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# Influence of seasonal variation in water quality on the microalgal diversity of sewage wastewater



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#### ABSTRACT

An investigation was undertaken to assess the variation in microalgal diversity vis a vis physicochemical characteristics of sewage wastewater at monthly time intervals. Diversity analyses revealed the presence of algal members belonging to all major divisions, with a predominance of Cyanophyta. Shannon-Wiener and Simpson's diversity indices illustrated low microalgal diversity in sewage wastewater. Highest chemical oxygen demand (COD) of 14,000 mg L<sup>-1</sup> was recorded in December 2012. Spectrometric analyses of sewage wastewater revealed the presence of heavy metals, with Cr ranging from 3 to 4 mg L<sup>-1</sup> being detected in all the samples collected over the year. A positive correlation was found between COD and total heavy metal concentration (r = 0.77). The indices of microalgal diversity showed a positive correlation with nutrients and a negative correlation with COD and heavy metal concentrations, implying the significant role of these factors in influencing the algal population. *Phormidium* sp. was the dominant genus present throughout the year.

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#### 1. Introduction

Water is indispensible for the sustenance of the life of mankind. In developing countries like India; many communities or tribes reside near various water resources and are directly or indirectly dependent on them 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 these water resources for future generations. Additionally, the impact of contaminated wastewater release on the overall health of aquatic bodies is also extremely important (Oliveira et al., 2007; Senthil et al., 2012; Yang et al., 2008), as untreated wastewater is usually very rich in nutrients (nitrogen, phosphorus) along with other contaminants (heavy metals, pesticides etc.). Discharge of 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 of its treatment is emerging as one of the major issues related to wastewater management.

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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 systems has been studied (Bernal et al., 2008; Chinnasamy et al., 2010). Temporal assessment studies can help to understand the extent of damage caused by mixing of untreated wastewater on the biota, and also enhance our knowledge of species diversity of contaminated wastewater, besides identifying microalgae which can tolerate and phytoremediate such contaminated sites (Renuka et al., 2013a, 2013b).

Nutrient composition of water bodies also determines the phytoplankton community structure. Imbalance in the nutrient ratio (N:P) can lead to the growth of certain allelochemical producing species, which suppresses 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-relation between algal communities and nutrient levels in wastewater still needs in depth analyses. Community structure generally fluctuates with the change in the nutrient composition of the wastewater (Borchardt, 1996). Therefore, it is important to study the microalgal dynamics in response to different environmental conditions

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Table 1	
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Monthly variation in the physicochemical characteristics of the sewage wastewater of canal during the year 2012.

