of water pollution (Torres et al., 2008), as they are the primary producers and have a key role in biotic and abiotic interactions of aquatic systems and possess the ability to survive in oligotrophic to eutrophic environments. They also play a role in nutrient sequestration and removal of other contaminants from wastewaters. Studies on microalgal diversity and their associations in the water bodies as biological indicators are helpful in the assessment of water quality (Shanthala et al., 2009). Microalgal diversity of wastewater system has also
been studied earlier (Bernal et al., 2008; Chinnasamy et al., 2010a); however, temporal assessment studies can help to understand the extent of pollution caused by mixing of untreated wastewater on the biota, and enhance our knowledge regarding the species diversity of contaminated wastewater. Microalgae can also be identified which can tolerate and phytoremediate such contaminated sites (Chinnasamy et al., 2010a).

Nutrient composition of water bodies also determines the phytoplankton community structure. Imbalance in the nutrient ratio (N: P) can lead to growth of certain allelochemical producing species, which suppress the growth of other organisms (Graneli et al., 2008). Water quality affects the abundance, species composition, productivity and physiology of these organisms (El-Sheekh et al., 2000). However, the complex inter-relations between the algal communities and nutrient levels in wastewater still needs in depth analyses. Since the community structure generally fluctuates with the changes in the nutrient composition of the wastewater (Borchardt, 1996), it is important to study the microalgal dynamics in response to different environmental conditions and fluctuations in the nutrient level of these wastewaters. However, reports on modulation of microalgal community structure due to qualitative and quantitative changes in nutrients in wastewater are scarce. The present investigation describes a systematic study in which physicochemical and nutrient characteristics of a wastewater channel and their inter-relationships with algal diversity were analyzed at monthly intervals, over a period of one year.

4.2. Materials and methods

4.2.1. Study area and sampling of wastewater

The details of sampling site are described in section 3.1. Sewage wastewater samples were collected in clean plastic bottles from January - December 2012, at monthly intervals from several points. Pooled samples were transported to laboratory, stored at 4°C and used for further analyses.

4.2.2. Selection of sampling time

Details regarding the criteria for selection of sampling time intervals are described in section 3.1 (on the basis of results, tabulated in Table 4.1).
4.2.3. Collection, identification and their diversity analyses

The details for collection and identification of microalgae, and their diversity analyses are given in section 3.7.

4.2.4. Analytical Procedures

Six samples of sewage wastewater were collected from different points within the channel and pooled for the analyses of physicochemical characteristics and heavy metals at monthly intervals, based on standard protocols. Quantification of physicochemical parameters viz. colour, temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, alkalinity, acidity, chlorides, carbonates, bicarbonates, calcium, hardness, free CO\textsubscript{2}, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate (NO\textsubscript{3}-N), nitrite (NO\textsubscript{2}-N), ammonia (NH\textsubscript{3}-N) and phosphate (PO\textsubscript{4}-P) was carried out using the standard methods for water and wastewater (APHA, 2005). Parameters viz. colour, temperature, pH, EC, TDS, salinity and DO were analysed in situ. Heavy metal analyses [Chromium (Cr), Cobalt (Co), Lead (Pb), Arsenic (As) and Cadmium (Cd)] was carried out by using WD-XRF spectrometer (Wavelength Dispersive X-Ray Fluorescence Spectrometer); Model: S8 TIGER, Make Bruker, Germany. All the calibration curves for heavy metal analyses were made using standard heavy metal solutions of Cr(NO\textsubscript{3})\textsubscript{3}, H\textsubscript{3}AsO\textsubscript{4}, Pb(NO\textsubscript{3})\textsubscript{2}, Co(NO\textsubscript{3})\textsubscript{2} and Cd(NO\textsubscript{3})\textsubscript{2} for Cr (III), As (V), Pb (II), Co (II) and Cd (II) respectively (Merck, Germany).

4.2.5. Statistical analyses

The statistical analyses were performed using the software Statistical package for Social Sciences (SPSS Version 16.0) as described in section 3.15.

4.3. Results

4.3.1. Water quality

Preliminary studies on the variations in physicochemical characteristics of sewage wastewater at 2 h intervals, revealed the highest EC, TDS, salinity (1930 $\mu$S cm\textsuperscript{-1}, 1350 mg L\textsuperscript{-1} and 983 mg L\textsuperscript{-1} respectively) at 12:00 noon. Similarly, the amount of nutrients (NO\textsubscript{3}-N, NH\textsubscript{4}-N and PO\textsubscript{4}-P) was also found to be highest (71.93, 30.64 and 3.76 mg L\textsuperscript{-1} respectively) at this time (Table 4.1). Therefore, the time interval between
11:30 a.m. - 12.30 noon was selected for collection of samples, on a monthly basis, during the present study.

**Table 4.1.** Diurnal variation in physicochemical characteristics of sewage wastewater of channel at different time intervals (October, 2011)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10 am</th>
<th>12 noon</th>
<th>2 pm</th>
<th>4 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature (visual</td>
<td>Turbid</td>
<td>Turbid</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>observations)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.1</td>
<td>23.3</td>
<td>23.9</td>
<td>23.4</td>
</tr>
<tr>
<td>pH</td>
<td>8.7</td>
<td>8.6</td>
<td>8.4</td>
<td>8.5</td>
</tr>
<tr>
<td>EC (μS cm⁻¹)</td>
<td>1330.0 ±9.0d</td>
<td>1930.0 ±11.0b</td>
<td>1803.0 ±4.0b</td>
<td>1720.0 ±7.0c</td>
</tr>
<tr>
<td>TDS (mg L⁻¹)</td>
<td>938.0 ±4.0d</td>
<td>1350.0 ±6.0a</td>
<td>1290.0 ±4.0b</td>
<td>1220.0 ±5.0c</td>
</tr>
<tr>
<td>Salinity (mg L⁻¹)</td>
<td>677.0 ±4.0d</td>
<td>983.0 ±3.0a</td>
<td>950.0 ±7.0b</td>
<td>896.0 ±6.0c</td>
</tr>
<tr>
<td>DO (mg L⁻¹)</td>
<td>1.0 ±0.001</td>
<td>0.4 ±0.001</td>
<td>1.1 ±0.002</td>
<td>0.4 ±0.000</td>
</tr>
<tr>
<td>PO₄-P (mg L⁻¹)</td>
<td>3.442 ±0.02b</td>
<td>3.764 ±0.001a</td>
<td>2.618 ±0.05c</td>
<td>2.319 ±0.01d</td>
</tr>
<tr>
<td>NO₂-N (mg L⁻¹)</td>
<td>33.792 ±0.9d</td>
<td>71.925 ±2.4a</td>
<td>38.903 ±1.9c</td>
<td>62.490 ±1.3b</td>
</tr>
<tr>
<td>NO₃-N (mg L⁻¹)</td>
<td>26.384 ±1.2c</td>
<td>30.639 ±0.9a</td>
<td>29.143 ±0.7b</td>
<td>29.118 ±1.02a</td>
</tr>
</tbody>
</table>

*Not detected; Values given are mean of n samples ± S.D., where n = 6 and superscripts (a, b etc.) indicate DMRT ranking in respective rows.

The various physicochemical characteristics showed a significant variation in terms of concentration and composition of different chemical constituents studied. The sewage wastewater was turbid in most of months of the study period. The pH of wastewater showed values ranging from 7.9 – 8.4. The highest EC, TDS, salinity, alkalinity, chloride, water hardness and Ca content were observed in February and these parameters (except alkalinity and Ca content) were lowest in September (Table 4.2 and 4.3). The highest free CO₂ and acidity were recorded in December (Table 4.2).

COD levels in sewage wastewater samples in our study showed a wide variation ranging from 1200 – 14000 mg L⁻¹ (Figure 4.1) and were higher from October to December (Figure 4.1). On the other hand, an opposite trend was recorded in BOD levels with relatively low values from October to December (Table 4.3). The
DO in sewage wastewater did not exhibit any distinct trend and was not detectable, in most of the months during the year of this investigation (Table 4.2).

The sewage wastewater in our study was found to be rich in nutrients and reflected a high variation in its enrichment levels during the study period. PO₄-P levels of the sewage wastewater did not show much variation during this period, however, the highest PO₄-P was observed in April (Table 4.4). NO₂-N levels in the sewage water were very low and were detectable only during the summer season, whereas, NH₄-N and NO₃-N levels in sewage wastewater revealed wide variation (Table 4.4, Figure 4.1a). The highest NH₄-N levels were observed in March (Table 4.4), while, the highest NO₃-N level was recorded in June (Figure 4.1a). High species richness in summer months (particularly in June) was correlated with the high NO₃-N level and low COD levels (Figure 4.1a).