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Month	рН	EC (μS cm <sup>-1</sup> )	TDS $(mg L^{-1})$	Salinity $(mg L^{-1})$	Alkalinity (mg L <sup>-1</sup> )	Hardness (mg L <sup>-1</sup> )	Ca (mg L <sup>-1</sup> )	$\frac{\text{DO}}{(\text{mg L}^{-1})}$	BOD $(mg L^{-1})$
November $8.0 \pm 0.10^{-11}$ $1450 \pm 13^{-11}$ $1020 \pm 15^{-11}$ $741 \pm 12^{-12}$ $283.3 \pm 15.3^{-11}$ $272.0 \pm 7.21^{-11}$ $56.7 \pm 1.2^{-11}$ $0.41 \pm 0.001^{-11}$ $0.91 \pm 0.05$	January February March April May June July August September October November	$\begin{array}{c} 8.1 \pm 0.12^{bc} \\ 8.4 \pm 0.11^{a} \\ 7.9 \pm 0.12^{d} \\ 8.2 \pm 0.11^{b} \\ 8.0 \pm 0.11^{cd} \\ 8.0 \pm 0.15^{cd} \\ 8.4 \pm 0.10^{a} \\ 8.0 \pm 0.10^{cd} \\ 8.4 \pm 0.10^{a} \\ 8.0 \pm 0.10^{cd} \\ 8.0 \pm 0.10^{cd} \\ 8.0 \pm 0.10^{cd} \end{array}$	$\begin{array}{c} 1745\pm8^{b}\\ 1917\pm19^{a}\\ 1615\pm15^{c}\\ 1501\pm13^{d}\\ 1603\pm15^{c}\\ 1404\pm18^{f}\\ 1498\pm19^{d}\\ 1390\pm23^{f}\\ 1045\pm8^{g}\\ 1908\pm9^{a}\\ 1450\pm13^{e} \end{array}$	$\begin{array}{c} 1240 \pm 14^b \\ 1360 \pm 19^a \\ 1150 \pm 25^c \\ 1060 \pm 12^d \\ 1130 \pm 11^c \\ 1010 \pm 18^f \\ 1040 \pm 14^{de} \\ 982 \pm 9^g \\ 739 \pm 16^h \\ 1035 \pm 13^{def} \\ 1020 \pm 19^{ef} \end{array}$	$\begin{array}{c} 898 \pm 11^b \\ 997 \pm 16^a \\ 838 \pm 13^c \\ 774 \pm 16^d \\ 823 \pm 19^c \\ 734 \pm 17^{ef} \\ 752 \pm 16^{de} \\ 713 \pm 15^f \\ 530 \pm 16^g \\ 990 \pm 14^a \\ 741 \pm 12^e \end{array}$	$\begin{array}{c} 330.0 \pm 14.6^{cd} \\ 376.7 \pm 12.0^{a} \\ 366.7 \pm 13.2^{a} \\ 341.7 \pm 15.8^{b} \\ 340.0 \pm 15.0^{b} \\ 283.3 \pm 12.0^{e} \\ 235.0 \pm 18.0^{f} \\ 218.3 \pm 17.6^{g} \\ 256.7 \pm 11.2^{f} \\ 308.3 \pm 17.6^{d} \\ 283.3 \pm 15.3^{e} \end{array}$	$\begin{array}{c} 437.0 \pm 15.0^{3} \\ 443.0 \pm 13.0^{3} \\ 310.0 \pm 15.0^{d} \\ 309.7 \pm 12.1^{d} \\ 395.0 \pm 15.0^{b} \\ 360.0 \pm 12.0^{c} \\ 299.3 \pm 11.1^{d} \\ 264.0 \pm 3.5^{f} \\ 222.7 \pm 1.2^{e} \\ 306.7 \pm 14.2^{d} \\ 272.0 \pm 7.21^{e} \end{array}$	$\begin{array}{c} 137.0 \pm 2.5^{a} \\ 141.3 \pm 7.5^{a} \\ 63.3 \pm 2.4^{fg} \\ 141.0 \pm 5.1^{a} \\ 57.5 \pm 1.5^{h} \\ 65.7 \pm 2.6^{e} \\ 79.3 \pm 1.2^{d} \\ 88.7 \pm 1.2^{c} \\ 58.6 \pm 1.9^{gh} \\ 114.0 \pm 4.0^{b} \\ 56.7 \pm 1.2^{h} \end{array}$	$\begin{array}{c} \text{ND} \\ \text{ND} \\ \text{ND} \\ \text{ND} \\ 1.08 \pm 0.001^c \\ 1.82 \pm 0.002^b \\ 2.55 \pm 0.01^a \\ 0.33 \pm 0.01^e \\ \text{ND} \\ 0.41 \pm 0.001^d \end{array}$	$\begin{array}{c} 2.00 \pm 0.01^{d} \\ 2.04 \pm 0.01^{d} \\ 1.83 \pm 0.01^{e} \\ 3.20 \pm 0.09^{a} \\ 2.98 \pm 0.09^{b} \\ 2.32 \pm 0.08^{c} \\ 1.97 \pm 0.09^{d} \\ 1.40 \pm 0.01^{g} \\ 3.30 \pm 0.09^{a} \\ 0.77 \pm 0.05^{i} \\ 0.91 \pm 0.05^{h} \end{array}$

ND - not detected; values given are mean of n samples  $\pm$  S.D., where n = 6 and superscripts (a, b...) indicate DMRT ranking within a column.

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 canal and their inter-relationships with algal diversity were analyzed at monthly intervals, over a period of one year.

#### 2. Materials and methods

#### 2.1. Study area and sampling of wastewater

Sewage wastewater samples were collected in clean plastic bottles from a canal, near the fields, belonging to the research farm of the Indian Agricultural Research Institute (IARI), New Delhi, situated at a latitude of 28°40'N and longitude of 77°12'E, altitude of 228.6 m above the mean sea level (Arabian sea) 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. The mean annual rainfall of Delhi is 650 mm, more than 80% of which generally occurs during the south-west monsoon season (July–September) with a mean annual evaporation of 850 mm.

### 2.2. Collection, identification and their diversity analyses

Microalgae were collected in sampling bottles (a minimum of six aliquots from different points within the canal and pooled) and 4% formaldehyde was added to preserve the samples. Three such replicates were taken for each monthly sampling. In a separate sampling bottle, 1% Lugol's iodine solution was added for quantification of algae. Microalgae were studied using the Neubauer hemocytometer under a light microscope (Axio Cam Cc1 Carl Zeiss Scope.A1) and calculated as

Table 2

Nutrient load and temperature of the sewage wastewater of the canal taken at monthly intervals (2012).

number of organisms  $mL^{-1}$ . Total number of different genera was considered for calculating total species richness and determined as described by Kindt and Coe (2005). Two diversity indices – Shannon-Wiener and Simpson's diversity index were used to analyze the microalgal diversity (Nayak et al., 2009; Shanthala et al., 2009). Identification of microalgae was done using standard monographs; Fritsch (1965a, 1965b); Cyanophyta (Desikachary, 1959; Komárek and Anagnostidis, 1999, 2005); Chlorophyta (Komárek and Fott, 1983; Krishnamurthy, 2000) and Bacillariophyta (Round et al., 1990).