The sewage wastewater in this study also showed the presence of heavy metals viz. Cr (III), Co (II), Pb (II), As (V) and Cd (II). The level of these heavy metals varied in terms of their presence and concentration throughout the year (Figure 4.1b). Cr (III) was observed throughout the year, with its concentration varying from 3 – 4 mg L⁻¹. Higher Cd concentrations were observed in September and November. The highest total heavy metal concentration i.e. Cr (4 mg L⁻¹), Co (4 mg L⁻¹), Pb (2 mg L⁻¹) and As (3 mg L⁻¹) was recorded in December. On the other hand, higher number of heavy metals was recorded in March, September, October and December (Figure 4.1b).

4.3.2. Microalgal diversity

The sewage wastewater samples in this study harboured microalgae belonging to the different divisions viz. Cyanophyta, Chlorophyta, Bacillariophyta, Xanthophyta and Euglenophyta (Plate 4.1, 4.2 and Figure 4.2a). Microalgae belonging to all divisions, except Xanthophyta and Euglenophyta were present in most of the months (Figure 4.2a). A total of 27 genera belonging to different divisions were recorded during the study period. The commonly distributed genera were Phormidium, Oscillatoria, Lyngbya, Limnothrix, Anabaena, Chroococcus, Ulothrix, Chlorella, Chlorococcum, Nitzschia and Navicula. Phormidium was the dominant genus, which was found
throughout the year (Plate 4.1 and 4.2). *Vaucheria* sp. was present only in December, while Bacillariophyta members were absent in this month. The highest species richness (17) was observed in June. Cyanophyta was the dominant division, accounting for 34 – 67% of total species richness with an average of 46% throughout the study period (Figure 4.2a).

**Figure 4.1.** Physico-chemical characteristics of sewage wastewater measured at monthly intervals. **a.** NO$_3$-N, COD and species richness (Upper case alphabets represent DMRT ranking for NO$_3$-N values and lower case alphabets represent DMRT ranking for COD values); **b.** COD and Heavy metal concentration.
Table 4.2. Monthly variation in the physicochemical characteristics of the sewage wastewater of channel during the year 2012

<table>
<thead>
<tr>
<th>Month</th>
<th>pH</th>
<th>EC (µS cm⁻¹)</th>
<th>TDS (mg L⁻¹)</th>
<th>Salinity (mg L⁻¹)</th>
<th>Alkalinity (mg L⁻¹)</th>
<th>Hardness (mg L⁻¹)</th>
<th>Ca (mg L⁻¹)</th>
<th>Free CO₂ (mg L⁻¹)</th>
<th>Acidity (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>8.1 ± 0.12a</td>
<td>1745 ± 8b</td>
<td>1240 ± 14b</td>
<td>898 ± 11b</td>
<td>330.0 ± 14.6d</td>
<td>437.0 ± 15.0c</td>
<td>137.0 ± 2.5c</td>
<td>418.33 ± 15.5c</td>
<td>687.5 ± 4.3c</td>
</tr>
<tr>
<td>February</td>
<td>8.4 ± 0.11a</td>
<td>1917 ± 19a</td>
<td>1300 ± 19e</td>
<td>997 ± 16d</td>
<td>376.7 ± 12.0a</td>
<td>443.0 ± 13.0b</td>
<td>141.3 ± 7.5b</td>
<td>149.6 ± 3.9c</td>
<td>432.5 ± 6.5a</td>
</tr>
<tr>
<td>March</td>
<td>7.9 ± 0.12d</td>
<td>1615 ± 15c</td>
<td>1150 ± 25f</td>
<td>838 ± 13f</td>
<td>366.7 ± 13.2a</td>
<td>310.0 ± 15.0d</td>
<td>63.3 ± 2.4f</td>
<td>8.5 ± 0.07d</td>
<td>30.83 ± 1.2f</td>
</tr>
<tr>
<td>April</td>
<td>8.2 ± 0.11b</td>
<td>1501 ± 13d</td>
<td>1060 ± 12e</td>
<td>774 ± 16d</td>
<td>341.7 ± 15.8e</td>
<td>309.7 ± 12.1d</td>
<td>141.0 ± 5.1b</td>
<td>8.77 ± 0.05c</td>
<td>12.17 ± 0.29c</td>
</tr>
<tr>
<td>May</td>
<td>8.0 ± 0.11d</td>
<td>1603 ± 15e</td>
<td>1130 ± 11f</td>
<td>823 ± 19d</td>
<td>340.0 ± 15.0e</td>
<td>395.0 ± 15.0e</td>
<td>57.5 ± 1.5d</td>
<td>8.02 ± 0.11c</td>
<td>14.17 ± 0.25c</td>
</tr>
<tr>
<td>June</td>
<td>8.0 ± 0.15d</td>
<td>1404 ± 18b</td>
<td>1010 ± 18g</td>
<td>734 ± 17g</td>
<td>283.3 ± 12.0h</td>
<td>360.0 ± 12.0g</td>
<td>65.7 ± 2.6g</td>
<td>32.12 ± 0.22d</td>
<td>23.0 ± 0.87g</td>
</tr>
<tr>
<td>July</td>
<td>8.4 ± 0.10a</td>
<td>1498 ± 19h</td>
<td>1040 ± 14i</td>
<td>752 ± 16i</td>
<td>235.0 ± 18.0i</td>
<td>299.3 ± 11.1h</td>
<td>79.3 ± 1.2h</td>
<td>4.99 ± 0.13i</td>
<td>27.67 ± 3.79h</td>
</tr>
<tr>
<td>August</td>
<td>8.0 ± 0.10d</td>
<td>1390 ± 23j</td>
<td>982 ± 9k</td>
<td>713 ± 15l</td>
<td>218.3 ± 17.6i</td>
<td>264.0 ± 3.5j</td>
<td>88.7 ± 1.2i</td>
<td>ND*</td>
<td>15.23 ± 0.45i</td>
</tr>
<tr>
<td>September</td>
<td>8.4 ± 0.10a</td>
<td>1045 ± 8l</td>
<td>739 ± 16m</td>
<td>530 ± 16n</td>
<td>256.7 ± 11.2j</td>
<td>222.7 ± 1.2k</td>
<td>58.6 ± 1.9i</td>
<td>6.01 ± 0.67i</td>
<td>47.0 ± 0.87i</td>
</tr>
<tr>
<td>October</td>
<td>8.0 ± 0.10d</td>
<td>1908 ± 9n</td>
<td>1035 ± 13m</td>
<td>990 ± 14o</td>
<td>308.3 ± 17.6d</td>
<td>306.7 ± 14.2c</td>
<td>114.0 ± 4.0c</td>
<td>17.2 ± 0.46c</td>
<td>65.83 ± 1.44c</td>
</tr>
<tr>
<td>November</td>
<td>8.0 ± 0.10d</td>
<td>1450 ± 13m</td>
<td>1020 ± 19l</td>
<td>741 ± 12m</td>
<td>283.3 ± 15.3m</td>
<td>272.0 ± 7.21d</td>
<td>56.7 ± 1.2b</td>
<td>2.71 ± 0.13f</td>
<td>43.67 ± 1.44f</td>
</tr>
<tr>
<td>December</td>
<td>8.2 ± 0.10b</td>
<td>1611 ± 16n</td>
<td>1140 ± 12o</td>
<td>822 ± 11p</td>
<td>310.0 ± 12.0o</td>
<td>377.5 ± 12.0o</td>
<td>65.6 ± 3.9c</td>
<td>1394.8 ± 20o</td>
<td>1107.5 ± 16p</td>
</tr>
</tbody>
</table>

*Not detected; Values given are mean of n samples ± S.D., where n = 6 and superscripts (a, b etc.) indicate DMRT ranking within a column
Table 4.3. Monthly variation in physicochemical characteristics of the sewage wastewater of channel during the year 2012