#### 2.3. Selection of sampling time

In a preliminary study, sampling of the sewage wastewater was performed at time intervals of 2 h from 10.00 a.m. to 4.00 p.m. in a day, to ascertain the progressive changes in the nature and amount of chemical constituents in the wastewater for selected physicochemical parameters. On the basis of data generated, the time duration between 11.30 a.m. and 12.30 noon was selected for the sampling of wastewater at monthly time intervals, when highest nutrient loading was detected. Sampling of water and microalgae were performed at the same time for the analyses of physicochemical and biological parameters respectively.

#### 2.4. Analytical procedures

Six samples of sewage wastewater were collected from different points within the canal and pooled for the analyses of physicochemical characteristics and heavy metals at monthly intervals. Quantification of physicochemical parameters viz. temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, alkalinity, acidity, chlorides, carbonate, bicarbonate, calcium, hardness, free CO<sub>2</sub>, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate (NO<sub>3</sub>–N), nitrite (NO<sub>2</sub>–N), ammonia (NH<sub>3</sub>–N)

Month	Water TEMPERATURE	Chlorides $(mg L^{-1})$	Carbonate (meq $L^{-1}$ )	Bicarbonate $(meq L^{-1})$	$NO_2-N$ (mg L <sup>-1</sup> )	$PO_4-P$ (mg L <sup>-1</sup> )	$NH_4-N$ (mg L <sup>-1</sup> )
January	$16.3 \pm 2.1^{g}$	$292.5 \pm 3.7^{c}$	$0.41\pm0.001^{a}$	$0.9\pm0.01^d$	ND	$3.16\pm0.06^d$	$8.46\pm0.50^{de}$
February	$20.5 \pm 1.5^{\rm f}$	$339.1 \pm 2.9^{b}$	ND	$0.9 \pm 0.001^{d}$	ND	$2.78 \pm 0.01^{e}$	$35.39 \pm 1.45^{a}$
March	$26.0 \pm 2.6^{de}$	$274.8 \pm 3.9^{d}$	ND	$1.4 \pm 0.001^{a}$	ND	$3.88 \pm 0.05^{b}$	$35.69 \pm 1.90^{a}$
April	$27.3 \pm 1.1^{cd}$	$254.9 \pm 5.4^{e}$	ND	$1.3 \pm 0.02^{b}$	ND	$4.56 \pm 0.02^{a}$	$9.40 \pm 0.35^{d}$
May	$32.9 \pm 2.9^{ab}$	252.7 ± 2.9 <sup>e</sup>	ND	$0.8 \pm 0.03^{e}$	$0.030\pm0.00^{ m d}$	$3.84 \pm 0.02^{b}$	$20.03 \pm 12.0^{\circ}$
June	$34.0 \pm 2.4^{a}$	$240.2 \pm 1.1^{f}$	ND	$0.8 \pm 0.012^{e}$	$0.025 \pm 0.001^{e}$	$3.44 \pm 0.04^{c}$	$29.20 \pm 1.6^{bc}$
July	$27.2 \pm 1.7^{cd}$	$242.8 \pm 2.8^{f}$	ND	$1.0 \pm 0.01^{c}$	$0.070 \pm 0.003^{a}$	$1.11 \pm 0.03^{h}$	$23.68 \pm 1.1^{bc}$
August	29.8 ± 1.3 <sup>bc</sup>	$251.3 \pm 1.4^{e}$	$0.167 \pm 0.01^{b}$	$0.8 \pm 0.03^{e}$	$0.017 \pm 0.001^{\rm f}$	$1.72 \pm 0.04^{g}$	$2.21 \pm 0.06^{e}$
September	$29.1 \pm 1.8^{cd}$	$90.4 \pm 0.8^{i}$	ND	$0.8 \pm 0.001^{e}$	$0.037 \pm 0.001^{\circ}$	$3.11 \pm 0.13^{d}$	$24.27 \pm 1.76^{bc}$
October	$23.0 \pm 2.3^{ef}$	$371.1 \pm 5.4^{a}$	ND	$0.8 \pm 0.01^{e}$	ND	$2.78\pm0.09^{ m e}$	$2.13 \pm 1.28^{e}$
November	$20.9 \pm 1.9^{f}$	$197.4 \pm 2.8^{h}$	ND	$0.8 \pm 0.01^{e}$	$0.059 \pm 0.003^{ m b}$	$3.92\pm0.09^{ m b}$	$19.18 \pm 1.86^{\circ}$
December	$15.6 \pm 2.2^{g}$	$229.0 \pm 4.9^{g}$	ND	$0.9\pm0.01^{d}$	ND	$2.6\pm0.02^{\rm f}$	$17.66 \pm 1.36^{\circ}$

ND – not detected; values given are mean of n samples  $\pm$  S.D., where n = 6 and superscripts (a, b...) indicate DMRT ranking within a column.

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