<table>
<thead>
<tr>
<th>Month</th>
<th>Nature (visual observations)</th>
<th>Chlorides (mg L⁻¹)</th>
<th>Carbonate (meq L⁻¹)</th>
<th>Bicarbonate (meq L⁻¹)</th>
<th>DO (mg L⁻¹)</th>
<th>BOD (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>Turbid</td>
<td>292.5 ± 3.7^c</td>
<td>0.41 ± 0.001^a</td>
<td>0.9 ± 0.01^d</td>
<td>ND*</td>
<td>2.00 ± 0.1^d</td>
</tr>
<tr>
<td>February</td>
<td>Turbid</td>
<td>339.1 ± 2.9^b</td>
<td>ND*</td>
<td>0.9 ± 0.001^d</td>
<td>ND*</td>
<td>2.04 ± 0.01^d</td>
</tr>
<tr>
<td>March</td>
<td>Turbid</td>
<td>274.8 ± 3.9^d</td>
<td>ND*</td>
<td>1.4 ± 0.001^a</td>
<td>ND*</td>
<td>1.83 ± 0.1^f</td>
</tr>
<tr>
<td>April</td>
<td>Turbid</td>
<td>254.9 ± 5.4^e</td>
<td>ND*</td>
<td>1.3 ± 0.02^b</td>
<td>ND*</td>
<td>3.20 ± 0.09^e</td>
</tr>
<tr>
<td>May</td>
<td>Turbid</td>
<td>252.7 ± 2.9^c</td>
<td>ND*</td>
<td>0.8 ± 0.03^e</td>
<td>ND*</td>
<td>2.98 ± 0.09^b</td>
</tr>
<tr>
<td>June</td>
<td>Turbid</td>
<td>240.2 ± 1.1^f</td>
<td>ND*</td>
<td>0.8 ± 0.012^c</td>
<td>1.08 ± 0.001^c</td>
<td>2.32 ± 0.08^c</td>
</tr>
<tr>
<td>July</td>
<td>Opaque</td>
<td>242.8 ± 2.8^d</td>
<td>ND*</td>
<td>1.0 ± 0.01^c</td>
<td>1.82 ± 0.002^b</td>
<td>1.97 ± 0.09^d</td>
</tr>
<tr>
<td>August</td>
<td>Clear</td>
<td>251.3 ± 1.4^c</td>
<td>0.167 ± 0.01^b</td>
<td>0.8 ± 0.03^e</td>
<td>2.55 ± 0.01^a</td>
<td>1.40 ± 0.01^d</td>
</tr>
<tr>
<td>September</td>
<td>Clear</td>
<td>90.4 ± 0.8^i</td>
<td>ND*</td>
<td>0.8 ± 0.001^f</td>
<td>0.33 ± 0.01^j</td>
<td>3.30 ± 0.09^j</td>
</tr>
<tr>
<td>October</td>
<td>Turbid</td>
<td>371.1 ± 5.4^a</td>
<td>ND*</td>
<td>0.8 ± 0.01^e</td>
<td>ND*</td>
<td>0.77 ± 0.05^i</td>
</tr>
<tr>
<td>November</td>
<td>Turbid</td>
<td>197.4 ± 2.8^b</td>
<td>ND*</td>
<td>0.8 ± 0.01^c</td>
<td>0.41 ± 0.001^d</td>
<td>0.91 ± 0.05^b</td>
</tr>
<tr>
<td>December</td>
<td>Turbid</td>
<td>229.0 ± 4.9^g</td>
<td>ND*</td>
<td>0.9 ± 0.01^d</td>
<td>ND*</td>
<td>1.70 ± 0.02^l</td>
</tr>
</tbody>
</table>

*Not detected; Values given are mean of n samples ± S.D., where n = 6 and superscripts (a, b etc.) indicate DMRT ranking in respective columns.
Table 4.4. Nutrient load and temperature of the sewage wastewater of the channel taken at monthly intervals (2012)

<table>
<thead>
<tr>
<th>Month</th>
<th>Water Temperature</th>
<th>NO$_3$-N (mg L$^{-1}$)</th>
<th>PO$_4$-P (mg L$^{-1}$)</th>
<th>NH$_4$-N (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>16.3 ± 2.1$^f$</td>
<td>ND*</td>
<td>3.16 ± 0.06$^d$</td>
<td>8.46 ± 0.50$^{bc}$</td>
</tr>
<tr>
<td>February</td>
<td>20.5 ± 1.5$^f$</td>
<td>ND*</td>
<td>2.78 ± 0.01$^c$</td>
<td>35.39 ± 1.45$a$</td>
</tr>
<tr>
<td>March</td>
<td>26.0 ± 2.6$^{de}$</td>
<td>ND*</td>
<td>3.88 ± 0.05$^b$</td>
<td>35.69 ± 1.90$^a$</td>
</tr>
<tr>
<td>April</td>
<td>27.3 ± 1.1$^{cd}$</td>
<td>ND*</td>
<td>4.56 ± 0.02$^a$</td>
<td>9.40 ± 0.35$^d$</td>
</tr>
<tr>
<td>May</td>
<td>32.9 ± 2.9$^{ab}$</td>
<td>0.030 ± 0.00$d$</td>
<td>3.84 ± 0.02$^b$</td>
<td>20.03 ± 12.0$^c$</td>
</tr>
<tr>
<td>June</td>
<td>34.0 ± 2.4$^g$</td>
<td>0.025 ± 0.001$^c$</td>
<td>3.44 ± 0.04$^c$</td>
<td>29.20 ± 1.6$^{bc}$</td>
</tr>
<tr>
<td>July</td>
<td>27.2 ± 1.7$^{cd}$</td>
<td>0.070 ± 0.003$^a$</td>
<td>1.11 ± 0.03$^h$</td>
<td>23.68 ± 1.1$^{bc}$</td>
</tr>
<tr>
<td>August</td>
<td>29.8 ± 1.3$^{bc}$</td>
<td>0.017 ± 0.001$^f$</td>
<td>1.72 ± 0.04$^g$</td>
<td>2.21 ± 0.06$^c$</td>
</tr>
<tr>
<td>September</td>
<td>29.1 ± 1.8$^{cd}$</td>
<td>0.037 ± 0.001$^c$</td>
<td>3.11 ± 0.13$^d$</td>
<td>24.27 ± 1.7$^{bc}$</td>
</tr>
<tr>
<td>October</td>
<td>23.0 ± 2.3$^{ef}$</td>
<td>ND*</td>
<td>2.78 ± 0.09$^c$</td>
<td>2.13 ± 1.28$^c$</td>
</tr>
<tr>
<td>November</td>
<td>20.9 ± 1.9$^f$</td>
<td>0.059 ± 0.003$^b$</td>
<td>3.92 ± 0.09$^b$</td>
<td>19.18 ± 1.86$^c$</td>
</tr>
<tr>
<td>December</td>
<td>15.6 ± 2.2$^g$</td>
<td>ND*</td>
<td>2.6 ± 0.02$^f$</td>
<td>17.66 ± 1.36$^c$</td>
</tr>
</tbody>
</table>

*Not detected; Values given are mean of n samples ± S.D., where n = 6 and superscripts (a, b etc.) indicate DMRT ranking within a column.

Only three divisions; Cyanophyta, Chlorophyta and Bacillariophyta were taken into account for indices, since only these three groups were present during all the months. Shannon – Weiner and Simpson’s diversity index ranged from 0.56 – 1.078 and 0.375 – 0.653 respectively, indicative of low microalgal diversity (Figure 4.2b). The lowest values of Shannon –Weiner index (0.56) and Simpson’s diversity index (0.375) were recorded in December.

4.3.3. Relationships between physicochemical characteristics, species richness and indices

The study revealed that heavy metals (number and/or their concentration) had a significant effect on the COD, and exhibited a positive correlation of COD level with Cr concentration ($r = 0.73$) and total heavy metal concentration ($r = 0.77$) (Figure 4.3a). COD levels negatively correlated with Shannon - Weiner index ($r = 0.76$), Simpson’s diversity index ($r = 0.79$) and with the species richness ($r = 0.67$) (Figure 4.3b, c and d). Species richness positively correlated with the NO$_3$-N ($r = 0.68$) and water temperature ($r = 0.63$) (Figure 4.3e and f).
Figure 4.2. Microalgal diversity evaluated at monthly intervals and represented as a. Per cent contribution of different divisions of microalgae to the total species richness; b. Diversity indices (Shannon-Weiner and Simpson's diversity index).
Plate 4.1. Microphotographs of dominant cyanobacteria present in sewage wastewater
Plate 4.2. Microphotographs of dominant microalgae present in sewage wastewater. 

- a. Chlorococcum sp.; 
- b. Chlorella sp.; 
- c. Scenedesmus sp.; 
- d. Ulothrix sp.; 
- e. Spirogyra sp.; 
- f. Vaucheria sp.; 
- g. Navicula sp.; 
- h. Nitzschia sp.; 
- i - j. Euglena sp.; 

Scale Bar represents 20 μm except 4.2a (10 μm), 4.2b and c (5 μm).
Figure 4.3. Correlation between physico-chemical parameters and microalgal diversity. a. COD and total heavy metal concentration; b. COD and Simpson’s diversity index; c. COD and Shannon–Weiner index; d. COD and Species richness; e. NO₃-N and Species richness; f. Water temperature and species richness.
4.4. Discussion

The release of untreated industrial and domestic wastewater into natural water systems is leading to eutrophication and proliferation of algal blooms, resulting in the deterioration of our aquatic resources (Yang et al., 2008). Remediation of these sites by any physical, chemical or biological method requires thorough knowledge of the physicochemical and biological characteristics. Microalgae are an important part of eutrophic water system and their composition/diversity is greatly influenced by the composition of nutrients and contaminants in the system. It is well known that the composition and concentration of the chemical constituents in the wastewater varies throughout the day and according to the season as well as depending on the release of wastewater from the industries or domestic drainage into the water channel. The use of native microalgae for the remediation of wastewaters is well documented (Chinnasamy et al., 2010a; Zhou et al., 2012a,b). Therefore, efforts towards bioremediation, especially combating aquatic pollution, should be coupled with deriving in-depth understanding of the micro/macro community dynamics and nutrient fluctuations as well as their inter-relationships. However, the reports on temporal change in microalgal community structure of wastewaters are scarce (Bernal et al., 2008; Chinnasamy et al., 2010a). The present study deals with the fluctuations in the physicochemical characteristics and microalgal diversity of sewage wastewater and their inter-relationships.

A wide variation in the physicochemical characteristics and microalgal diversity of sewage wastewater was observed during the study period. Similarly, wide variations in the physicochemical characteristics of sewage wastewater collected from different sites in Sivakasi, Tamil Nadu, India, were reported (Krishnan et al., 2007). In the present investigation, the lowest values of water hardness and chloride content were recorded in the month of September, which may be due to heavy precipitation during this period. DO is also an important parameter in water quality assessment and the levels of DO (3 – 5 mg L\(^{-1}\)) are an indicator of healthy state of water (Patil and Patil, 2010). In the present study, DO was not detectable in most months of the study period, indicating high pollution load in the sewage wastewater.

Sewage wastewater in this study also exhibited high NO\(_3\)-N, NH\(_4\)-N and COD levels. The amount of NO\(_3\)-N was much above the permissible limit (5 mg L\(^{-1}\)) given
The sewage wastewater also showed the presence of heavy metals (Cr, Co, Pb, As and Cd) which exceeded the permissible limits for drinking water, as prescribed by the WHO (Kumar and Puri, 2012). However, the level of these heavy metals varied in terms of their presence and concentration throughout the year. The observed variation in heavy metal concentrations may be due to the fluctuations in the nature of discharge during the study period. Previous workers have also reported the occurrence of heavy metals in contaminated freshwater bodies - Cr, Cd, Hg and Zn (Ogoyi et al., 2011). Oliveira et al. (2007) reported the presence of Cr, Cd, Cu, Pb, Hg, Mn, and Zn in urban effluents. Chen et al. (2000) found that high concentration of Pb in eutrophic lakes could be due to adjacent agricultural areas or road traffic and exhaust – gases. In the present study, the presence of Pb in sewage wastewater could be due to the run off from the adjacent agricultural fields into the channel. Analyses revealed that water quality parameters, nutrient levels and heavy metal concentration of sewage wastewater show a high degree of variation, which is indicative of fluctuations in the input of contaminants and precipitation during the time period of the study.

Microalgal diversity of the sewage wastewater in the present study revealed the presence of members belonging to different Divisions viz. Cyanophyta, Chlorophyta, Bacillariophyta, Xanthophyta and Euglenophyta. Cyanophyta was the dominant group throughout the study period and accounted for 34 – 67% of total species richness with an average of 46%. This is in agreement with the published work of various researchers, who observed the predominance of Cyanophycean members in different wastewater environments (Vasconcelos and Pereira, 2001; Badr et al., 2010; Martins et al., 2010). Vasconcelos and Pereira (2001) reported that cyanobacteria frequently dominated the facultative and maturation pond of wastewater treatment plant and constituted 15.2 - 99.8% of the total phytoplankton density. Similarly, Badr et al. (2010) also observed the frequent dominance of cyanobacteria in wastewater treatment plant with 2.2 - 97.8% of total phytoplankton density. On the other hand, Chlorophyta was observed as the dominating group in carpet mill effluent where pH ranged from slightly acidic to neutral 6.54 – 7.18
(Chinnasamy et al., 2010a). Ghosh et al. (2012) observed that 34% of the population comprised Chlorophycean members, followed by cyanobacteria (28%) in Santragachi lake (pH 6.8 – 7.2), West Bengal, India.

pH is one of the major factors controlling growth, establishment and the overall diversity of different groups. The neutral- alkaline pH is known to support faster growth and establishment of Cyanophycean species, than that of other microalgal groups (Nayak and Prasanna, 2007). In the present investigation, the predominance of Cyanophycean genera can be directly related to the neutral to alkaline pH (7.4 – 8.5) of sewage wastewater. Senthil et al. (2012) also reported cyanobacteria as one of the dominant groups of algae in rubber industry effluent with pH ranging from 7.28 – 7.85.

Species diversity is usually used in monitoring the ecological changes, and expressed through indices. These indices are derived from quantitative data and expressed as scores. There are many types of indices used in ecological studies, but the Shannon–Wiener Index is the most commonly employed, as it is not greatly affected by sample size, and is useful in indicating the pollution and trophic status of aquatic bodies (Spellerberg, 2008; Ghosh et al., 2012). In the present study, the lowest values of Shannon –Weiner index (0.56) and Simpson’s diversity index (0.375) were recorded in the month of December. This is well supported by the data on the higher pollution load (COD and total heavy metal concentration) in this month. Diversity indices were negatively correlated with the COD levels. Shanthala and co-workers (2009) also observed lesser diversity of phytoplankton and a negative correlation of diversity indices with the pollution level. Although, the NO$_3$-N level was high during this period (December), the observed high COD levels might be responsible for low species diversity in this month. On the other hand, the highest species richness was recorded in summers (particularly in June), which is correlated with high NO$_3$-N and low COD level. Bernal et al. (2008) also reported a significant negative correlation of microalgal biomass with COD and a positive correlation with NO$_3$-N level. Similarly, Kim et al., (2004) also observed the existence of a correlation between the prevalence of cyanobacterial species and levels of pollution. However, in this study, species richness was also positively correlated with the water temperature, which was reflected by the higher species diversity observed in the summer season. Similarly, Muller (1994) found a positive correlation of biomass and growth of algae with water
temperature. In another study, higher temperature was found to support the growth and density of epipelic algae in the Balikli Dam Reservoir (Kolayli and Sahin, 2009). Therefore, factors other than nutrient composition can also be responsible for the change in community structure. The biology of such complex wastewater systems is probably dependent upon the interaction among the different types of factors such as climate, concentration of nutrients and heavy metals, besides species interaction and production of chemicals by algae (Chinnasamy et al., 2010a).

The N: P ratio is an indicator of the limiting factors of growth of algae in eutrophic water bodies (Yang et al., 2008). When the P concentration in water is low vis a vis N, it can be a limiting factor for inducing eutrophication or algal blooms (Zhao, 2004). Algal species, which have the potential to compete and survive in these nutrient limiting conditions, dominate the scenario. Such nutrient conditions can also stimulate the production of allelochemicals in certain microalgal species and exert an adverse effect on other algae, thereby inhibiting their growth (Graneli et al., 2008). Sewage wastewater in the present study was found to be very rich in N, and N:P >16 indicate that P is the limiting factor for the growth of microalgae. Such conditions can lead to the dominance of certain microalgal species (particularly cyanobacteria), well known for the production of allelochemicals and toxin production, which may enhance their competitive ability (Graneli et al., 2008).

In the present study, the indices of microalgal diversity showed a positive correlation with nutrients and a negative correlation with COD and heavy metal concentrations. The highest microalgal species richness was recorded in the month of June, due to high NO₃-N and low COD levels. Low values of Shannon-Weiner and Simpson’s diversity indices revealed that sewage wastewater was heavily polluted, resulting in low microalgal diversity. Phormidium sp. is a robust organism, as it was present throughout the year and could tolerate high COD and heavy metal concentrations.